A Technical Report for the

ALA PROGRAMME: PHASE II

Third Assessment Report of Faunal and Floral Biodiversity in Sainte Luce's Forest Corridors and Littoral Forest

July 2023

Summary

Madagascar's littoral forests face a variety of threats, including unsustainable logging, increased reliance on natural resources, and climate change. The Sainte Luce littoral forest (SLLF) fragments in the Anosy region, southeast Madagascar, are no exception. The SLLF supports disconnected populations of four Endangered lemur species; numerous amphibian and reptilian species including endemic and locally endemic species; and a rich diversity of floral and invertebrate species.

To increase habitat connectivity, SEED Madagascar's Ala Programme, with the support of local stakeholders, has planted five forest corridors to connect littoral forest remnants with a larger protected forest fragment, S8 North. Monitoring of lemur, amphibian, reptile, and invertebrate populations within the corridors and littoral remnants has been conducted to gain a more detailed understanding of species richness, diversity, and geographic range of disconnected groups of species. This research has been complemented by floral research to monitor Programme progress holistically.

Between May 2019 and May 2023, SEED's Conservation Research Programme was responsible for conducting ecological monitoring of the forest corridors and littoral forest remnants. In this time, 118 nocturnal Visual Encounter Sampling (VES) surveys for lemurs were completed in the corridors and adjoining forest remnants. During this period, there were four observations of lemurs in the corridors (three observations of *C. thomasi* and one *E. collaris*). With multiple observations of *A. meridionalis* and *C. thomasi* in the forest remnants adjoining the corridors, further research into the use of corridors by lemurs will help to gain a more detailed understanding of species richness, diversity and the range of disconnected groups of species. The lemur VES surveys were stopped in October 2022 and were replaced by camera traps, enabling continuous monitoring of the corridors.

Between July 2019 and May 2023, 206 herpetofauna biodiversity surveys were completed with 1,398 observations of 47 species made across all study locations. The most frequently observed species were *Phelsuma lineata* (gecko) and *Mantidactylus tricinctus* (frog). Most observations of species were made in the forest remnants. While Corridor 4 had the highest number of observations, Corridor 3 showed the highest species richness of the corridors, with nine species observed. New herpetofauna species continue to be discovered within the corridors, so it is important that monitoring continues.

Between August 2019 and May 2023, 54 invertebrate biodiversity surveys were conducted, with a total of 1,636 observations of 21 different orders made across the corridors and forest remnants. Orthoptera and Hymenoptera were the most observed orders in the corridor quadrats, whereas Arachnida, Isoptera and Blattodea were the orders observed most frequently in the forest remnant quadrats.

Between July 2019 and May 2023, 162 survival and growth surveys were completed across 10 quadrats in the Ala Programme's five forest corridors. *Acacia* are successfully growing in all of the forest corridors, increasingly providing shade and structure for the natives in Corridors 1 to 4. In June 2023, the mean whole corridor survival rate of native plant species in Corridors 1 to 4 was 91%, which represents a significant increase in comparison to last year. As the Ala Programme's forest corridors become more established in the landscape and average tree height, circumference, and canopy cover increase, it is expected that biodiversity within the corridors will increase.

1 Introduction

1.1 Background

Madagascar is one of the world's highest conservation priorities (Myers et al., 2000). As a megadiverse country (Convention on Biological Diversity, n.d.), Madagascar supports circa 5% of global biodiversity (Ministry of Environment and Forests Madagascar, 2014) and has some of the highest levels of endemism worldwide (Helmstetter et al., 2021). However, forest loss continues to threaten Madagascar's unique flora and fauna with 4.36 million hectares of forest cover lost through deforestation between 2001 and 2021 (Global Forest Watch, 2022). As a result, lemurs have been categorised as one of the world's most threatened groups of mammals (IUCN, 2020), with habitat loss and fragmentation threatening populations across Madagascar (Schwitzer et al., 2013).

Additionally, Madagascar is one of the most impoverished and least developed countries globally, ranking 173/191 in the Human Development Index (UNDP, 2022). 81% of Madagascar's population live below the international poverty line of $2.15 per capita per day (World Bank, 2022). With more than 60% of the population living in rural communities and dependent on natural resources (Landlinks, 2020), many communities are vulnerable to the effects of forest loss.

1.2 Littoral Forests

Littoral forest ecosystems are one of the most threatened ecosystems in Madagascar (Watson et al., 2010) and are considered a national conservation priority (Ganzhorn et al., 2001). Habitat fragmentation and degradation continue to endanger this ecosystem and the myriad of genetically diverse species it supports (Consiglio et al., 2006; Krishnan et al., 2013). The Anosy region in southeast Madagascar contains some of the few remaining viable littoral forests, including the Sainte Luce littoral forest (SLLF). Littoral forests support unusually rich and diverse ecological communities (Bollen & Donati, 2006), including 13% of the country's native flora (Consiglio et al., 2006). The SLLF is one of three larger fragmented littoral forests left in the region, comprising 17 fragments and supporting a large variety of plant species, 83% of which are endemic to Madagascar (Rabenantoandro et al., 2007). Additionally, the SLLF is home to populations of various Threatened species, such as four Endangered lemur species: the Red-collared brown lemur (*Eulemur collaris*), the Southern woolly lemur (*Avahi meridionalis*), the Anosy mouse lemur (*Microcebus tanosi*), and the Thomas' dwarf lemur (*Cheirogaleus thomasi*) (Donati et al., 2020a; Donati et al., 2020b; Donati et al., 2020c; J. Ganzhorn et al., 2020).

However, the SLLF is threatened by a variety of factors. The SLLF supports a highly impoverished and growing community (SEED Madagascar, 2021) who depend on the forests for natural resources (Bollen & Donati, 2006). Irregular rainfall and frequent droughts have depleted groundwater sources (Ashraf et al., 2021), exacerbating edge effects[1] and increasing vulnerability to fire (Cochrane & Laurance, 2002). SLLF fragments are especially vulnerable because they have dry, fire-prone edges which are predominantly adjoined to grasslands that are often burned as part of a traditional land management technique, called *tavy* (Cochrane & Laurance, 2002; Schwitzer et al., 2013). In addition to community use, proposed sand mining operations within many parts of the SLLF by QIT Madagascar Minerals (QMM) threaten to remove 661.80ha of littoral forest, increasing the risk of extinction of locally endemic species (Bollen & Donati, 2006; Temple et al., 2012; Watson et al., 2010).

1.3 Ala Programme

As a response to the threats to the SLLF, SEED Madagascar (SEED) initiated the Ala Programme (*ala* meaning forest in Malagasy). The Programme aims to improve habitat connectivity and species dispersal between Sainte Luce Fragment 8 (S8) and nearby forest remnants (R1, R2, R3, R4) through the creation of five forest corridors (C1, C2, C3, C4, C5). Further, the Programme aims understand and meet community forest resource needs, build

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[1] Edge effect involves microclimatic variations (light, temperature, and humidity) in the forest edge that become unsuitable for organisms adapted to forest interior conditions (Aragón et al., 2015).

sustainable, locally led forest management structures, and contribute to the body of international conservation knowledge regarding forest corridors as a conservation strategy.

Forest corridors facilitate the movement of forest-dependent species between patches of otherwise disconnected habitat. This has been found to increase habitat connectivity, habitat permeability (Andriamandimbiarisoa et al., 2015; Caro et al., 2009; Christie & Knowles, 2015), and mitigate the effects of habitat fragmentation (Christie & Knowles, 2015). Forest corridors can increase gene flow between previously disconnected habitats, supporting larger population sizes (Caro et al., 2009; Pardini et al., 2005; Wan et al., 2018).

Through the establishment of forest corridors, the Ala Programme aims to increase and improve habitat connectivity for the conservation of three Endangered nocturnal lemur species (*Avahi meridionalis*, *Cheirogaleus thomasi*, and *Microcebus tanosi*) which cannot traverse the open land created by fragmentation. The Programme will also conserve a large number of invertebrate and herpetofauna species in the SLLF, including the Critically Endangered *Guibemantis diphonus* (frog) (IUCN SSC Amphibian Specialist Group, 2020) and the Critically Endangered *Phelsuma antanosy* (gecko) (Jenkins et al., 2011a), as well as leaf litter-dependent snake, skink, and gecko species.

The fragment of S8 and its proximal forest remnants was designated as a community protected area as part of QMM's mining offset zone (Temple et al., 2012) and provides crucial lemur habitat. *Avahi meridionali*s have been recorded in the forest remnants surrounding S8, making it imperative to connect R1-R4 to S8 to reduce the risk of inbreeding and other detrimental consequences of isolation. Increasing forest remnant habitat availability may also be crucial for the protection of *Eulemur collaris*. Moreover, an increase in local lemur populations will increase native seed dispersal, which will in turn improve chances of long-term survival for the forest itself.

2 Methodology

Data collected since the beginning of the Ala Programme has yielded a variety of interesting and important observations. However, due to the global COVID-19 pandemic, in-situ capacity was reduced from March 2020, and not all lemur, herpetofauna, and invertebrate surveys were completed as scheduled. Data collection resumed at a normal frequency from January 2022.

2.1 Study Site

The Ala Programme conducts research in five forest corridors between forest fragments in the rural community of Sainte Luce, southeast Madagascar (24° 46' S, 47° 10' E) (Figure 1). The five corridors span 3.72ha and connect a total area of 111ha of littoral forest. Data collection is led by SEED's Conservation Research Programme (SCRP) and started in May 2019, following the planting of C1-C4. C5 was planted in April 2022; floral monitoring is ongoing here and faunal monitoring will start once the corridor is more established.

2.2 Data Collection

Three methodologies have been used for assessing faunal biodiversity within C1-C4: Visual Encounter Sampling (VES), catch-and-release sampling, and camera traps. Additionally, two methodologies have been used for assessing floral biodiversity within C1-C5: survival and growth surveys in quadrats and whole corridor survival surveys. This report summarises the findings of data collected by the Ala Programme over the last four years, from May 2019 until May 2023.

