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MS ENVIRONMENTAL BIOLOGY CAPSTONE PROJECT

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by

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CHAPTER 1. LITERATURE REVIEW

Introduction

Climate change and habitat alteration on a global scale are the largest threats to biodiversity (Rands et al., 2010). These effects may be most prevalent in the tropics where much of the Earth's biodiversity exists. Primates generally inhabit tropical and subtropical forests and grasslands that can be disproportionately impacted by anthropogenic habitat degradation and climate change (Estrada et al., 2017; Haddad et al., 2015). A thorough examination of how primates adapt to changing ecosystems can provide insight into the effects of climate change and habitat loss, and help identify areas of special conservation need.

Effects that may cause adaptations in primate populations can take the form of natural climatic changes such as seasonality (Brugiere et al., 2002; Gould & Gabriel, 2015; Talebi & Lee, 2010) or extreme weather events (Behie & Pavelka, 2013). Other impacts can be anthropogenic habitat destruction such as deforestation (Chaves et al., 2012; Schreier et al., 2022), overgrazing (Ménard et al., 2013), and anthropogenic climate change (Beeby et al., 2023). Deforestation leads to habitat fragmentation which increases edge effects, reducing tree size and diversity, and thus food availability for many primates (Arroyo-Rodríguez & Mandujano, 2006; Bolt et al., 2018; Haddad et al., 2015; Schreier et al., 2022). Warming temperatures can intensify seasonal changes, affecting plant growth, fruiting and flowering times, and plant diversity (Beeby et al., 2023). This can further impact food availability for primates. Changes in the quantity and nutritional value of consumed foods consequently alters overall primate behavior. Warmer climates also generally increase daily resting energy expenditure and metabolism in animals, leading to reduced body size (Bethge et al., 2017). This may compound how primates alter behavior in changing environments, specifically regarding how they consume and expend energy. Two primary ways primates may alter their energetic habits are to change their diet or foraging strategies and/or alter their activity budgets (time spent feeding, resting, and traveling).

In general, primates are adapted to have diets that are specialized enough to maximize energy intake while limiting the amount of energy required to obtain and digest that food given the resources available (Altmann, 2006). Prioritizing high-energy foods may affect activity budgets because primates could consume less to meet the same nutritional requirements and would therefore be able to rest more and spend less time traveling in search of food. In anthropogenically affected environments where there are fewer resources and lower quality food (Arroyo-Rodríguez & Mandujano, 2006; Schreier et al., 2022), this strategy may be adapted to spend more time feeding to obtain the same amount of nutritional value; however, that could reduce how much time primates spend resting. When primates maximize energy intake by consuming higher quality foods and/or spending more time feeding while also maximizing resting behavior and minimizing traveling, that is an "energy-maximizing strategy." Environments with particularly limited resources may be so thin that primates must expend more energy to find food, but still feed less often (Ménard et al., 2013). "Energy-minimizing strategies" are those that minimize travel and maximize rest when resources are inadequate for primates.

Optimizing diet and altering activity budget are not mutually exclusive strategies. Variations in strategies differ across species and locations, where patterns may not be clear when examining just one species in a single location or at the same time of year. Therefore, synthesizing many examples of primate behavior in limited-resource environments may provide insight into general energy allocation tendencies across species and how habitat alteration is affecting them. Knowledge of common trends in primate behavior in disrupted habitat can help inform more consistent conservation practices around the world.

Feeding Strategies

Primate physiology, habitat type, and climate play key roles in determining diet. Primate diets follow "Optimal Foraging Theory" (Altmann, 2006; Felton et al., 2009a, Zhao et al., 2013). This theory explains the various strategies that primates display when it comes to their food selection. The first, and perhaps most common pillar of optimal foraging theory, is an energy maximizing diet where primates choose foods highest in macronutrients (carbohydrates, lipids, and protein) with an emphasis on fats and soluble carbohydrates without sacrificing for greater foraging exertion or digestion time. High-energy plant foods are ripe, fleshy fruits, decreasing in nutrition to dry fruits and seeds, flowers and young leaves, and mature leaves being lower quality (Brugiere et al., 2002). Other strategies of optimal foraging theory are to select a balanced diet by consuming many different plant types and/or selecting specific foods that maximize micronutrient intake. Finally, primates may choose a diet that specifically avoids food high in plant secondary metabolites (PSMs) including cellulose and tannins that are higher in more woody species or in mature leaves. PSMs require higher energy input to digest and may have toxic effects in the digestive tract (Altmann, 2006; Felton et al., 2009a, Zhao et al., 2013).

Ecosystem changes can alter the amount and types of food available and change the way primates behave. Energy maximizers include spider monkeys (*Ateles* spp.) who consume primarily ripe fruits (Talebi & Lee, 2010; Zhao et al., 2013), but can alter their diet to a less nutritious, folivorous (leaf-eating) one in response to leaner times when there is less fruit available (Talebi & Lee, 2010). However, Peruvian spider monkeys (*Ateles chamek*) in Bolivia, while thought to be energy maximizing purists, consumed more protein than expected and less carbohydrates and lipids than what would be predicted following an energy maximizing diet (Felton et al., 2009b). This shows a more balanced approach to diet than was previously thought. In a Mexican forest fragment with fewer, smaller trees, black-handed spider monkeys (*Ateles geoffroyi*) consumed different species, more low-energy leaves, and less fruit compared to monkeys in a continuous forest (Chaves et al., 2012). Capuchins (*Sapajus* spp.) are omnivorous frugivore-insectivores that generally maximize energy intake. Capuchins in a heavily impacted urban forest in Brazil consumed high amounts of processed human foods and relied on more high-energy human food than forest-sourced foods during the less-abundant dry season (Gonçalves et al., 2022).

Primate species living in forests consisting of trees that are highly variable in fruit production from year to year (mast fruiting) alter their diets as well (Brugiere et al., 2002; Cui et al., 2020). Areas with highly variable or unpredictable fruiting seasons support fewer, smaller primate species in forests worldwide (Brugiere et al., 2002). Rhesus macaques (*Macaca mulatta*) in China feed on highly seasonal, dry oak (*Quercus* spp.) fruit, and in a year with little to no fruit production (non-mast), researchers found that they significantly reduced carbohydrate and fat intake (Cui et al., 2020). The reduction of high-energy foods resulted in more sedentary females and a significantly lower birth rate. Likewise, four primate species in central Africa that depend on seasonal tree species reduced ripe fruit consumption and increased dry fruit and leaf consumption in dry seasons (Brugiere et al., 2002).