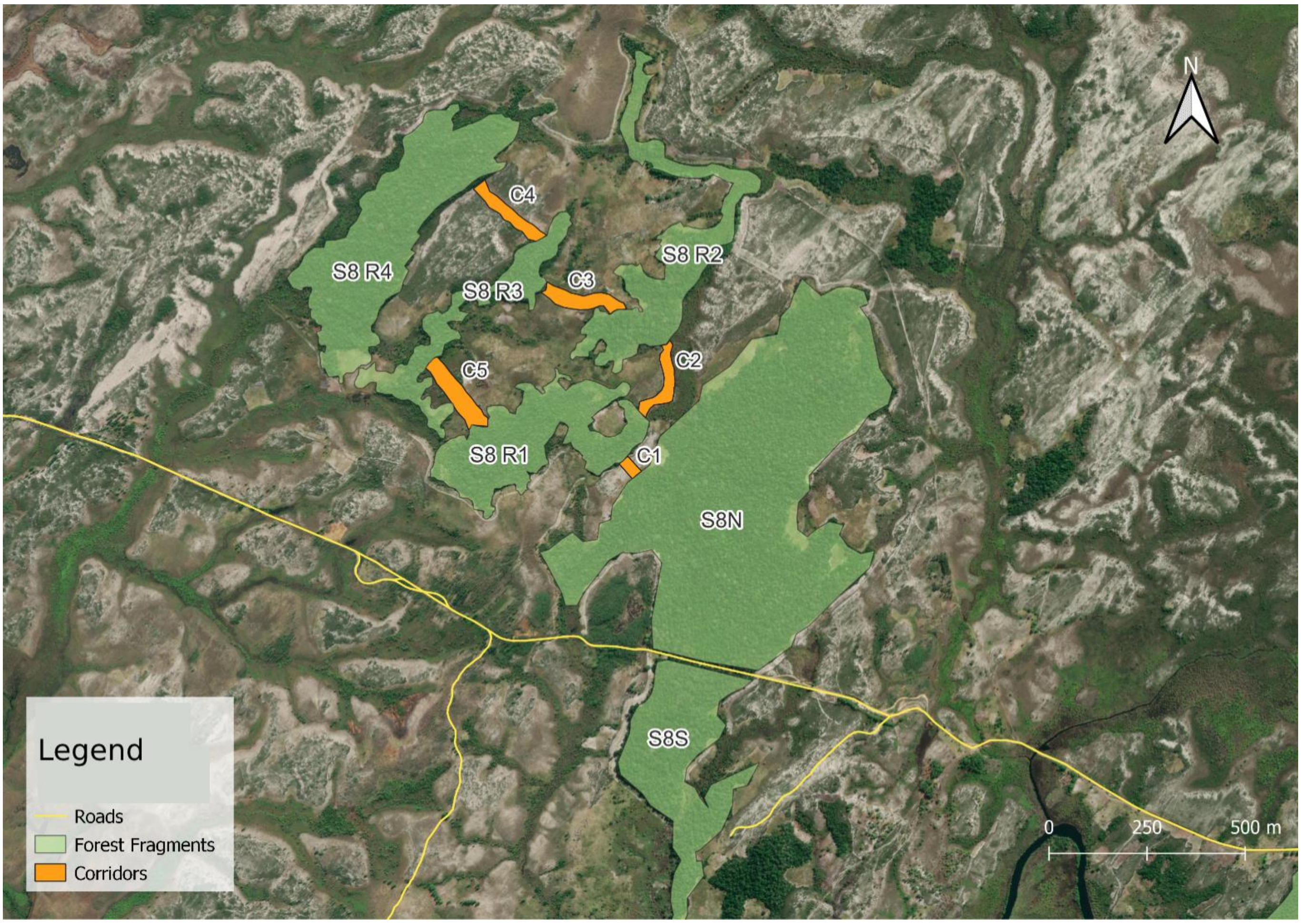

Figure 1: Ala Programme study site.

2.2.1 Lemur and Herpetofauna Visual Encounter Sampling

VES is a way of systematically monitoring an area with standardised effort. It is a robust technique that is often used for long-term datasets to estimate the density of different species (Flint & Harris, 2005; Furnas et al., 2019). VES surveys were conducted using a distance sampling methodology to monitor lemur and herpetofauna diversity and abundance over time. Distance sampling is often used to make estimates of population size and density, and its applications are well-suited to the requirements of the Ala Programme (Fewster et al., 2009). The purpose of this research was to a) establish a baseline population estimate of lemur and herpetofauna species within forest remnants; b) assess how primarily nocturnal lemur populations respond to increasing connectivity as corridors mature; and c) assess the effectiveness of forest corridors for lemur conservation.

Under the Ala Programme, SCRP recorded all observations of lemur and herpetofauna species along a set transect (Hutchens & Deperno, 2009). Transects were established along the length and centre of C1-C4. Transects were also established and maintained within neighbouring forest remnants, R1-R4. Forest remnants were surveyed to provide an ecological baseline that corridors could be compared to. The larger littoral forest fragment, S8 North, was also surveyed as part of SCRP's Long-Term Monitoring Programmes. S8 North is joined to R1 by C1, so it was important to understand lemur abundance and richness of this forest fragment to aid comparison between forest remnants, forest corridors, and this larger littoral forest fragment. Surveys took place in each corridor, remnant, and fragment once every three months. Surveys were conducted during the day for diurnal species of herpetofauna, and at night for nocturnal species of lemur and herpetofauna. Surveys in the forest remnants ended in December 2021 after an ecological baseline was established. Lemur and herpetofauna VES surveys have continued in S8 as part of SCRP's Long-Term Monitoring Programmes.

Lemur VES was conducted after sunset by at least one experienced SCRP staff member and one local expert guide, who moved along each transect at a slow speed (~1km/hr). Torches were used to aid detection. Prior to each survey, contextual data were recorded, including date, number of observers, start time, weather, forest fragment, transect number, lunar phase, and transect direction. At the end of the survey, the time and any additional notes on the survey were included. When an observation was made, several environmental and biological variables were recorded, including time of sighting, species sighted, GPS coordinates of sighting location, distance from transect (m), number of individuals, group spread, sex of lemurs (if able to be determined), tree species occupying, height above ground (m), height of tree occupying (m), circumference of tree (cm), canopy cover, and any additional information of interest. Tree height (distance from tree base to canopy apex) (m) and canopy cover (approximate proportion of intact canopy at the lemur's location) (%) were visually estimated, and tree circumference (cm) was measured using a tape measure at the height of the surveyor's chest. To minimise the disturbance to lemurs, transects were walked by small groups.

Statistical analyses were conducted to check for statistically significant differences in environmental variables between lemur species. Checks for normality were completed, and appropriate tests for each variable run. For normally distributed continuous variables (e.g. height above ground and height of tree occupying), an independent t-test was used. For non-normally distributed continuous variables (e.g. circumference of tree), a Kruskal-Wallis rank sum test was used. For non-normally distributed categorical variables (e.g. canopy cover), Pearson's Chi-Squared test for association was used. Statistically significant relationships were concluded if an alpha value ($p<0.05$) for each test was achieved.

Herpetofauna VES was conducted using the same methodology. Many of the same measurements were taken, with additional metrics recorded, including life stage (juvenile, sub-adult, or adult) and microhabitat (such as in the leaf litter, under a dead branch, on a tree trunk, and others).

Lemur VES surveys in the corridors ended in October 2022 and from this point on lemur populations have been surveyed using camera traps.

2.2.2 Invertebrate Catch-and-Release Surveys

Monitoring invertebrate richness and abundance in the corridors and adjacent forest remnants over time will enable a more rapid assessment of how species composition changes in the corridors as they mature. Invertebrate surveys will contribute to the overall biodiversity monitoring of the Ala Programme as well as SCRP's wider understanding of local invertebrate biodiversity.

Invertebrate biodiversity surveys were conducted in a total of 20 10mx10m longitudinal quadrats, situated in C1-C4, R1-R4, and S8. Three quadrats were positioned within each corridor's interior – one at each end of the corridor and one in the centre. Two more quadrats were positioned within adjoining forest remnants for each corridor, to serve as ecological baselines that corridor invertebrate biodiversity could be compared to.

To monitor invertebrate presence and abundance, each quadrat was surveyed for a total of 10 minutes using VES. The forest or corridor floor and vegetation were scanned for invertebrates, capturing individuals throughout the time period for identification. Three to five SCRP data collectors conducted each survey. Effort was then standardised to account for any change in the number of data collectors. Prior to the survey, contextual data was recorded, including date, start and finish time, number of observers, weather, and percentage cloud cover. When an invertebrate was spotted (excluding flying species), it was collected in a jar or net and identified by an SCRP data collector to morpho-species. Oftentimes, species could only be identified to order, as many species in the SLLF are undescribed. A photographic guide sheet of the different species previously observed by SCRP was used to aid identification, standardise observations, and identify potential new species. A morphological species code (e.g., arach3) was assigned to each species in the identification guide, which was recorded on the data sheet with a tally made for each individual of that species caught. If a species was absent from the identification guide, it was recorded as a new species and identified to order level (e.g., blatt new). New species were photographed and described so that they could be identified and added to the guide. Once identification was complete, each individual was released on the outside of the quadrat to prevent double sampling. Handling time was kept to a minimum to reduce stress for the captured individual.

2.2.3 Camera Trap Trial

In October 2022, SCRP started using camera traps to monitor lemur activity within the corridors, replacing the VES of nocturnal lemur species. Camera traps allow for continuous monitoring of the corridors while reducing anthropogenic disturbance. A trial was completed to identify optimal camera position and settings. Four different locations were selected within C2 and C3 to position the cameras. At the start of the trial camera traps were placed on the forest edge, angled into the corridors. C2 and C3 were selected for their high levels of public foot traffic, indicating how noticeable the camera traps would be once installed. Four different settings were trialled: single photos, a burst of three photos, time-lapse with single photos, and 30-second videos. Camera traps were installed with the highest levels of sensitivity and low time delays between triggers to increase likelihood of capturing species using the corridors. Once installed, cameras were left for three weeks after which the footage was downloaded, reviewed, and then cameras re-installed. Images or videos with no meaningful information in them (i.e. no wildlife) were deleted. Images or videos containing people that we did not have permission to obtain were also deleted. Findings from the trial were evaluated between data collection periods.

From April 2023, there was a phased increase in coverage of camera traps in the corridors. This started with one camera trap per corridor being moved from the forest edge into the middle of the corridor, after obtaining permission from the landowners. In May 2023, there was further expansion in coverage, where camera traps were installed in both C1 and C4, with one placed on the forest edge and one in the middle of the corridor, following the same method as was utilised in C2 and C3.

2.2.4 Survival and Growth

Survival and growth data of *Acacia Mangium* (*Acacia*) and native seedlings (natives) were recorded for seedlings within quadrats. Measurements for survival and growth were collected at initial planting, and at months one, three, six, and 12 after planting for Acacia, and at months one, three, nine and 12 after planting for natives. Data collection on survival and growth continues four times a year for natives, and once a year for *Acacia*. Since the Ala Programme's forest corridors were planted at different times, the date of the first data collection within quadrats differed for each forest corridor (Annex 1). Survival and growth data were collected for labelled seedlings within fixed quadrats in C1-C5. There are two quadrats per corridor, one at the edge and one in the middle, all measuring 10mx10m. All quadrats were visited during daylight hours, with data for survival and growth collected at the same time. General data were recorded, such as quadrat code, seedling identification number, species, and other abiotic factors such as weather and rainfall. Mean survival and seedling condition were recorded through visual cues, such as if the seedlings were alive or dead, wilting, or lost leaves. Seedling condition was rated from 1 to 4 (Table 1). Mean seedling growth over time was calculated by measuring height and Diameter at Breast Height (DBH). Height was measured from the root collar to the apex with a tape measure, if the *Acacia* became too tall to be measured height was visually estimated. The DBH was measured at the middle of the trunk for seedlings <1.30m and at breast height for seedlings ≥1.30m in height (Batcheler, 1985).

Table 1: Descriptions of each of the conditions assigned to the seedlings.

Condition	Description
1	Dead: Seedling has wilted, lost its leaves and turned brown or is not visible
2	Poor: Severe signs of either damage, pest infestation, discolouration, fungi or abnormal growth
3	Fair: Signs of either damage, pest/infestation, discolouration, fungi or abnormal growth
4	Excellent: No signs of damage or pest/infestation, no discolouration or fungi growth and growing steadily.

Additionally, mean whole corridor survival rates were calculated for natives in C1-C4. Seedling survival was recorded via visual observation. The mean whole corridor survival rate was then calculated by dividing the alive seedling count at monitoring intervals by the seedling count at initial planting, and then multiplying by 100. After monitoring was conducted, and the number of dead natives recorded, a replant event was held to return the number of alive natives in the corridor to the seedling count at initial planting.