Another primate dietary strategy is to avoid PSMs and cellulose (leaves and other lower-quality foods). A meta-analysis of 43 species showed a strong avoidance of high PSM foods (Windley et al., 2022). However, primates like gorillas, chimpanzees, howler monkeys and some lemurs may prefer higher PSM diets and have guts adapted to metabolize more undigestible foods (Windley et al., 2022; Zhao et al., 2013). Black howler monkeys (*Alouatta nigra*) already living in a Belizean forest fragment suffered a massive population decline and reduction of resources following a hurricane in 2001 (Behie & Pavelka, 2013). While howler monkeys are highly folivorous and can process PSMs, fruit is a major part of their diets. Behie and Pavelka's (2013) study showed that fruit consumption dropped considerably after the storm, and was related to population decline, malnutrition, and high stress (cortisol) levels. The population did not recover until fruit consumption reached prior levels.

Endangered ring-tailed lemurs (*Lemur catta*) can also consume highly folivorous diets (Gould & Gabriel, 2015) and are not as deterred by high-PSM foods compared to other primate species (Brugiere et al., 2002). A study of ring-tailed lemur populations in two forest fragments in Madagascar showed that 1) lemurs consumed significantly more fruit than leaves in the wet season in both fragments and equal amounts of fruit and leaves in the dry season and 2) in the larger, more continuous fragment they consumed more fruit and less leaves in the wet season, and more flowers in the dry season than in the smaller, more broken fragment (Gould & Gabriel, 2015). Many studies show that primates alter their feeding strategies to maximize dietary intake across species and geography.

Activity Budgets

Environmental impacts affect food availability and the quality of food available. Habitat destruction by both natural and anthropogenic effects reduces plant cover overall, fragmentation reduces tree size and diversity (Arroyo-Rodríguez & Mandujano, 2006; Bolt et al., 2018; Schreier et al., 2022), and drier years/seasons reduce fruit production (Beeby et al., 2023; Gould & Gabriel, 2015). This can alter the way primates allocate their time and energy. Most primate behavioral studies focus on the activities of resting, feeding, and traveling since these activities comprise the majority of their activity budgets. Most studies also include social behavior as an activity, but this review focuses on resting, feeding, and traveling behaviors since these are directly related to energy consumption and expenditure.

Across two distinct years, woolly spider monkeys (*Brachyteles arachnoides*) in a forest fragment in Brazil exhibited behavioral variability across seasons (Talebi & Lee, 2010). In both years, the amount of time spent feeding increased and resting decreased during dry winter months, and there were no discernable patterns in traveling behavior. The spider monkeys also rested more during the hot, humid middle of the day when resting energy expenditure is at its peak, and traveled and fed more in the mornings and late afternoons when energy expenditure is lower (Talebi & Lee, 2010).

Mantled howler monkey (*Alouatta palliata*) activity in a small forest fragment differed across forest interior and forest edge (Schreier et al., 2022). Monkeys at the edges, where trees were significantly smaller and less diverse, were energy minimizers by resting significantly more and traveling significantly less. There was no difference in time spent feeding across forest zones. However, when *A. palliata* from the same small fragment were compared to a large, continuous forest over ten times the size, monkeys spent significantly more time feeding and traveling, and significantly less time traveling in the forest fragment (Miller et al., in prep). When comparing across populations as opposed to within a population, energy preferences shifted from minimizing energy expenditure to maximizing both energy intake and expenditure in the less abundant areas.

Other environmental influencers may also affect activity patterns in primates. Endangered ashy red colobus monkeys (*Piliocolobus tephrosceles*) are highly folivorous and have a four-chambered stomach adapted to digesting PSMs (Kibaja et al., 2023). Kibaja et al. (2023)

examined activity budgets and home ranges of *P. tephrosceles* in two distinct habitats in Tanzania: a forest mosaic and a dry savanna woodland. While the woodland had more diverse tree species, the forest mosaic had larger and more dense trees, suggesting higher food availability. The monkeys in the dry woodland traveled more and had larger home ranges, but also rested more and fed less.

Ménard et al. (2013) compared barbary macaque (*Macaca sylvanus*) activity budgets across three forests with varying levels of anthropogenic impact in northern Africa. The habitats ranged from an extremely overgrazed forest with lower resource availability to a protected national park, and a third moderately grazed area. In the most heavily modified habitat, the macaques altered their diets, expanded their home range, and spent more time foraging and traveling than in the protected area. However, they decreased the total amount of time spent feeding. They also spent more time traveling in the hot summer months when monkeys in the protected forest increased time spent resting. Overall, macaques in the moderately affected habitat spent the most time feeding and spent less time resting than in the more protected area.

Discussion

Primates react to changes in food availability by altering their diet (e.g. Chaves et al., 2011; Gonçalves et al., 2022; Gould & Gabriel, 2015). Quality food availability regulates primate populations because less-abundant areas support smaller, fewer primate species and when primates experience times without ripe fruit, populations suffer (Behie & Pavelka, 2013; Brugiere et al., 2002; Cui et al., 2020). Differences in habitat type or seasonality impact food availability. Plant species may have longer fruiting intervals where year-to-year availability is highly variable, or dry seasons may reduce overall plant food abundance (e.g. Cui et al., 2020; Gould & Gabriel, 2015). Other impacts include anthropogenic habitat destruction resulting in fragments, reduced size or connectivity of fragments, or natural disturbances such as hurricanes. Fragments compared to large, continuous habitat or small fragments compared to larger ones generally harbor less food availability and lower quality food overall (Bolt et al., 2018, Chaves et al., 2011, Schreier et al., 2022).

Across primate species, it appears that high-energy, ripe fruit availability is the primary driver of diet selection (Chaves et al., 2012; Cui et al., 2020; Gould & Gabriel, 2015). When it is available, primates consume fruit at much higher rates than lower energy food sources such as unripe fruit, leaves, or bark. Leaner circumstances are represented by dry seasons, non-mast years, smaller/fragmented habitat, or following an extreme weather event. Primates must reduce the amount of carbohydrates and lipids consumed and increase consumption of higher protein, higher cellulose, and higher PSM foods in these times. This may have detrimental effects on some primate populations; for example, increased cortisol production and limited population growth in black howler monkeys (Behie & Pavelka, 2013) or reduced fecundity in rhesus macaques (Cui et al., 2020). Abundant food resources and larger, more connected habitat in the absence of disturbance are the more optimal and historically more natural conditions for primates. Therefore, the trends across species suggest that primates prefer an overall energy-maximizing diet regardless of habitat type or location. Diet choice and energy available in food can affect time spent feeding and energy expenditure, therefore affecting activity patterns.

Activity budgets vary across seasons, levels of anthropogenic disturbance, and across primate species overall. However, there are major consistencies across both habitat type and species. In most examples examined here, the amount of time spent feeding tended to increase in seasons or locations with lower food availability/quality. A meta-analysis of 135 studies on fragment size across all regions where primates live showed a significant correlation between diminishing patch size and increased feeding (Carratero-Pinzón et al., 2016). This coincides with patterns in reducing the quality of food consumed and that primates across habitat types require greater consumption of lower quality foods when resource availability is limited in order to meet their dietary requirements.