2.2.5 Opportunistic Sightings

In addition to the five aforementioned methods of data collection, opportunistic sightings of species are recorded outside of scheduled surveys or specific data collection periods, if the species is not seen frequently in the corridors. In such incidence, the species name, number of individuals, sex (if identifiable), location, and date are recorded. Such sightings are particularly helpful at identifying individuals that are not recognised within herpetofauna or invertebrate surveys. Opportunistic observations are not included in data analysis but are noted in this report as they do contribute to understanding of species diversity within the corridors.

3 Results

3.1 Lemur VES

Between May 2019 and October 2022, 118 nocturnal VES surveys for lemurs were completed, in C1-C4 and R1-R4. During this period, there were two observations of *C. thomasi* in C1 and one observation in C4, as well as one observation of *E. collaris* in C2. Two of the three nocturnal lemur species present in the area, *A. meridionalis* and *C. thomasi*, were seen in all four of the forest remnants. *A. meridionalis* was observed most frequently in R1 and R4 (n=4), whilst *C. thomasi* was observed most frequently in R4 (n=11) (Table 2 and Figure 2).

Table 2: Total number of VES surveys and observations for lemurs in the Ala Programme's forest corridors and the surrounding forest remnants between May 2019 and October 2022.

Location	Total Number of Surveys	Total Number of Observations
C1	17	2 (2 *C. thomasi)*
C2	15	1 (1 *E. collaris*)
C3	17	0
C4	18	1 (1 *C. thomasi*)
R1	12	6 (4 *A. meridionalis,* 2 *C. thomasi*)
R2	13	6 (3 *A. meridionalis,* 3 *C. thomasi*)
R3	13	3 (2 *A. meridionalis,* 1 *C. thomasi*)
R4	13	15 (4 *A. meridionalis,* 11 *C. thomasi*)

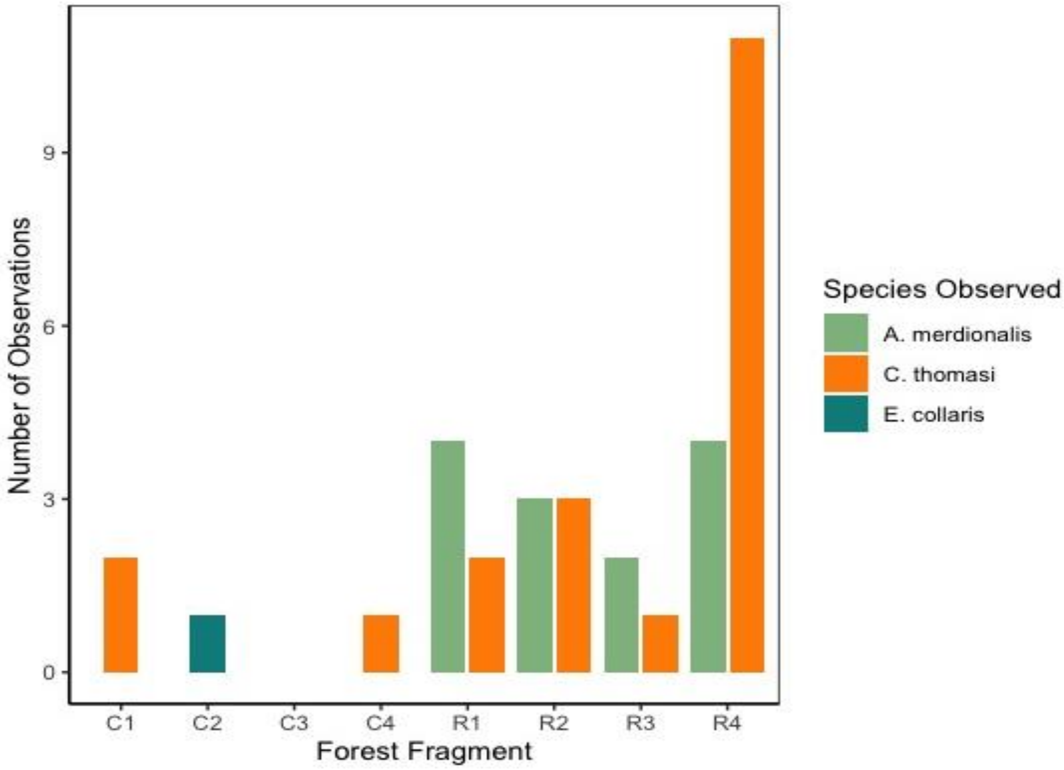

Figure 2: Number of observations of lemurs in the Ala Programme's forest corridors and the surrounding forest remnants between May 2019 and October 2022.

Lemurs were observed in 27 different species of tree during surveys (Figure 3), with most observations being made in *Ampoly* (n=3), *Makaragna* (n=3), and *Nato* (n=3). *A. meridionalis* were observed in 12 species of tree and were found marginally more frequently in *Haramboanjo* (n=2) and *Nato* (n=2). *C. thomasi* were observed in 19 species of tree and were found most frequently in *Makaragna* (n=3). On average, observations were made 7.47m off the ground and 7.40m from the transect. *A. meridionalis* were found on average marginally higher (7.77m) than *C. thomasi* (6.95m), closer to the transect (6.41m) than *C. thomasi* (7.64m), and in taller trees (11.15m) than *C. thomasi* (9.75m) (Table 3). There was one observation of *E. collaris* in C2, where five individuals were seen. *E. collaris* was observed in one species of tree, *Ampoly*, that was 8m tall, and 15m from the transect; this is further away from the transect and in a shorter tree than the other two species of lemur.

Following a check for normality, lemur height and tree height variables were found to be normally distributed, whereas tree circumference and canopy cover values were non-normally distributed. Therefore, a mixture of parametric and non-parametric statistical tests were used to analyse these environmental variables. A Welch two sample t-test was used to compare the mean height lemurs were observed above ground and the mean height of trees lemurs were observed in for *A. meridionalis* and *C. thomasi*. It was found that there was no statistical difference in the height above ground (t(30.4)=0.95288, p-value=0.348) or in the height of tree (t(31)=1.469, p-value=0.1521) between the these two species. A Kruskal-Wallis test was used to compare the differences between canopy cover and tree circumference for each of the species. It was found that there was no difference in the canopy cover ($Chi^2(1)$= 0.22656, p-value=0.6341) or between tree circumference ($Chi^2(1)$=0.54344, p-value=0.461) between the two species. Further data collection is required to verify the significance of differences in habitat use.

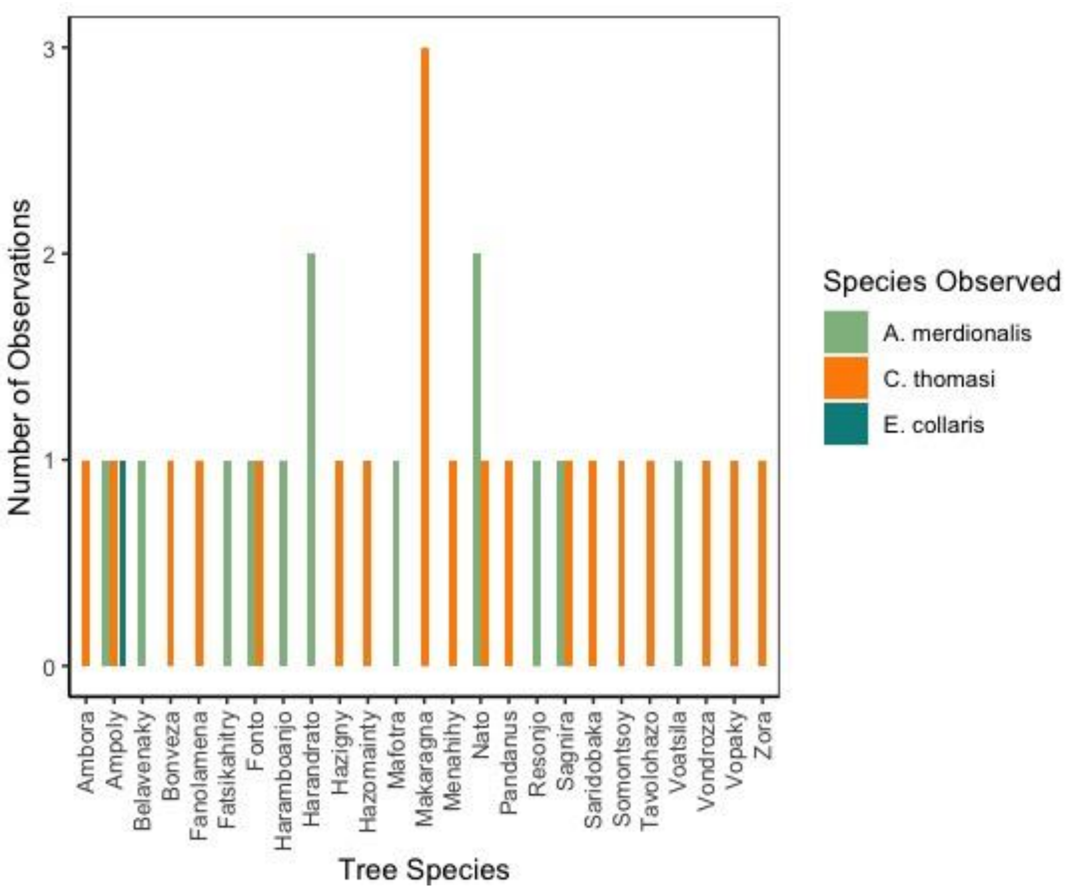

Figure 3: Number of lemur observations in different tree species in the Ala Programme's forest corridors and the surrounding forest remnants between May 2019 and October 2022.

Table 3: Summary of environmental variables collected from all lemur observations Ala Programme's forest corridors and the surrounding forest remnants between May 2019 and October 2022.

Species	Number of Observations	Mean Tree Height (m)	Median Tree Height (m)	Mean Tree Circumference (cm)	Median Tree Circumference (cm)	Mean Canopy Cover (%)	Median Canopy Cover (%)
A. meridionalis	13	11.15	11.00	43.85	43.00	75.00	75.00
C. thomasi	20	9.75	10.00	44.66	35.50	80.00	87.50
E. collaris	1	8.00	8.00	24.00	24.00	25.00	25.00
Total	34	10.24	10.00	43.74	39.00	76.47	75.00

3.2 Herpetofauna VES

Between July 2019 and May 2023, 206 herpetofauna biodiversity surveys were completed (Table 4). 121 surveys were conducted within the forest corridors (59 diurnal and 62 nocturnal surveys) and 85 surveys were conducted in the forest remnants (44 diurnal and 41 nocturnal surveys). 1,398 observations of 47 species were made in this period across all study locations (transects in C1-C4, R1-R4, and S8) (Figure 4). One species of gecko (*Phelsuma lineata)* (n=213) and one species of frog (*Mantidactylus tricinctus)* (n=204) were the most frequently observed species. During 44 surveys no observations of herpetofauna species were made. Most observations of herpetofauna were made in the forest remnants (n=1,168), rather than the corridors (n=230). Of these observations, 36 species were observed in the forest remnants, compared to 13 species observed within the corridors. C1 had the lowest number of observations of herpetofauna out of all the corridors (n=20), while all the others had higher and relatively similar numbers of observations (C2: n=65, C3: n=71 and C4: n=74).