Also, although not a consistent pattern across species, and variable across levels of disturbance, species may also reduce time spent resting and/or increase time spent traveling in areas with lower food quality (e.g., Ménard et al., 2013; Miller et al. in prep; Talebi & Lee, 2010). This may show a need to travel more in search of food and an inability to rest as often when abundance is low. Increases in traveling and feeding behavior are directly correlated with a decrease in resting. These examples further highlight the preference of primates to maximize energy intake regardless of species or climate, as opposed to conserving energy when resources are limited.

When compared between forest edge and interior, mantled howler monkeys did not follow the aforementioned trends, showing no difference in feeding, increasing rest, and decreasing travel in the edge (Schreier et al., 2022). However, this same population compared to monkeys inhabiting a larger forest, time spent feeding increased, resting decreased, and traveling increased in the fragment. This trend is similar to other primate species at a larger scale (e.g., Talebi & Lee, 2010), but may also demonstrate a different reaction to further diminished resources when in a small fragment *and* on the forest edge where feeding trees may be especially small and less diverse.

Similarly, while barbary macaques in the most overgrazed site in Africa traveled more than in protected areas and more during the dry season, they contradicted patterns of increased feeding in disturbed areas by feeding less than at the other two sites (Ménard et al., 2013).

However, the macaques in the moderately impacted site fed more and rested less than in the most protected area. This may be another example where resources are especially affected in the most overgrazed site, and therefore primates cannot maximize energy intake like in other areas. When comparing the second-most impacted site to the protected one, activity is similar to other species.

Conclusion

Primates alter their diets and activity budgets in response to environmental change (e.g., Brugiere et al., 2002; Behie & Pavelka, 2013; Carretero-Pinzón et al., 2016; Schreier et al., 2022). Environmental change drives changes in plant growth and therefore food availability for primates (Beeby et al., 2023; Bolt et al., 2018). The quantity and quality of food available for primates is a substantial driver of behavioral changes in primates. This review demonstrates trends in primate species worldwide that suggest that primates will maximize energy intake in terms of both their diet choice and activity budgets. It also demonstrates that in times of lower food availability, primates must eat lower quality foods, but spend more time feeding, thus maintaining energy consumption as much as possible.

Seasonal differences in climate, cyclical fruiting periods of plants, and natural disturbances present expected variability in fruit production, the preferred food source of many primate species. However, global climate change may cause these natural cycles to intensify and become more unpredictable (Beeby et al., 2023). Anthropogenic effects on habitat include deforestation and fragmentation and grazing impacts on vegetation. These effects are comparable to dry seasons or low-production years in that they similarly reduce high-quality food availability and alter primate behavior. As forests continue to be cleared and fragmented, and agricultural use increases (Arroyo-Rodríguez & Mandujano, 2006; Estrada et al., 2017; Marsh et al., 2013), these impacts may compound with increased climatic uncertainty, further reducing important high-energy food resources for primates. Limiting these resources inhibits primate populations and can negatively impact their overall health and future success (Behie & Pavelka, 2013; Cui et al., 2020). Trends identified in this review can assist in conservation efforts by emphasizing the benefits of identifying primates' primary food resources, areas of high food abundance, and times of high quality food production; therefore prioritizing those species and locations when implementing protections for primate species around the globe.

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CHAPTER 2. GRANT PROPOSAL

Section 1. Abstract

Thinning treatments of Douglas fir in the Colorado front range are becoming more necessary to reduce wildfire risk and return forests to their natural state. However, thinning increases non-native plant species in newly open areas. Therefore, herbicide treatments such as indaziflam may be necessary to mitigate unwanted effects of thinning treatments. Also, plant community structure differs across northern and southern slopes, so the effects of thinning and herbicide treatment may differ. I propose a study in Stanley Park (Jefferson County, CO) where I will assess the effects of thinning and indaziflam treatments on understory vegetation both separately and in combination with one another across north and south-facing aspects. This study may improve the management of front range forests by providing information about non-native species invasion post-thinning treatment, the efficacy of indaziflam in these areas, potential negative effects on native species, and differences in all these factors across slope positions.

Section 2. Objective, Hypotheses, Anticipated Value, Literature Review

Objective

This study will assess the effects of indaziflam on native and non-native plants in an alpine forest near Evergreen, Colorado (Stanley Park) across thinned and unthinned areas, as well as across north and south-facing slopes. It will also investigate whether indaziflam can more effectively prevent invasion associated with disturbance and tree removal if applied before invasion has occurred. The site will then be revisited yearly to assess both short and long-term indaziflam effects at this site across thinning treatments and aspect.

Hypotheses/Predictions

1. Indaziflam will be more effective in north-facing areas (relative reduction of target species presence and percent cover, and length of efficacy) and non-native species abundance will be highest in the south-facing, thinned, and unsprayed area because indaziflam successfully reduces non-native plant cover and greater water retention allows native species to continue to outcompete non-native species.
2. Non-native species will have higher occurrence and abundance in thinned areas than in unthinned areas across each aspect and indaziflam-treatment area because thinned areas are more vulnerable to invasion.
3. Native species richness and percent cover will increase in thinned areas across both aspects and indaziflam treatments because thinning increases overall understory plant species richness and percent cover, and indaziflam has been shown to positively affect native species abundance.

Anticipated Value

Wildfire management is of utmost concern for land managers, farmers, and the public worldwide (Rodman et al., 2020; Clark et al., 2020). Fire suppression in the front range has contributed to a Douglas fir-dominated forest structure that increases the risk of high-intensity forest fires (Addington et al., 2018). Therefore, there is a growing need to reduce Douglas fir cover in the front range. However, reducing canopy cover with thinning treatments disturbs the understory and provides space for non-native species to invade (Demarest et al., 2023; Pappas et al., 2022). Non-native, early-season annuals such as *Bromus* and *Alyssum* spp. increase fuel loads and outcompete native species (Clark et al., 2020; Meyer-Morey et al., 2021). These species necessitate the development of products that target non-native understory species, but there may be negative effects of using herbicide on native species richness and abundance.

Indaziflam successfully reduces targeted early-season annual species (e.g. Courkamp et al., 2022a; Sebastian et al., 2017), but may also reduce native species richness (Courkamp et al., 2022b; Meyer-Morey et al., 2021). While there are many studies of indaziflam's effects on native and target species richness and abundance, there is a lack of empirical data on its effects compared between north and south-facing slopes, or how it affects understory plant species after thinning treatments. This study may determine the potential benefits of applying indaziflam in conjunction with thinning treatments to restore front range forest communities most effectively. Also, as a multi-year study, we will investigate the effects on the native seed bank by the presence and abundance of native species in years post-treatment compared to pre-treatment. Minimizing potential fire impacts and establishing best practices for non-native species treatments will contribute to the current and future success of restoration projects throughout the front range and beyond.

Literature Review

Climate change and expanding human populations have altered fire regimes and increased the risk of high-intensity wildfires that can impact both the natural and human environment (Rodman et al., 2020). There is a growing need to actively manage forest ecosystems to limit the threat of large-scale fires, protect native species, and keep people safe. Fire suppression along the front range has allowed shade-tolerant Douglas fir (*Pseudotsuga menziesii*) to establish rapidly in the understory of ponderosa pine (*Pinus ponderosa*) forests (Addington et al., 2018). Where once there were interspersed tree species with large canopy openings and rich understory vegetation, there are now dense stands of Douglas fir closing the canopy and shading out native understory plants. By invading openings and extending the forest crown, firs increase the risk of highly destructive crown fires in areas that historically experienced low-moderate intensity wildfires that would leave old-growth forest intact, maintain openings, and recycle nutrients to the understory (Addington et al., 2018).

Denser canopies reduce understory species cover (Addington et al., 2018). In order to maintain/restore the historical range of variability and prevent high-severity wildfire in front range forests, managers have increasingly implemented thinning treatments of Douglas fir. While some studies report no change in understory vegetation after thinning treatments (Fornwalt et al., 2008), others report an increase in overall species richness and abundance (Demarest et al., 2023; Pappas et al., 2022). However, thinning also increases non-native species invasion into newly colonizable areas (Nelson et al., 2008) and may negatively impact rare species (Pappas et al., 2022). In general, north-facing slopes have higher soil water retention and support greater species richness than south-facing slopes (Tyagi et al., 2023). Also, while firs are more common on north-facing slopes, they can be found on south-facing slopes in moisture-rich areas (Addington et al., 2018). Therefore, further treatment of non-native plant species with herbicide will inevitably increase in thinned areas, but thinning and herbicide treatment effects on native and non-native species may vary between north and south-facing slopes due to differences in community composition.

Indaziflam is a cellulose biosynthesis inhibiting herbicide that targets the shallow seed bank, thus impacting short-lived, cold-season annuals (Clark et al., 2020; Courkamp et al., 2022a; Sebastian et al. 2017). It limits the growth of germinating seeds early in the growing season and allows later-season, native perennials to establish. *Bromus tectorum* and *Alyssum* spp. are non-native, early-season annual species that outcompete native grasses and forbs across the

U.S. and are of special concern in the front range (Clark et al., 2020; Meyer-Morey et al., 2021). *Bromus* and *Alyssum* spp. facilitate the spread of wildfires, which are of growing concern in dry, front-range forests (Clark et al., 2020; Meyer-Morey et al., 2021; Rodman et al., 2020). Many studies report the efficiency of indaziflam in reducing these non-native species with little effect on native species richness and percent cover (e.g. Courkamp et al., 2022a; Sebastian et al., 2017). However, indaziflam also reduces native forbs as well as seed bank richness (Courkamp et al., 2022a; Meyer-Morey et al., 2021)**.** Another recent study reported higher native grass recruitment in more moisture-rich areas after indaziflam treatment (Terry et al., 2021). Therefore, more research is needed on the effects of indaziflam on native species, and how these effects may differ across aspects with varying moisture availability.

Section 3. Methods

Study site

Stanley Park is an 80-acre park in Jefferson County near Evergreen, CO. At about 8,000 feet elevation, it is a mixed-conifer forest dominated by ponderosa pine and Douglas fir. It is bisected by a small ridgeline creating two distinct areas, a north and a south-facing slope. The north-facing slope is characterized by more dense Douglas fir patches and understory dominated by more moisture-dependent and shade-tolerant species. The south-facing slope contains more dispersed trees, more ponderosa pine relative to Douglas fir, and an understory of more abundant non-native annual species. Denver Mountain Parks (DMP) is planning a thinning treatment in 2024, similar to those implemented by DMP in neighboring parks (Cub Creek and Bell Parks) to reduce Douglas fir in the crown and in the understory (Perri et al., 2018).

Data collection

We will divide the study site along the ridgeline into north and south-facing areas using aspect layers in ArcGIS. Within each aspect zone, DMP will delineate where the proposed thinning will occur. We will randomly place twelve 10m radial plots in both north and south-facing areas (24 total) with six plots in each of the thinned and unthinned areas. In each radial plot, we will center four $1m^2$ quadrats at the 5m mark along cardinal direction transects extending from plot center for a total of 48 quadrats in each north and south-facing area (96 total), and 24 in each thinning treatment area within aspect zones.

Prior to indaziflam and/or thinning treatments, we will survey the entirety of the radial plots recording each species for presence/absence and richness data (Carter et al., 2021) and sample the quadrats by recording each species and it’s percent cover (e. g., Courkamp et al., 2022; Fornwalt et al., 2008; Meyer-Morey et al., 2021). After the initial surveys, we will randomly select three of the six radial plots in each area for indaziflam treatment to be sprayed throughout. This will produce three radial plots containing 12 total quadrats in each of the four treatment areas in each aspect zone: 1) unthinned, unsprayed (control); 2) thinned, unsprayed; 3) unthinned, sprayed; 4) thinned, sprayed.

Data Analysis

We will use richness data from radial plots collected pre-treatment to create overall species lists for the north and south-facing slopes, where we will compare richness and occurrence (number of plots species is present) across aspects with t-tests and binomial generalized linear models (GLMs), respectively, using R statistical software and the lme4 package (R Core Team, 2021). We will calculate pre-treatment species richness and overall abundance of both native and non-native species to compare quadrat data across aspects using t-tests. One and two years after thinning and indaziflam treatments, Regis University environmental biology students will collect and compare the same data in both radial plots using GLMs and quadrats using t-tests within each of the four treatment types and compare them to: 1) Pre-treatment data from the same plots and quadrats; 2) The other three treatments in the same aspect zone using pairwise t-tests with Bonferroni-adjusted p-values; 3) The equivalent treatment area from the opposite aspect zone.

Potential Negative Impacts

Indaziflam can potentially harm native plants and seedbanks, which could be detrimental if any threatened or rare species were impacted (Courkamp et al., 2022a; Meyer-Morey et al., 2021). There may also be negative impacts on soil microbes, affecting nitrogen cycling and soil fertility in the future, although these impacts aren't largely supported (González-Delgado et al., 2022). Finally, impacts on wildlife are not well understood, but may cause harm if ingested.