Table 4: Number of herpetofauna biodiversity surveys completed, number of observations, and number of species observed within the Ala Programme's corridors and forest remnants between July 2019 and May 2023.

Location	Number of Diurnal Surveys	Number of Nocturnal Surveys	Total Number of Surveys	Number of Observations	Number of Species Observed
C1	16	17	33	20	5
C2	12	13	25	65	7
C3	15	14	29	71	9
C4	16	18	34	74	7
R1	11	10	21	300	25
R2	11	10	21	103	19
R3	11	10	21	173	20
R4	11	11	22	595	29

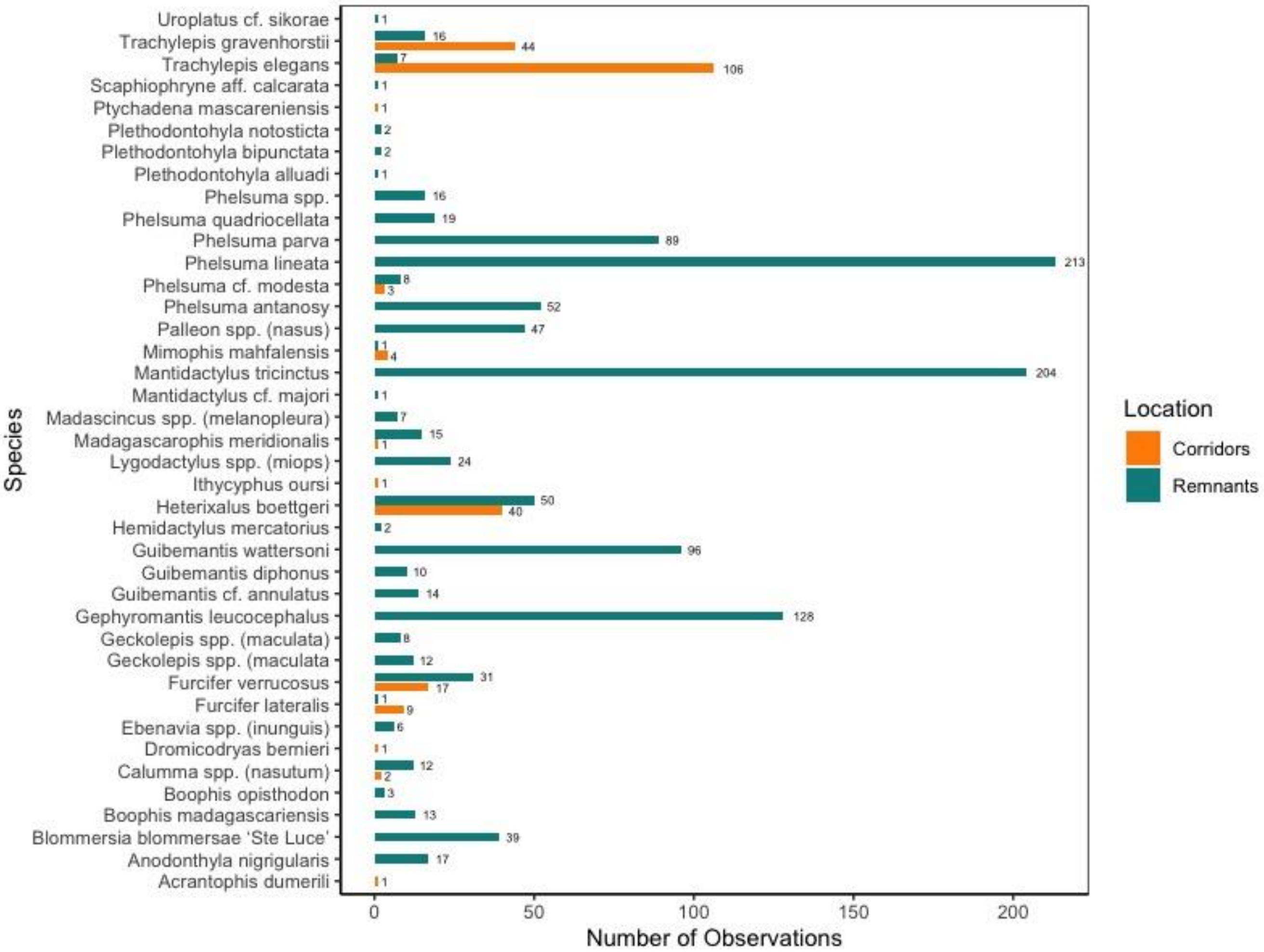

Figure 4: Number of observations of each herpetofauna species within the Ala Programme's forest corridors and forest remnants between July 2019 and May 2023.

The most frequently observed species in the corridors were skinks, mainly *Trachylepis elegans* (n=106) and *Trachylepis gravenhorstii* (n=44), as well as a reed frog, *Heterixalus boettgeri* (n=40) (Figure 5). C3 presented the highest levels of abundance and species richness[2] (S), with a total of 71 observations made (S=9). C1 had the lowest levels of abundance and species richness, with a total of 20 observations made (S=5). To date, *Acrantophis dumerili* and *Dromicodryas bernieri* have only been observed in C2; *Calumma spp. (nasutum), Ithycyphus oursi, Madagascarophis meriodionalis* and *Phelsuma cf. modesta* have only been observed in C3; and *Mimophis mahfalensis* and *Ptychadena mascariensis* have only been observed in C4 (Figure 5). The discovery rates of new herpetofauna species within C2, C3 and C4 have steadily increased over time, C1 showed similar rates of discovery of new herpetofauna species at the beginning and over time this has plateaued (Figure 6). However, no new species have been seen in C4 since the 18th survey (10/04/2021). There were no new species observed in C1 between the seventh survey (28/05/2020) and the 32nd survey (27/04/2023), when *F. lateralis* was seen in C1 for the first time.

[2] Species richness is the number of species in a given region (Moore, 2013).

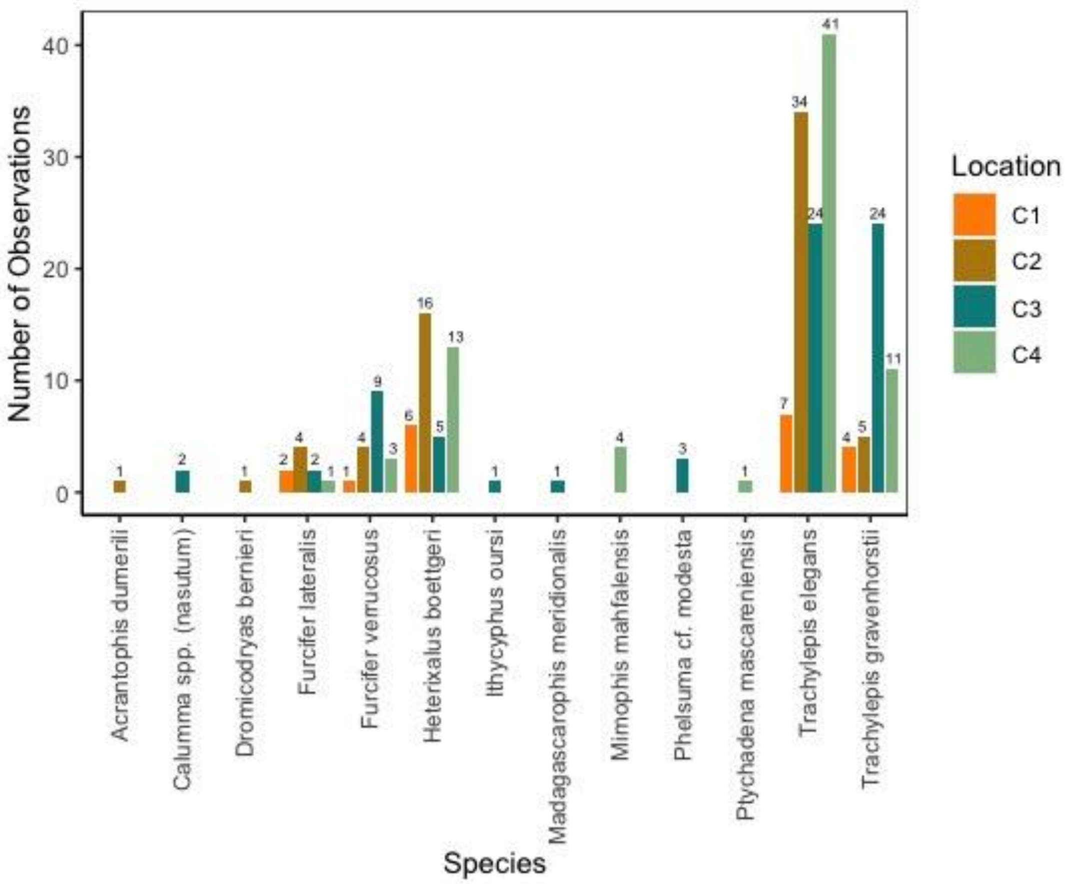

Figure 5: Herpetofauna species observed within the Ala Programme's forest corridors between July 2019 and May 2023.

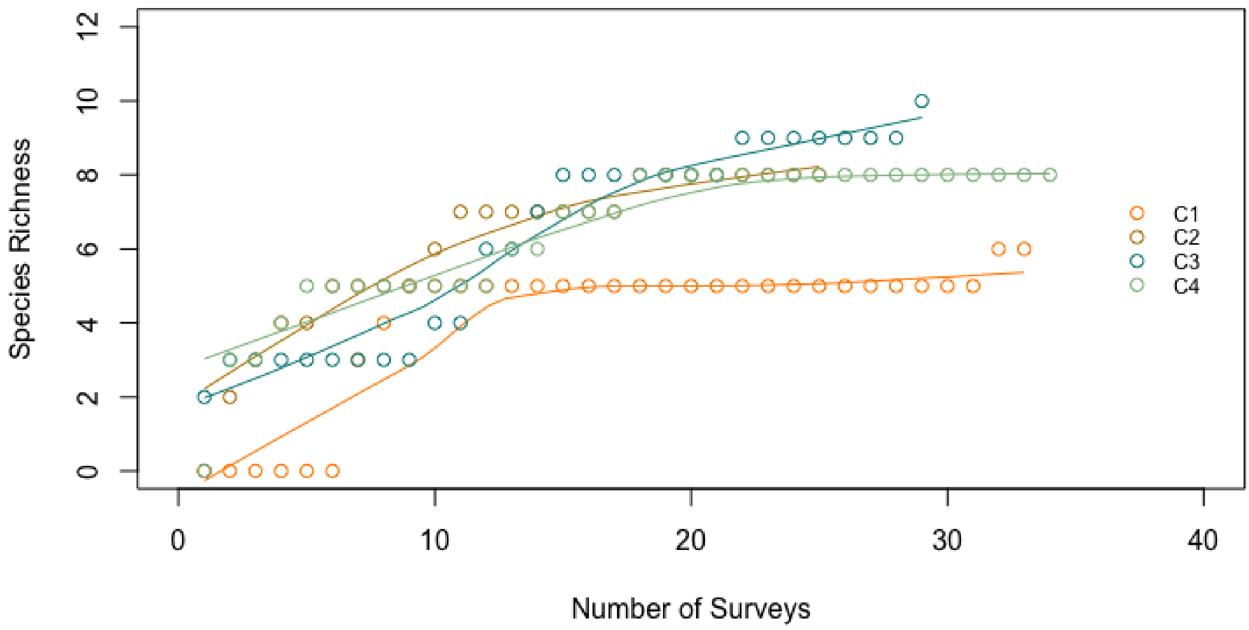

Figure 6: Species Accumulation Curves (SACs) showing the total number of herpetofauna species observed between July 2019 and May 2023 within the Ala Programme's forest corridors. Line fitted by locally estimated scatterplot smoothing (LOESS).

A suite of habitat data was also collected for each observation. On average, observations were made 1.84m from the transect in the corridors, which was further than the average distance from the transect in the forest remnants (1.27m). In the corridors, observations were made on average 29.63cm off the ground, which was lower than the average height off the ground of observations in the forest remnants (76cm). Additionally, in the corridors, most observations were made within forest habitats (n=1,046), with fewer observations made in swamps (n=173), open grassland (n=114), and stream habitats (n=2). Overall, herpetofauna were observed in approximately 24 different microhabitats including: leaf litter, on and inside wooden structures (e.g. trees and tree holes, dead wood and branches), in swamps and water, and on specific floral species (*Acacia mangium, Ravenala madagascariensis*, and *Pandanus sp.*). The average canopy cover for all observations was 57.68%, with a lower mean canopy cover for observations made in the corridors (17.79%) than in the forest remnants (65.49%).

3.3 Invertebrate Catch-and-Release Surveys

Between August 2019 and May 2023, a total of 54 invertebrate biodiversity surveys were conducted. Across all of the study locations (quadrats in C1-C4, R1-R4, and S8), a total of 1,636 observations of 21 orders were made. Most invertebrate observations were made in C1 and the adjoining forest remnants (n=578), with the fewest observations made within C2 (n=294) (Table 5 and Figure 7).

Table 5: Number of surveys and observations made within each of the Ala Programme's forest corridors and the adjoining forest remnants between August 2019 and May 2023.

Location	Number of Surveys	Number of Observations	Number of Orders Observed[3]
C1 (in corridor)	16	343	15
C1 (in forest remnant)	17[4]	235	16
C2 (in corridor)	11	178	11
C2 (in forest remnant)	11	116	16
C3 (in corridor)	11	207	12
C3 (in forest remnant)	11	123	14
C4 (in corridor)	15	235	14
C4 (in forest remnant)	15	199	16

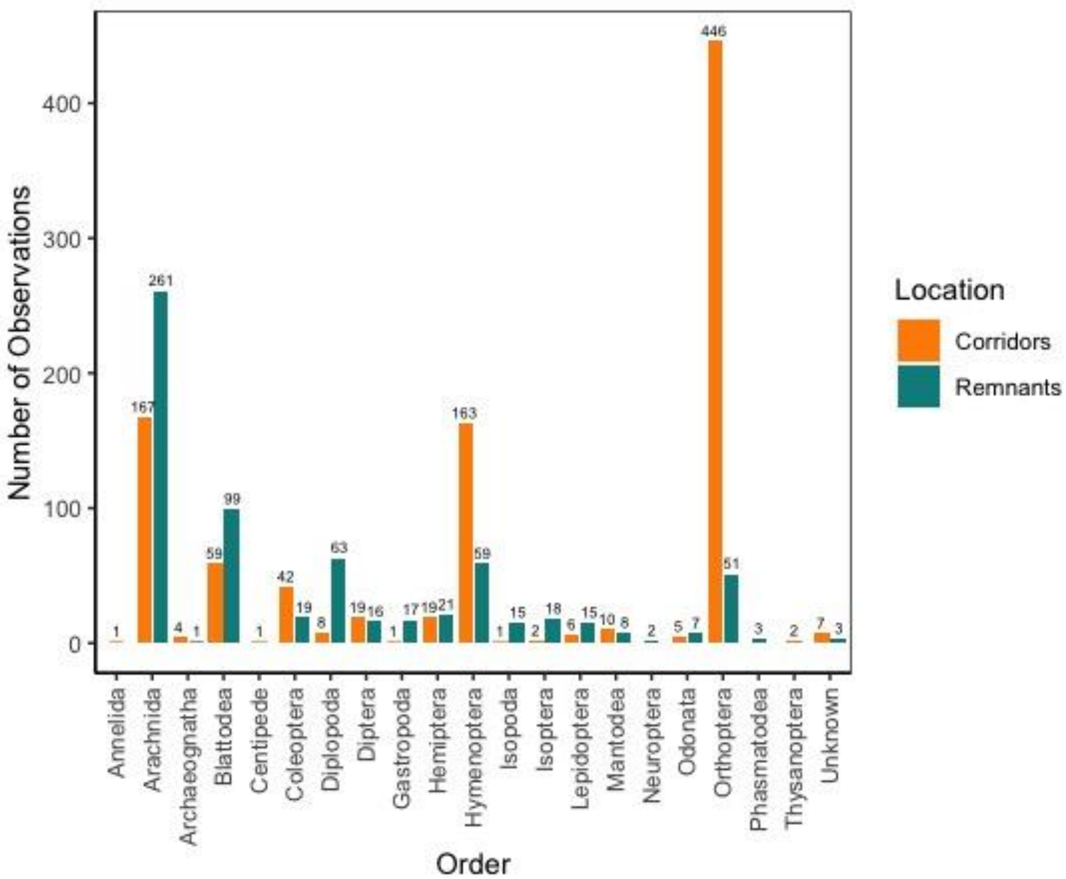

Figure 7: Total number of observations of each invertebrate order within the Ala Programme's forest corridors and forest remnants between August 2019 and May 2023.

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[3] Including Unknown Orders.

[4] C1 forest remnant has an additional survey as one additional invertebrate survey was conducted in S8N.

Species of grasshopper and cricket (Orthoptera) were the most frequently observed order (n=497), followed by spiders and scorpions (Arachnida) (n=428), and wasps, bees and ants (Hymenoptera) (n=222). 963 observations of 19 orders were made within the corridors, and 531 observations of 18 orders were made within the forest remnants. Of these observations, approximately 185 morpho-species were identified, with Arachnida being the most species rich order (S=49) (Table 6). Corridors and forest remnants were also rich in Orthoptera (S=37), Coleoptera (S=14) and Hymenoptera (S=9) species. Orthoptera were the most commonly observed order in the corridors (n=446), making up the majority of the observation of this order across all quadrats. Hymenoptera and Arachnida were also abundant in the corridors.

Table 6: Total numbers of morpho-species observed within each order and number of observations of each invertebrate order made within the Ala Programme's forest corridors and adjoining forest remnants between August 2019 and May 2023.

Order	Number of Observations in Corridors	Number of Observations in Remnants	Total Observations	Number of morpho-species observed per Order[5]
Annelida	1	0	1	1
Arachnida	167	261	428	49
Archaeognatha	4	1	5	1
Blattodea	59	99	158	11
Chilopoda	1	0	1	1
Coleoptera	42	19	61	14
Diplopoda	8	63	71	7
Diptera	19	16	35	7
Gastropoda	1	17	18	6
Hemiptera	19	21	40	9
Hymenoptera	163	59	222	9
Isopoda	1	15	16	2
Isoptera	2	18	20	3
Lepidoptera	6	15	21	8
Mantodea	10	8	18	5
Neuroptera	0	2	2	2
Odonata	5	7	12	4
Orthoptera	446	51	497	37
Phasmatodea	0	3	3	2
Thysanoptera	2	0	2	1
Unknown	7	3	10	2
Total	**963**	**678**	**1641**	**185**

A Jaccard Similarity Test, run using the 'vegan' package in R Studio, assessed the similarity in invertebrate species richness and abundance between quadrats in corridors and forest remnants. Corridors and forest remnants were found to be relatively similar (Jaccard Similarity Index value = 0.522)[6].

[5] For new and unknown morpho-species that have yet to be categorised to their own morpho-species, all were categorised to 'Unknown' morpho-species.

[6] Jaccard Similarity Index value of 0 indicates that the two groups are completely different and have no species in common, whilst an index value of 1 indicates that the two groups are identical.

New species of invertebrate continue to be observed in each of the four corridors. C1 and C4 have higher invertebrate species richness than C2 and C3, which show similar increases in the total number of species observed (Figure 8). This can be expected as C2 and C3 were more recently planted (October 2020 and November 2019 respectively) than the other two corridors. Newly identified species of invertebrate regularly need to be categorised, with the largest numbers in C1 (n=95) and C4 (n=70), and similarly lower numbers in C2 (n=46) and C3 (n=38). Ahead of any future data collection, the categorisation of newly identified morpho-species in each order will be completed. Regularly updating the Invertebrate Guide that is used to identify the different species in each quadrat promotes learning within the Programme.

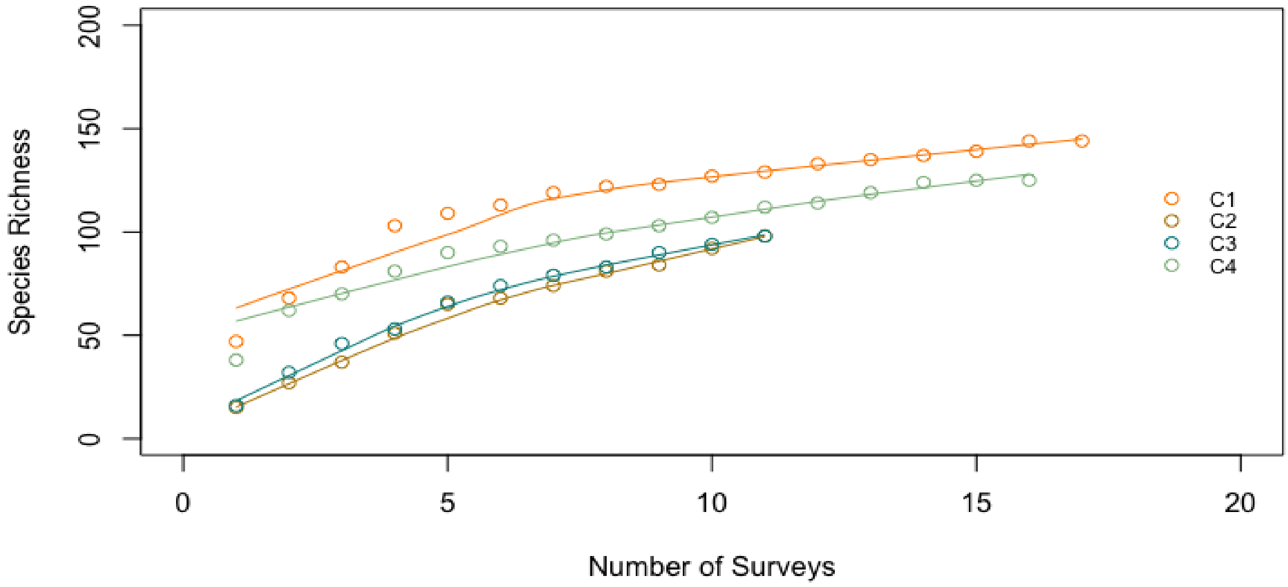

Figure 8: Species Accumulation Curves (SACs) showing the total number of invertebrate morpho-species observed between August 2019 and May 2023 within the Ala Programme's forest corridors. Line fitted by locally estimated scatterplot smoothing (LOESS).