Project Schedule

Date	***Deliverables***
June 10-11, 2024	Create maps in ArcGIS, delineate areas, and identify treatment sites
June 12-July 3, 2024	Data Collection and indaziflam treatment
July 8-October 24, 2024	Data cleaning, analysis of north vs. south-facing slopes, and preparation of first report
September-October, 2024	Thinning treatments in both aspect zones
October 25, 2024	Initial findings report submitted to Boulder County Open Space
Summer 2025 and 2026	Follow-up studies repeating data collection from 2024 and data analysis of treatment combinations

Section 4. Budget

Item	*Price*
One quart of Rejuvra (indaziflam formulation) https://www.intermountainturf.com/vegetation-management/selective-herbicides/rejuvra-herbicide-2.html	365.93
Field King four-gallon no-leak professional backpack sprayer (for indaziflam application) https://www.homedepot.com/p/Field-King-4-Gal-Professional-No-Leak-Backpack-Sprayer-190328/205713890	84.97
Garmin GPSMap 64xs https://www.garmin.com/en-US/p/669284	364.99
Colorado 4WD mileage reimbursement rate of $0.62/mile driving 60 miles per day for three weeks (15 weekdays) during initial surveys (30 miles each way from Regis University) https://osc.colorado.gov/financial-operations/fiscal-rules-procedures/mileage-reimbursement-rate	558.00
Field equipment replacement (flagging, measuring tapes, quadrats, batteries, etc.)	300.00
Researcher stipend: $25.00/hour for four weeks (160 hours) of labor by two researchers for preparing preliminary resources for data collection, surveying the site, applying indaziflam, and for analysis and preparation of the initial report	8,000.00
Total	**9,673.19**

Subsequent years' research would require no further financial input as Regis University graduate students in Environmental Biology would perform follow-up studies as part of their graduate research.

Section 5. Qualifications of Researchers

Please see attached CV and letter of support for additional requirements (after literature cited)

Literature Cited

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Reilly Miller

rmiller016@regis.edu
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3047 W 47th Ave. #211 Denver, CO 80211
760-685-5686

Skills

Proficient in ArcGIS and statistical analyses in R. Field research and data collection using various protocols and programs (Zoomonitor, Survey123, Excel, Collector, DIMA, Terrasync etc.). Supervisory experience. SCUBA certified (Open Water, Deep Water, Nitrox) with over 80 dives logged. CPR/First Aid.

Education

Master of Science: **Environmental Biology**, **Regis University – 2022-2024**
Coursework covering ecological modeling using R software, Advanced ecology, Aquatics, Behavioral ecology, Conservation/restoration, ArcGIS, NEPA policy, Wetland delineation, and Scientific writing/grant proposals.

Bachelor of Science: **Animal Science**, **Cal Poly San Luis Obispo – 2011-2015**
Extensive coursework in Anatomy/Physiology, Animal Nutrition, Genetics, and Chemistry, as well as Environmental Sciences and Natural Resources.

Research/Work Experience

Regis University – Mantled Howler Monkey Activity and Spatial Cohesion in a Forest Fragment vs. Large Forest in Costa Rica – Dr. Amy Schreier – 2022-24.

- Studied mantled howler monkeys' activity and spatial cohesion at La Selva Biological Station in northeastern Costa Rica. Spent 1 month recording feeding, resting, and traveling behavior as well as the number of, and distance to neighbors using instantaneous scan sampling. Identified plant species used as food sources. Analyzed data in R and presented findings at various conferences/symposiums. Manuscript will be submitted for publishing in 2024. Wrote grant proposal and received a university grant to conduct research.

Regis University – Indaziflam Effects on Native and Non-native communities in Colorado Grasslands – Dr. Mike Ghedotti, Dr. Tyler Imfeld, and Dr. Daniela Rivarola – Academic Year 2022-23

- Conducted a vegetation study to examine the effects of an herbicide (indaziflam) on various grassland communities around Denver. Specifically, the reduction of *Bromus tectorum* and *Alyssum simplex* and the effects on other plants in the community. Collected data in sprayed and un-sprayed areas in three parks by identifying plants, estimating percent cover of each species, and collecting vegetation and soil samples to measure plant, fungal, and bacterial biomass. I analyzed the data and presented a poster at the Front Range Student Ecology Symposium (FRSES) at CSU in Fort Collins. I also presented our findings to Denver Mountain Parks, who manage the treated areas.

Regis University – Bull Asian Elephant Activity Budget at Denver Zoo – Dr. Amy Schreier – 2022-23

- Assisted with two behavioral studies on male Asian elephants at Denver Zoo. Behavioral Ecology, Fall 2022: watched videos to record behaviors of different male elephants while they were housed in various pairings at night. Performed and presented preliminary analyses on this data. Summer 2023: Returned as research associate and collected daytime data on a new elephant introduced to the herd. The behaviors are to be compared both to the elephants already established in the herd, as well as to elephants who arrived at the zoo in a pair as opposed to solo.

Veterinary Assistant – Banfield North Oceanside – 760.639.5210 (Megan Cromwell/Cedric Koch/Khea Labagnoy) – April 26, 2021 to April 23, 2022 – 30 Hrs/Week

- Handled and helped treat dogs and cats as well as some exotic species. Assisted in diagnoses, surgery, restraint, laboratory tests, and interacted with customers on a daily basis. Performed cephalic and jugular blood draws, intramuscular and subcutaneous injections, and analyzed fecal, urine, ear swab, and blood samples under a microscope and using machinery.

Botany Crew Lead – Great Basin Institute/USFS Spring Mountains – GBI Las Vegas (Katy Gulley/Benjamin Jacobs – kathryn.gulley@usda.gov benjamin.jacobs@usda.gov) – May 11 through October 29, 2020 – Americorps – 40 Hrs/Week

- Surveyed for host plants of endemic butterfly species in the Spring Mountains National Recreation Area. Compiled comprehensive species lists from multiple sites and recorded Threatened, Endangered, and Sensitive (TES) plant species throughout the range. Described habitat communities, mapped data in ArcMap, and wrote multiple reports for the Forest Service and Fish and Wildlife on these findings. I was a crew leader and had supervisory responsibilities in the field and taught proper identification and data collection.

Biological Science Technician – USGS – WERC Henderson, NV – 702.564.4500 (Todd Esque/Felicia Chen – tesque@usgs.gov fchen@usgs.gov) – April 1 through December 20, 2019 – GS-05 – 40 Hrs/Week

- Tortoise Monitoring crew member with USGS. Consistently tracked Mojave Desert tortoises with radio telemetry to study movement across populations. Performed QA/QC on data and was involved in writing reports on our various projects. Performed visual health assessments on animals, collected oral swabs to test for bacterial disease, collected ticks from animals, drew blood via sub-carapacial venipuncture, and assisted in dissections. Performed comprehensive vegetation assessments on a salt marsh in central Nevada and in Guadalupe Canyon in CA, near Palm Springs. Learned to identify native and invasive plant species throughout desert regions. Mapped Joshua trees via satellite imagery to determine the entirety of the range for that species.