3.4 Camera Trap

3.4.1 Trial

Camera traps were installed in C2 and C3 between October and December 2022 as part of the camera trap trial. From the footage collected, single photos and 30-second videos performed best in the trial, providing two alternative forms of information on lemur presence and behaviour. Single photo settings outperformed three-photo burst settings, with three-photo bursts not providing additional information but greatly increasing the quantity of footage collected. Time-lapse footage did not provide additional information as the target species are not active throughout the day; the time when this function performed best. From the end of the trial period the camera traps have been set up with two different settings: single photos with a 10 second delay between photos, and 30 second videos with a 10 second delay between videos. There has been an increase in the time delay between photos and videos to try and reduce the volume of footage collected by excessive triggering.

3.4.2 Observations

In November 2022, during the camera trap trial, there was one observation of *C. thomasi* in C3 (Figure 9). This demonstrates that the camera traps are able to collect data on the presence of lemur species. From January 2023, the camera traps were deployed, using the settings that performed best during the trial, and the data recorded is being cleaned and analysed. No further observations of lemurs in the forest corridors have been made in analysed footage so far.

Figure 9: Image of C. thomasi from camera traps in C3, November 2022.

3.5 Survival and Growth

3.5.1 Acacia

The *Acacia* in quadrats in the Ala Programme's forest corridors show a very high mean survival rate from initial planting to the last date of data collection, at 97% (Table 7). The *Acacia* in C3 are the tallest, at 5.03m, followed by C4, and then C1. The large average height of *Acacia* in C3 is surprising as these trees were planted more recently than those in C1 and C4. The average *Acacia* height is shortest in C5, 0.77m, which is as expected due to its recent planting date. The *Acacia* in all corridors shows a steady increase in height over time (Annex 2).

Table 7: Descriptive statistics for the Acacia in each of the Ala Programme's forest corridors at the most recent data collection period.

Location	Date of Data Collection	Minimum Height (m)	Maximum Height (m)	Mean Height (m)	Percentage Alive (%)	Most Common Condition
C1	14/06/2022	1.00	6.00	3.13	96.43	3
C2	03/03/2022	0.30	3.00	1.19	100.00	4
C3	21/02/2022	3.75	7.00	5.03	100.00	4
C4	23/07/2022	1.10	8.00	3.87	100.00	4
C5	17/02/2023	0.00	1.62	0.77	98.08	4

3.5.2 Natives

At least 50% of the natives in quadrats in C1-C4 were alive at the most recent point of data collection (Table 8). It is important to note here that survival data is inclusive of replants, and therefore the percentage of alive natives is influenced by replanting events. C1 quadrats show the highest number of alive natives at the most recent time of data collection, as well as the tallest plants. Natives in C1 and C3 quadrats are generally in better condition (3, Fair) than natives in C2 and C4 quadrats (1, Dead). C3 contains the greatest mean height of natives, at 0.28m. Natives in the quadrat in the middle of C2 show a higher survival rate than those in the edge quadrat. In C3, the natives in the edge quadrat show a higher survival rate than those in the middle quadrat. There is little difference in the number of alive natives between the edge and middle quadrats in C1 and C4.

Table 8: Descriptive statistics for the natives in quadrats in each of the Ala Programme's forest corridors at the most recent instance of data collection.

Location	Date of Data Collection	Minimum Height (m)	Maximum Height (m)	Mean Height (m)	Percentage Alive (%)	Most Common Condition
C1	15/04/2023	0.03	0.73	0.14	90.91	3
C2	20/04/2023	0.03	0.40	0.18	60.78	1
C3	11/05/2023	0.06	0.72	0.28	70.21	3
C4	13/05/2023	0.06	0.39	0.17	54.35	1

In terms of whole corridor survival, in 2021, it was assumed all natives planted in 2020 were dead. This was attributed to a drought ongoing in the Anosy region of Madagascar at the time. As a result, a complete replant of native seedlings was conducted in April 2021 (Table 9). One year on from this planting, in April 2022, C1-C4 had a mean native survival rate of 12%. Although low, this was expected given no replants were conducted throughout the year. In May 2022, another large replant event was conducted (n=4,218) to return native seedling numbers back to the seedling count at initial planting. Subsequent replants were done throughout Phase II, Year 2, in July 2022 (n=129), and February (n=1,504), March (n=671), and June 2023 (n=495), to maintain floral species diversity. Data from June 2023 revealed C1-C4 had a mean native survival rate of 91%, which represents a dramatic improvement from Phase II, Year 1. Mean native survival rates at the latest monitoring interval, are highest in C3 (100%), followed by C1 (93%) and C2 (88%), and are lowest in C4 (83%). Survival rate over time remained most consistently high in C1.

Table 9: Survival rate of natives planted in April 2021 in C1-C4 at monitoring intervals.[7]

	Corridor 1 (n=360)	Corridor 2 (n=1,830)	Corridor 3 (n=1,050)	Corridor 4 (n=1,610)
Month 1: May 2021	93%	71%	85%	96%
Month 3: July 2021	72%	43%	58%	46%
Month 9: January 2022	53%	30%	28%	34%
Month 12: April 2022	10%	16%	8%	10%
Month 13: May 2022	20%	0%	0%	0%
Month 15: July 2022	98%	98%	96%	97%
Month 22: February 2023	79%	70%	58%	38%
Month 23: March 2023	92%	82%	97%	82%
Month 26: June 2023	93%	88%	100%	84%

3.6 Opportunistic Sightings

There have been two opportunistic sightings in the Ala Programme's forest corridors. In April 2022, one *Fossa fossana* (Spotted Fanaloka) was observed on the edge of C2, near a wetland that borders the corridor. Additionally in April 2023, a group of four *E. collaris,* two males and two females, were observed in C1 moving towards R1 when observers were there for botanical survival and growth surveys.

4 Discussion

4.1 Lemur Biodiversity

C. thomasi has been observed in two corridors, C1 and C4, which could be attributed to the earlier planting dates of C1 and C4. There have been no observations of *A. meridionalis* in the corridors to date, although they have been observed in forest remnants within close proximity to the corridors. When considering lemur observations in both corridors and forest remnants, it was found that *A. meridionalis* were observed higher in the canopy than *C. thomasi*. Therefore, it is possible that the corridors are not yet at a height that *A. meridionalis* would use. Further research into the difference in habitat requirements for different lemur species could help to inform

[7] Replants of dead natives were conducted at all monitoring intervals apart from Months 3, 9, and 12.

understanding of how different lemur species would use the forest corridors. The final lemur VES survey in the forest remnants was conducted in December 2021, after it was decided that sufficient sampling had taken place and an understanding of lemur populations in R1-R4 was gained. From January 2022, lemur VES surveys were still conducted in C1-C4 until camera traps were set up.

Since October 2022, camera traps have been used to monitor the corridors for lemur populations. The image of *C. thomasi* on the edge of C3 indicates that there are lemurs within the forest remnants showing interest in the corridors. In April and May 2023, camera trap coverage was expanded both in terms of the number of corridors covered and the area within corridors covered. It is hoped the movement of camera traps into the middle of the corridors will indicate if species are using the corridors to their facilitate movement between S8 and R1-R4. Camera traps have successfully been used to monitor and identify arboreal primates in the canopy, including small gliding mammals in Borneo (Haysom et al., 2021), and monitor the response of larger primates to human disturbance in Peru (Whitworth et al., 2019). Therefore, it is expected camera trap data from the forest corridors will provide further information on their use by different lemur species.

4.2 Herpetofauna Biodiversity

The Ala Programme's corridors presented relatively low herpetofauna species richness compared to the forest remnants. Four species were found in C1-C4; *F. lateralis, F verrucosus, T. elegans*, and *T. gravenhorstii*, with a total of 13 species observed across the four corridors. The two species of skink (*T. elegans* and *T. gravenhorstii*) and three of the species of snake *(D. bernieri, M. meridionalis,* and *M. mahfalensis*) are ground dwelling species that can be found in both arid and humid habitats (Glaw & Vences, 2007; Raxworthy, 2011a; Raxworthy, 2011b; Vences, 2011). Two of the species of chameleon that were observed in the corridors *(F. lateralis* and *F. verrucosus*) also favour arid and disturbed land, however they require herbaceous vegetation such as low bushes (Jenkins et al., 2011b; Raxworthy, 2014). While species including Boettger's reed frog (*H. boettgeri*) and Mascarene grass frog (*P. mascariensis*) do require temporary or permanent water sources in order to breed, these species are also generalists and live in a variety of habitats (IUCN SSC Amphibian Specialist Group, 2016a). Interestingly, a species of day gecko *(P. modesta*) and chameleon (*C. nasutum*) were observed in C3. *C. nasutum* is a widely distributed species that is typically found in primary humid forest, secondary vegetation, and on forest edges, while *P. modesta* is widely distributed in arid parts of southeast Madagascar and typically observed on *Ravenala* (Glaw & Vences, 2007). The broad ecological niches of these species make them suited to the developing and open nature of the vegetation within the corridors.

The observations of arboreal species such *as C. nasutum, F. lateralis, F. verrucosus, I. oursi,* and *P. modesta* indicate the corridor's potential suitability for other similar species. Data collection over a longer period of time will confirm if these observations are indicative of a suitable habitat for arboreal species or if they were simply coincidental.

C1 had a significantly lower number of herpetofauna observations than the other corridors. It is noted that the *Acacia* trees in C1 are shorter than those in the other corridors, and that there is more bare ground in C1. The shorter height of trees and greater proportion of bare ground in C1 may be limiting the number of habitats suitable for herpetofauna within the corridor. In particular, the lack of leaf litter in the corridor could mean that there is not the habitat for leaf litter dwelling species, such *as G. leucocephalus* (IUCN SSC Amphibian Specialist Group, 2016b), which were seen in large numbers in the forest remnants, but to date have not been seen in the forest corridors.

In December 2021, the final VES survey of herpetofauna populations was conducted in R1-R4, as it was decided that sufficient data had been collected and a baseline population established. Herpetofauna VES surveys are continuing in C1-C4 to monitor how herpetofauna species richness and abundance change overtime. Following trends in species accumulation to date, it is anticipated that as the corridors mature, they will support an increasing diversity of herpetofauna species.

4.3 Invertebrate Biodiversity

Invertebrate assemblages in C1-C4 were found to be relatively diverse and abundant. A greater number of invertebrate observations were made within quadrats in the corridors than in the forest remnants, with a similar number of orders observed in both the forest remnant and corridor quadrats. It is important to note the greater number of invertebrate observations in the corridor quadrats, compared to the remnant quadrats, is likely due to there being one additional quadrat in the corridors than in the forest remnants.

The orders observed more frequently within the corridors (Orthoptera and Hymenoptera) typically prefer open matrix ecosystems, whereas those observed more frequently in the forest remnants (Arachnida, Isoptera, and Blattodea) are associated with closed matrix ecosystems and with denser canopy covers. It is hoped that as the corridors continue to grow more shade and organic matter is provided by an established canopy the invertebrate community will more closely resemble invertebrate communities observed in the forest remnants.

Invertebrate biodiversity surveys continue in the corridors and forest remnants to monitor how invertebrate assemblages change with time.