Botany Field Technician – Great Basin Institute/Nevada Dept. of Wildlife – GBI – 775.674.5475 (Sara Violett/Jessica Saenz – jsaenz@thegreatbasininstitute.org) – May 1 through November 20, 2018 – Americorps – 40Hrs/Week

- Americorps position with GBI in conjunction with NV Dept. of Wildlife and US Forest Service. Performed "AIM Protocol" and experimental methods to determine type, abundance, and density of vegetation in sagebrush communities. Learned to identify hundreds of native and invasive plant species throughout Nevada and eastern California. Performed soil stability tests on post-burn sites. Navigated 4-wheel-drive roads and off-trail hiking using GPS. Received contract extension to continue working as part of the soil crew to process samples for chemical testing.

Aquatics Crew Lead (Wildlife Technician) – USFS – High Sierra Ranger District - 559.855.5355 (Stephanie Barnes – slbarnes@fs.fed.us) **–** May 1 through October 12, 2017 – GS-05 (GS-07 equivalent) – 40 Hrs/Week

- Crew leader for the District Aquatics Biologist. Independently supervised and instructed two crew members on proper identification of aquatic species, specifically, endangered Sierra Nevada Yellow-Legged Frogs and Yosemite Toads at all life stages. Instructed and performed proper surveying practices, data collection & entry, and safety in the Sierra Nevada. Handled and researched threatened and endangered species, frequently interacted with and informed the public about our forest and species, and assisted with restoration and mitigation in conjunction with Fish & Wildlife and others. I received three awards from the forest's District Ranger for my performance and exceeding target goals as a GS-05 performing tasks of a GS-07.

Biological Science Technician -- US Forest Service – High Sierra Ranger District -- 559.855.5355 (Stephanie Barnes/ Cathy Brown – UC Davis – cathybrown@fs.fed.us) – June 13 through September 30, 2016 – GS-05 – 40 Hrs/Week

- Field technician on back-country crew for amphibian monitoring project. Backpacked 10-15+ miles per day to back-country locations throughout the Sierra Nevada to find endangered Yellow-Legged frogs and Yosemite Toads, for which I had a license to handle. Surveyed meadow, stream, and lake habitats to assess habitat condition and determine presence and abundance of the animals. Collected data and swabbed adults for skin samples to test for Chytrid fungus and gather genetic material.

Cal Poly San Luis Obispo – Bull Test and Quail Management Enterprises – Academic Year 2014-15

- In the Bull Test opportunity, my duties were to feed and monitor the bulls' health and growth, and to calculate what increase of feed to deliver based on these observations. While managing the quail, in addition to monitoring health, growth, and feed amount, I collected and incubated eggs, and monitored from hatchling to adulthood.

Harnas Wildlife Refuge – Namibia – August 21-September 9, 2014

- Volunteered on a wildlife refuge in Africa in the summer prior to my senior year at Cal Poly. I collected behavioral data on a captive pack of African wild dogs documenting social, feeding, and locomotive behaviors. This data was to be used to determine a viable pack for re-release to the wild. I also tracked cheetahs using radio telemetry and recorded their locations and types of kill (species, sex, size, etc.), as well as the health status of the cheetahs (body condition score).

REGIS UNIVERSITY

December 12, 2023

Boulder County Parks and Open Space
5201 St. Vain Rd.
Longmont, CO, 80503

To whom it may concern,

It is my pleasure write a letter in support of the proposal "*Indaziflam effects on native and non-native species across north and south-facing slopes in a thinned alpine forest"* being submitted to BCPOS Small Grants by Reilly Miller, Master's student at Regis University.

Reilly Miller is an outstanding student, with a solid scientific background and extensive fieldwork experience, as clearly shown on his resume. This proposal is part of a bigger project aimed at understanding the effects of wildfire mitigation strategies (thinning in particular) on wildlife and vegetation. Although the research will be conducted in Jefferson County due to an ongoing cooperation between Denver Mountain Parks and Regis University, the knowledge expected from this research will provide valuable information to Boulder County since thinning is the most widely used fire mitigation strategy along the Front Range. Furthermore, the introduction of non-native plant species that later become invasive following a disturbance (such as thinning) is a shared problem along the Front Range as well.

In conclusion, after reviewing Reilly Miller's proposal, I fully approve and support it as Reilly Miller seeks external funding to support this research designated to determine if the early use of indaziflam as a pre- thinning treatment prevents later plant invasion, a process that has never been tested.

Sincerely,

Daniela Rivarola

M. Daniela Rivarola, PhD.
Assistant Professor at Regis University
3333 Regis Blvd.
Denver, CO, 80221

CHAPTER 3. JOURNAL MANUSCRIPT

Mantled howler monkeys (*Alouatta palliata*) alter activity and spatial cohesion in a Costa Rican forest fragment

Reilly L. Miller[1], Francesca V.E. Kaser[1], Ryan E. Belmont[1], Michael Ennis[1], Kristofor A. Voss[1], Laura M. Bolt[2], Amy L. Schreier[1]

[1]Department of Biology, Regis University, Denver, Colorado, U.S.

[2]Department of Anthropology, University of Toronto Mississauga, Mississauga, Ontario, Canada

Abstract

Habitat loss due to deforestation is a primary threat to global biodiversity. Clearing tropical rainforests for agriculture leads to forest fragmentation. Forest fragments contain fewer large trees and provide lower food availability for primates compared to continuous forests. Mantled howler monkeys inhabit the increasingly fragmented rainforests of Central and South America and may need to alter their activity and spatial cohesion to mitigate competition and preserve energy in fragments where there is lower quality food. We compared howler monkey activity and spatial cohesion across a small forest fragment (La Suerte Biological Research Station, LSBRS) and a large, continuous forest (La Selva Research Station) in northeastern Costa Rica. We predicted that monkeys at LSBRS would rest more, feed more, travel less, and be less spatially cohesive compared to La Selva to contend with fewer resources and higher competition in the small fragment. Using instantaneous scan sampling at two-minute intervals during 30-minute focal samples, we recorded activity and counted the number of individuals within 5

meters of the focal animal. The probabilities of observing each behavior differed significantly across sites. As predicted, monkeys at LSBRS fed more than those at La Selva, but contrary to our predictions, they rested less and traveled more. The mean number of individuals within 5 m was significantly lower at LSBRS compared to La Selva. The ability to modify their activity and spatial cohesion in response to fragmented forests provides insight into how primates can contend with fewer resources and higher competition in changing ecosystems worldwide.

Introduction

Deforestation and habitat fragmentation are among the greatest drivers of declining wildlife populations worldwide (Haddad et al. 2015). Home to much of the world's biodiversity, tropical rainforest ecosystems are especially vulnerable to such degradation. Over 90% of global deforestation is due to agricultural expansion (Pendrill et al. 2022), which is the primary cause of habitat loss in the tropics (Estrada 2015). Deforestation decreases rainforest continuity, leaving behind smaller patches of segregated forest fragments. Fragmentation and overall habitat loss decrease available resources and increase distances to other viable habitats and the amount of forest edge; all of these factors have substantial negative impacts on species diversity (Haddad et al. 2015). Larger mammalian species have a higher risk of population declines (Cardillo et al. 2005; Tomiya 2013) and animals with populations that are spatially clustered and have specific dietary requirements appear to be particularly impacted by habitat fragmentation (Turner 1996). In general, primates are large-bodied, social mammals most prevalent in tropical forests and require complex food resources; consequently, global primate populations are declining, and many species are at risk of extinction (Estrada and Garber 2022; Estrada et al. 2017). Determining how primates respond to anthropogenic change is therefore critical to understanding how to preserve these charismatic species moving forward.

Primates typically require high plant species diversity and abundance of mature trees for food (Arroyo-Rodríguez et al. 2007; Arroyo-Rodríguez and Mandujano 2009; Dunn et al. 2009). Fragments resulting from deforestation contain a higher proportion of forest edge and lower quality vegetation than larger forests (Arroyo-Rodríguez and Mandujano 2006; Bolt et al. 2018). Because of these edge effects, differences in vegetation are further modified near forest edges; trees are smaller (lower diameter at breast-height – DBH), provide less canopy cover, and the prevalence of secondary vegetation and tree mortality are greater in forest edges compared to forest interior (Arroyo-Rodríguez and Mandujano 2006; Bolt et al. 2018). Therefore, forest fragments generally provide primates with less food than larger continuous forests. Maximizing dietary intake appears to be an important driver of primate behavior across many species (e.g. Cui et al. 2020; Dunn et al. 2010; Gould and Gabriel, 2015; Rimbach et al. 2014; Windley et al. 2022), so changes in food availability in degraded landscapes may cause primates to alter their feeding strategies, overall activity, and social cohesion.

In general, primate feeding strategies follow "Optimal Foraging Theory," where many species preferentially consume the most energy-rich foods when available, balance their diets by consuming a diversity of resources, and avoid foods high in plant secondary metabolites (PSMs) that can be toxic or undigestible (Altmann 2006; Zhao et al. 2013). High-energy foods include ripe fruit, seeds, and young leaves while lower quality foods include mature leaves or fibrous foods that are high in PSMs (Brugiere et al. 2002). While some species such as ring-tailed lemurs *(Lemur catta)* and howler monkeys (*Alouatta spp.*) may be adapted to consume more high-PSM diets, they will still follow optimal foraging theory by consuming more ripe fruit when available (Dunn et al. 2010; Gould and Gabriel 2015; Windley et al. 2022). When these high-energy foods are not available, primates may alter their diets by consuming more low-energy foods in order to maximize dietary intake. For example, in a Mexican forest fragment, Central American spider monkeys (*Ateles geoffroyi*) that typically prefer high-energy foods consumed different species, more low-energy leaves, and less fruit than monkeys in a continuous forest (Chaves et al. 2012). Also, after canopy cover and tree density were reduced in a forest fragment in Belize, black howler monkeys (*Alouatta pigra*) altered their diets and ate species that were not preferred prior to the changes in forest structure (Behie and Pavelka 2005).

Alterations in diet due to reduced food quality and availability in forest fragments (Chapman et al. 1992) may also require primates to alter their group sizes and spatial cohesion and/or redistribute energy requirements by changing the amount of time they spend feeding, resting, and traveling to mitigate feeding competition, maximize energy intake, and/or reduce energy expenditure (Boyle and Smith 2010; Chapman 1995; Chaves et al. 2011; Dunn et al. 2010). Spider monkeys in Mexico increased the time spent feeding and decreased time spent traveling in smaller fragments (Chaves et al. 2011). Northern bearded saki monkeys (*Chiropotes satanas chiropotes*) in forest fragments in Brazil reduced their group sizes and spent more time resting and less time traveling than populations in continuous forests (Boyle and Smith 2010). Additionally, chacma baboons (*Papio ursinus*) living along an urban-natural interface in South Africa altered their traveling behavior and reduced spatial cohesion by spreading farther apart and forming smaller subgroups in more urban settings (Bracken et al. 2022). In Colombia, red howler monkeys (*Alouatta seniculus*) traveled less, fed and rested more, and ate lower quality food in a forest fragment compared to a continuous forest (Stevenson et al. 2015).

Howler monkeys (*Alouatta* spp.) are large-bodied primates living in Central and South American rainforests (Altmann 1959). Mantled howler monkeys (*Alouatta palliata*) live in large, spatially cohesive, multimale-multifemale groups of ~10-15 individuals (Ryan et al. 2008; di

Fiore et al. 2011; Schreier et al. 2023). They are folivore-frugivores whose diet depends on leaves and fruit from large, diverse tree species (Asensio et al. 2007; di Fiore et al. 2011; Garber and Kowalewski 2015). While they are not as deterred by high PSM foods as other primates (Windley et al. 2022) and much of their diet consists of leaves, they prefer to eat nutrient-rich foods such as flowers and ripe fruit when they are available (Dunn et al. 2010; Bolt et al. 2021). Because leaves contain toxins that are hard to digest, howler monkeys generally minimize energy expenditure by spending most of their activity budget resting (di Fiore and Campbell 2007; Milton 1980). Like many primate species, forest fragmentation negatively impacts the quality of mantled howler monkey habitat (Arroyo-Rodríguez and Mandujano 2006) and may require populations to alter their activity and spatial cohesion. Populations of mantled howler monkeys in Mexico reduced group size, increased their time spent resting, and decreased traveling in smaller fragments compared to a larger one (Juan et al. 2000). Furthermore, mantled howler monkeys in Hacienda La Pacifica, Costa Rica also reduced group sizes in response to fragmentation (Clarke et al. 2002), and another population in Mexico increased the time spent resting and feeding, and reduced traveling in response to fragmentation Asencio et al. 2007)

As with most primate species, mantled howler monkey populations are declining and are currently rated as “Vulnerable” on the IUCN red list (Cortes-Ortíz 2021). More than half of mantled howler monkey populations live outside protected areas (Estrada et al. 2015, 2017). Global rainforest area continues to decline with Central and South America losing the highest proportion of their forests each year (Estrada and Garber 2022; Marsh et al. 2013). The vulnerable status of mantled howler monkeys and their range in highly fragmented areas makes their conservation and protection a priority, and conservation practices must depend on how the animals behave in forest fragments compared to large, continuous forests. Because most primates live in the tropics, depend on abundant food sources, and much of the world's deforestation occurs in rainforests, the effects of habitat loss on howler monkeys in fragments in Costa Rica can be broadly applied to primate species inhabiting rainforests worldwide.