4.4 Floral Biodiversity

The *Acacia* in C1-C4 are well established, where the most common condition is 4, Excellent, or 3, Fair, and the mean tree height in each of the forest corridors is over 1m. The *Acacia* in C5 is younger and has a shorter average height, however here too the most common condition is 4. In all of the forest corridors, very high percentages of *Acacia* were found to be alive at the last period of data collection. *Acacia* can provide shade for slower growing and shade-dependent native tree species, fix nitrogen into a nutrient deficient substrate (Koutika and Richardson, 2019), and can facilitate the reconnection of a range of species in fragmented habitat (Andriamandimbiarisoa et al. 2015, Chazdon et al. 2020, Gillies and Cassady St. Clair, 2008, Luckett et al., 2004, Schlaepfer, Sax and Olden, 2011, Singh et al. 2001). As such, it is expected that mature *Acacia* in the forest corridors will provide the structure, shade, and soil nutrients needed for natives to grow. As expected, the natives in the Ala Programme's forest corridors do not show as high survival rates over time in comparison to *Acacia*. However, 91% of natives were alive across C1-C4 at the most recent set of data collection.

Acacia is now well established in all corridors and no further replants will be conducted. Consequently, the Ala Programme has shifted its focus towards increasing native survival and growth rates. Replants of natives have been concluded as per the original planting schedule, yet, will continue to take place when necessary to promote species diversity.

Additionally, seedling planting and corridor management strategies have been reviewed. The planting strategy has been revised to increase water retention and reduce drainage rates. From June 2023 onwards, holes in which native seedlings are planted will be dug to $20cm^3$. It is also expected that planting seedlings in a larger hole will help them to establish and grow roots more easily, due to the soil being loosened.

Throughout April and May 2023, a new strategy for corridor management was developed and refined. In the Ala Programme: Phase II, Year 3, native seedlings will be watered once a month throughout the dry season. It is anticipated watering will be necessary for approximately six months (July-December), however watering schedules will be adjusted depending on the rain. Manure will be applied to seedlings once every two months throughout the dry season, and once a month throughout the rainy season. Application will be increased during the rainy season to account for increased decomposition and runoff rates. Lastly, mulch will be added to seedlings in corridor areas where leaf litter coverage is low. Mulch application is intended to reduce evaporation rates after seedlings have been watered, so it will be particularly focused on areas where levels of sunlight reaching the ground are high (areas of C1, C2, and C4, and C5 when it is planted with natives). Mulch will be applied to native seedlings after the initial watering, and again at regular intervals as it decomposes.

Acacia will also be monitored monthly to check for self-seeding and any signs it is impeding native growth. In Phase II, Year 3, it is expected that *Acacia* will need thinning to a small extent in some areas. Removing whole trees from the centre of corridors will likely have the best impact on native growth by allowing low levels of sunlight to reach the ground. However, *Acacia* will be thinned conservatively, as they are still providing benefits in the corridors such as shade, leaf litter coverage, nitrogen fixation, and a reduction in wind velocity.

5 Conclusion

A variety of faunal species have been observed within the Ala Programme's forest corridors, both during and outside of surveys, with new species being observed frequently. While most herpetofauna and invertebrate species observed have been generalist species that are well suited to open matrix habitats, since January 2022 there have been four arboreal herpetofauna species observed. The number of lemurs that have been observed in the corridors both during and outside of surveys has increased over time, and there have been additional observations of *C. thomasi* and *M. tanosi* near the corridor edges. It is hoped that as tree size in the corridors increases, lemurs will utilise the corridors more to facilitate their movement, and more observations will be made.

Acacia is establishing well in all corridors, and increasingly providing shade, leaf litter coverage, and soil nutrients. Mean native survival rates have increased dramatically in comparison to Phase II, Year 1. A corridor management strategy, intended to promote native growth rates, will be implemented from June 2023 onwards. It will be particularly interesting to see how the abundance, richness, and species of fauna and flora observed in each of the corridors changes over time as the corridors develop and become more established, with larger trees, increased vegetation, ground cover and leaf litter.

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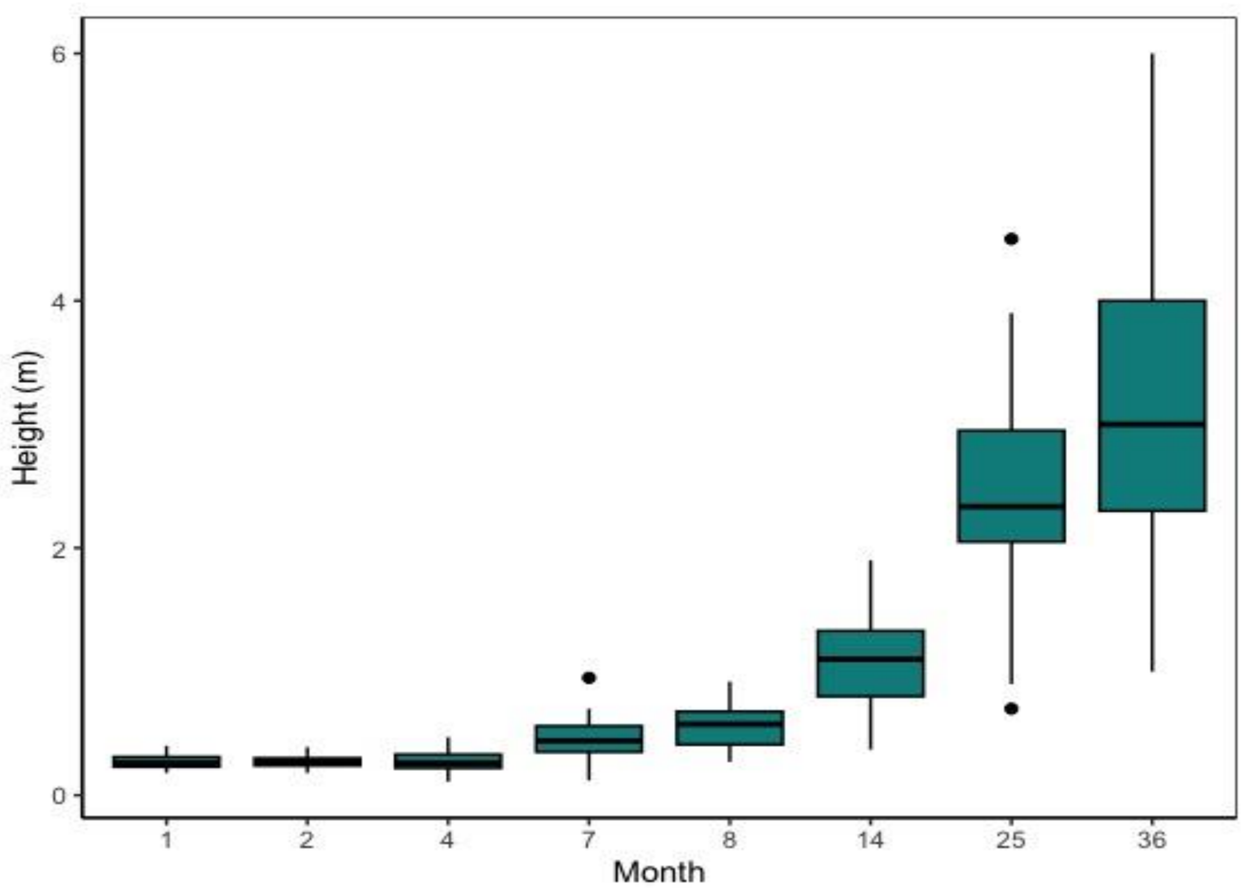
7 Annex

Annex 1

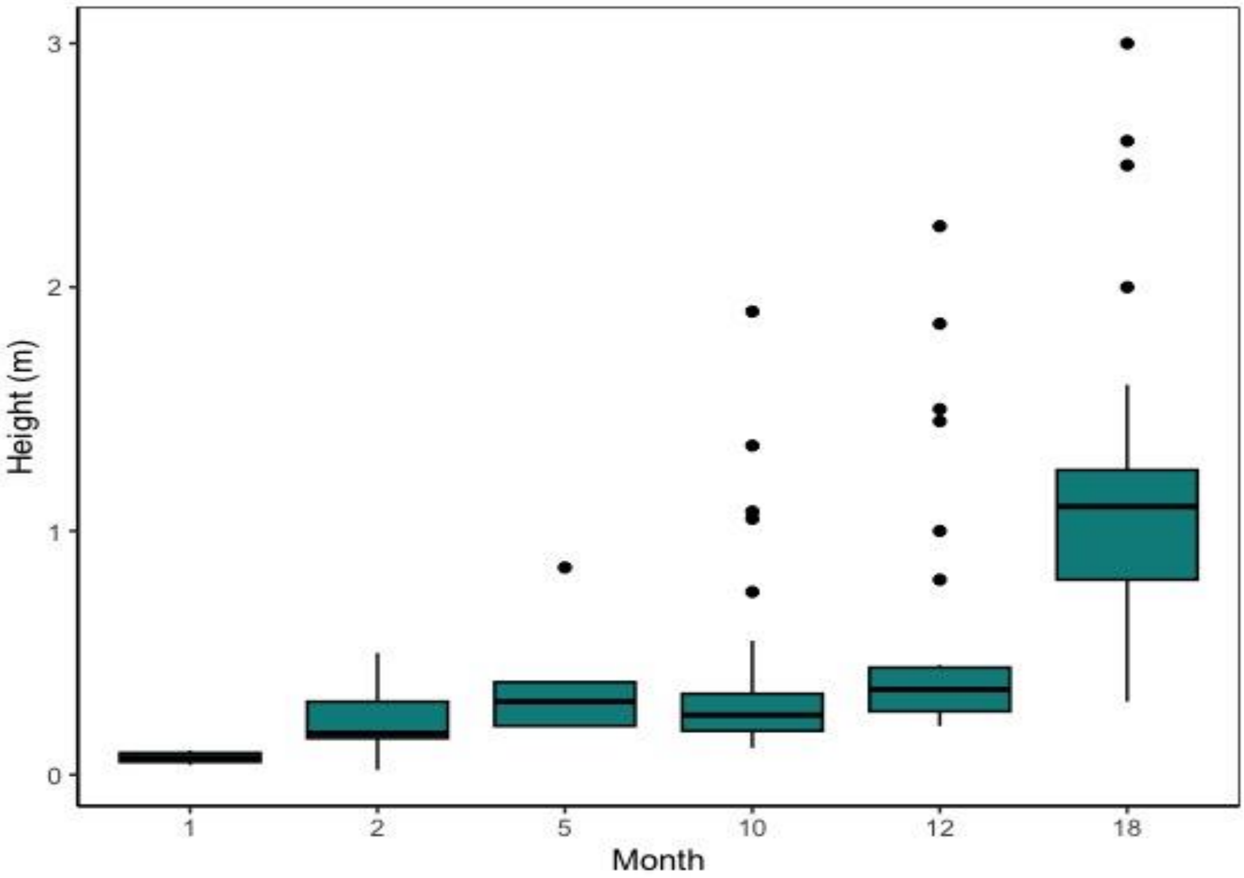
Table of dates of first plants and first data collection for *Acacia* and natives in each of the Ala Programme's forest corridors.

Corridor	Date When First Planted: *Acacia*	Date of First Data Collection: *Acacia*	Date When First Planted: Natives	Date of First Data Collection: Natives
C1	July 2019	16/07/2019	January 2020	08/02/2020
C2	July 2020	01/10/2020	January 2021	05/02/2021
C3	November 2019	28/11/2019	January 2020	14/02/2020
C4	August 2019	02/09/2019	January 2020	07/02/2020
C5	March 2022	03/09/2022	-	-

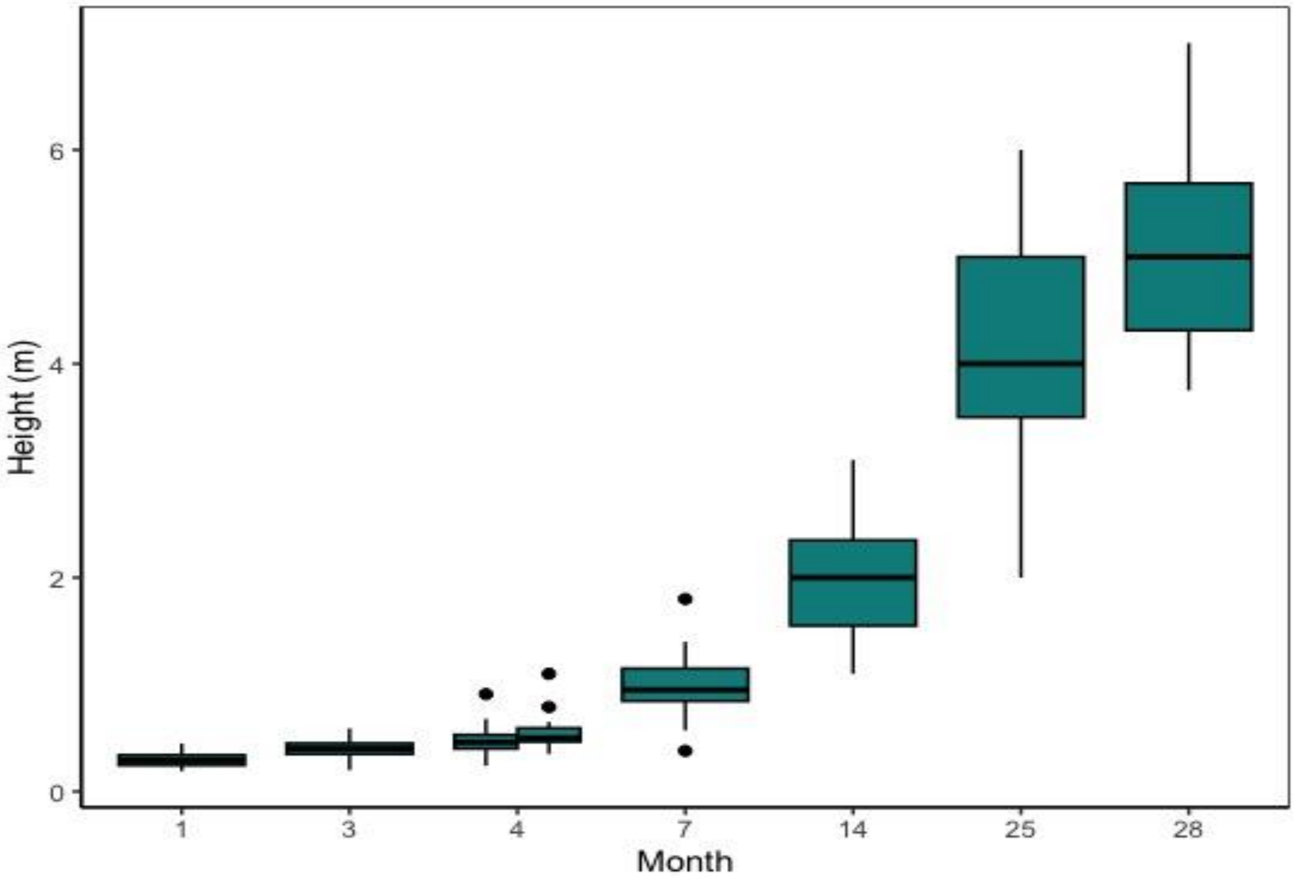
Annex 2



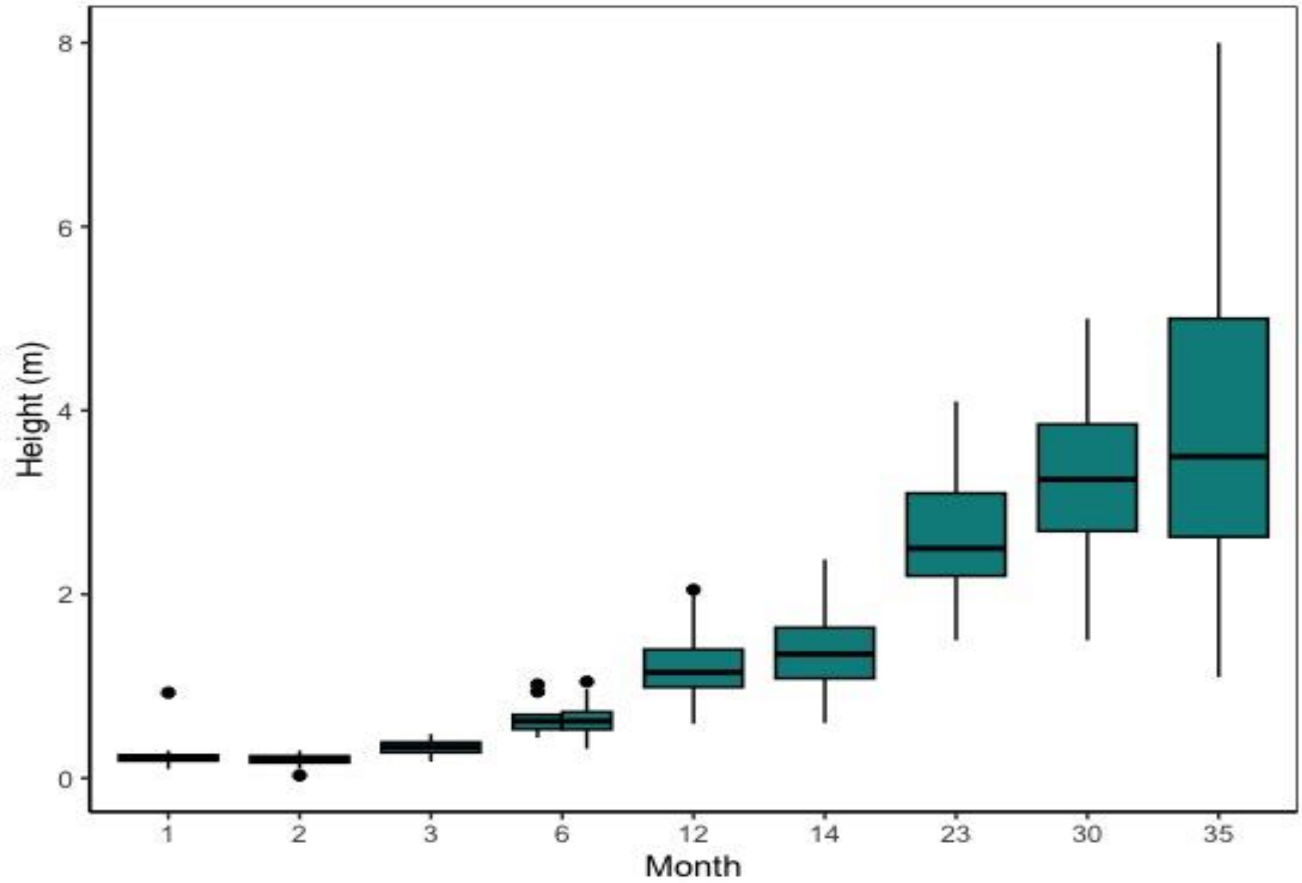
Boxplot showing the growth of Acacia *in C1 over time.*



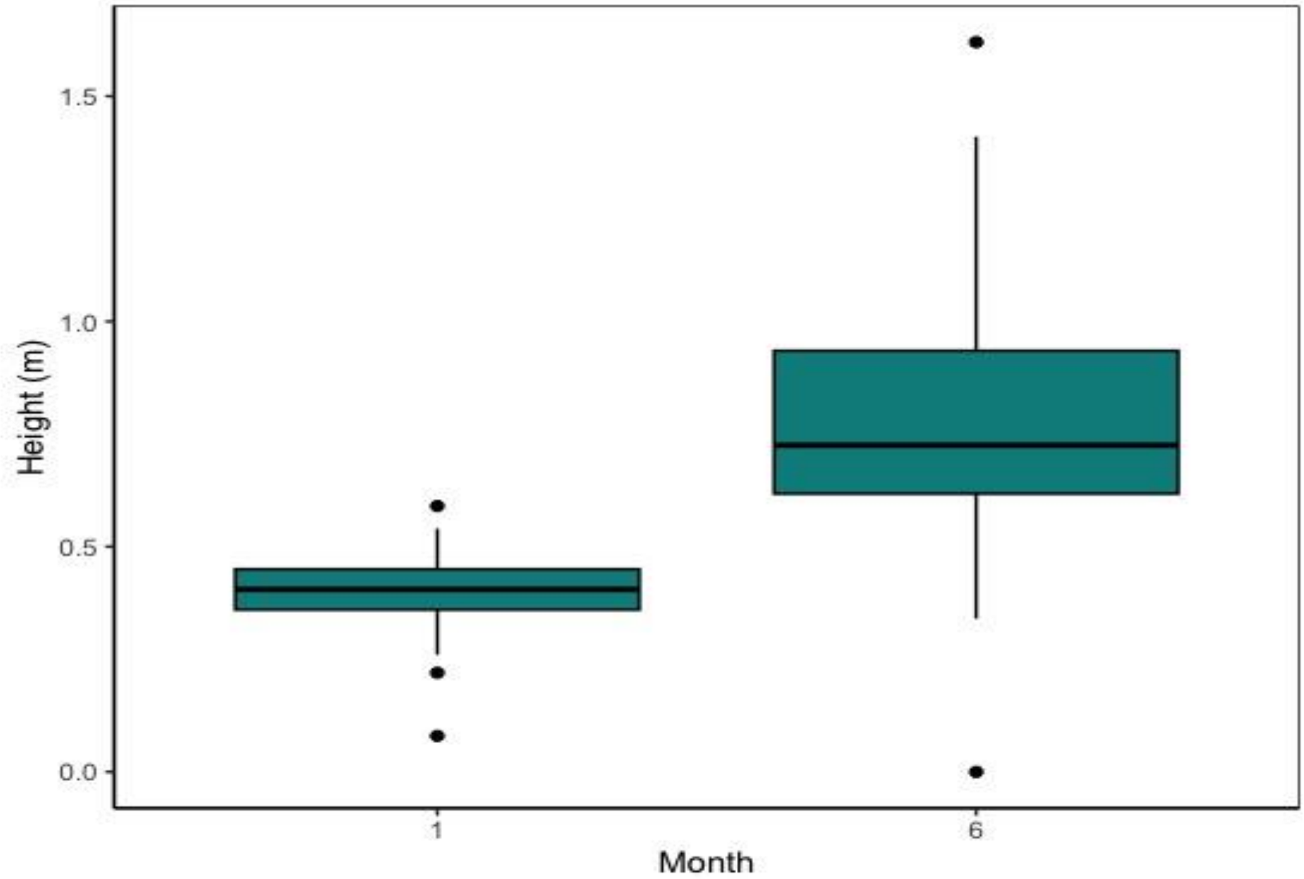
Boxplot showing the growth of Acacia *in C2 over time.*



Boxplot showing the growth of Acacia *in C3 over time.*



Boxplot showing the growth of Acacia *in C4 over time.*



Boxplot showing the growth of Acacia *in C5 over time.*