In this study, we compared mantled howler monkey activity budgets and spatial cohesion across populations at La Suerte Biological Research Station (LSBRS) and La Selva Research Station (La Selva) in northeastern Costa Rica. LSBRS is a small fragment that is almost completely surrounded by plantations and cattle ranches (Pruetz & Leasor 2002; Schreier et al. 2021), while La Selva is more than ten times larger and is directly adjacent to Braulio Carrillo National Park, forming a continuous forest for an extended area. Accordingly, La Selva contains larger trees that provide more food and habitat to primate species compared to LSBRS (Schreier et al. 2024). Previous studies at LSBRS found that howler monkeys at the forest edge where trees are smaller and less abundant did not alter their activity or spatial cohesion when compared to forest interior (Schreier et al. 2021). However, the combination of extreme population pressure and the small size of LSBRS may obscure variation in behavior and spatial cohesion between forest zones. Therefore, we compared monkeys at LSBRS to a larger, more continuous forest in order to evaluate larger-scale behavioral responses across forest types.

Due to the differences in habitat quality and population density between LSBRS and La Selva, we hypothesized that mantled howler monkeys would maximize feeding behavior, minimize energy expenditure, and modify social cohesion to reduce feeding competition in the fragment compared to the large forest. We predicted that they would spend more time feeding at LSBRS than La Selva in order to meet their nutritional requirements. Because feeding more at LSBRS would require them to eat a higher abundance of low-quality food that is difficult and energy-intensive to digest, we also predicted that the monkeys would spend more time resting and less time traveling at LSBRS in order to conserve energy compared to in the continuous forest at La Selva. We also predicted that the monkeys at LSBRS would reduce their spatial cohesion, and therefore feeding competition, by limiting the number of neighbors within 5 m and increasing the distance to nearest neighbors. Regional and population-level differences in adaptations suggest flexible responses to anthropogenic change (Arroyo-Rodríguez and Dias 2010; Clarke at al. 2002; Schreier et al. 2021, 2022), and similarities may identify key conservation priorities for vulnerable primates moving forward.

Methods

Study Sites

We conducted this study at two tropical rainforest sites in northeastern Costa Rica. La Suerte Biological Research Station (LSBRS; 10°26’ N, 83°46’ W) is a small, 3 km^2 forest fragment surrounded by cattle ranches and coconut plantations (Molina 2015; Bolt et al. 2018; Schreier et al. 2021). Located in the same region of Costa Rica as LSBRS, La Selva Biological Station (La Selva; 10°25’ N, 84°00’ W) encompasses 16 km^2 of tropical rainforest (Matlock and Hartshorn 1999). The southern border of La Selva is continuous with Braulio Carrillo National Park which consists of ~440 km^2 of cloud and tropical rainforest (Bell and Donnelly 2006). The same three species of primates inhabit both forest sites: mantled howler monkeys, white-faced capuchins (*Cebus imitator*), and Central American spider monkeys (*Ateles geoffroyi*). The population density of mantled howler monkeys at LSBRS is estimated at 109.5 individuals/km^2, one of the highest densities ever recorded for the species, while the population density of 21.3 individuals/km^2 at La Selva is much lower and more typical of mantled howler population density reported across their geographic range (Bolt et al. 2022; Schreier et al. 2024).

Data Collection

We collected data from 5:00 to 18:00 at both sites. We located a group of howler monkeys at dawn and stayed with it until they returned to a sleeping site or for as long as possible. We used scan sampling of focal individuals for 30-minute sampling periods and collected activity and spatial cohesion data at 2-minute intervals (Altmann 1974; Schreier et al. 2021, 2022). At each 2-min scan, we recorded the focal monkey's behavior (i.e., resting, feeding, traveling, other). We recorded spatial cohesion by estimating the distance from the focal individual to its nearest neighbor (in meters) utilizing distance classes of 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 m or >10 m (Irwin 2007; Schreier et al. 2021, 2022), and recorded the number of neighbors within a 5 m radius of the focal individual (Schreier et al. 2021, 2022). To collect equal amounts of data on each sex, we alternated between adult males and adult females. We also collected data on juveniles but not on dependent infants. We collected data spanning both wet and dry seasons at each site. At LSBRS we collected a total of 874.5 hours of data between 2017 and 2020: May-August 2017 and 2018, and December-January 2018-19 and 2019-20. At La Selva we collected a total of 603 hours of behavioral data across three field seasons: November 2018-February 2019, and May-June 2022 and 2023.

Data Analysis

We fit generalized linear mixed models (GLMM) to assess the effect of forest site (LSBRS and La Selva) on each activity and spatial cohesion measure. We used each 30-minute sampling period as a random effect in the models to account for intrinsic autocorrelation between observations within the same sample. For feeding, resting, and traveling behaviors, we ran the models with binomial response variables (1=behavior observed, 0=any other behavior was observed) assuming they followed a binomial distribution where the log(odds) was linearly related to predictors. For spatial cohesion measures, we assumed the number of neighbors within 5 m followed a Poisson distribution where the log(mean) was linearly related to predictors. We assumed a normal distribution of distance to nearest neighbor (*d*) after performing a transformation of log(*d*+0.1) where the mean was linearly related to predictors.

We analyzed all data with R software version 4.2.1 (R Core Team 2022). We used the lme4 package (Bates et al. 2015) to fit GLMM, and we used the multcomp package (Hothorn et al. 2008) to conduct general linear hypothesis tests in order to determine confidence intervals and p-values, and significance was set at $p<0.05$.

Results

Mantled howler monkeys altered their activity budgets across forest size. As predicted, the probability of howler monkeys feeding at LSBRS (0.028, 95% CI: 0.023-0.034) was significantly higher ($p<0.001$) than at La Selva (0.017, 95% CI: 0.013-0.022; Fig. 1A). Contrary to our predictions, however, the probability of howler monkeys traveling was significantly higher ($p<0.001$) at LSBRS (0.066, 95% CI: 0.059-0.073) than at La Selva (0.032, 95% CI: 0.027-0.036; Fig. 1B), and the probability of resting at LSBRS (0.773, 95% CI: 0.745-0.798) was significantly lower ($p<0.001$) than at La Selva (0.876, 95% CI: 0.853-0.895; Fig. 1C).

Mantled howler monkeys reduced spatial cohesion across forest size, but we did not observe a significant effect on the distance to nearest neighbor. However, following our predictions, the mean number of neighbors within 5 m of the focal individual was significantly lower ($p<0.001$) at LSBRS (0.89, 95% CI: 0.83-0.96) than at La Selva (1.27, 95% CI: 1.17-1.38; Fig. 2A). The median distance to nearest neighbor did not differ significantly ($p=0.862$) across sites (LSBRS: 1.71 m, 95% CI: 1.59-1.83 m; La Selva: 1.66 m, 95% CI: 1.52-1.81 m; Fig. 2B).

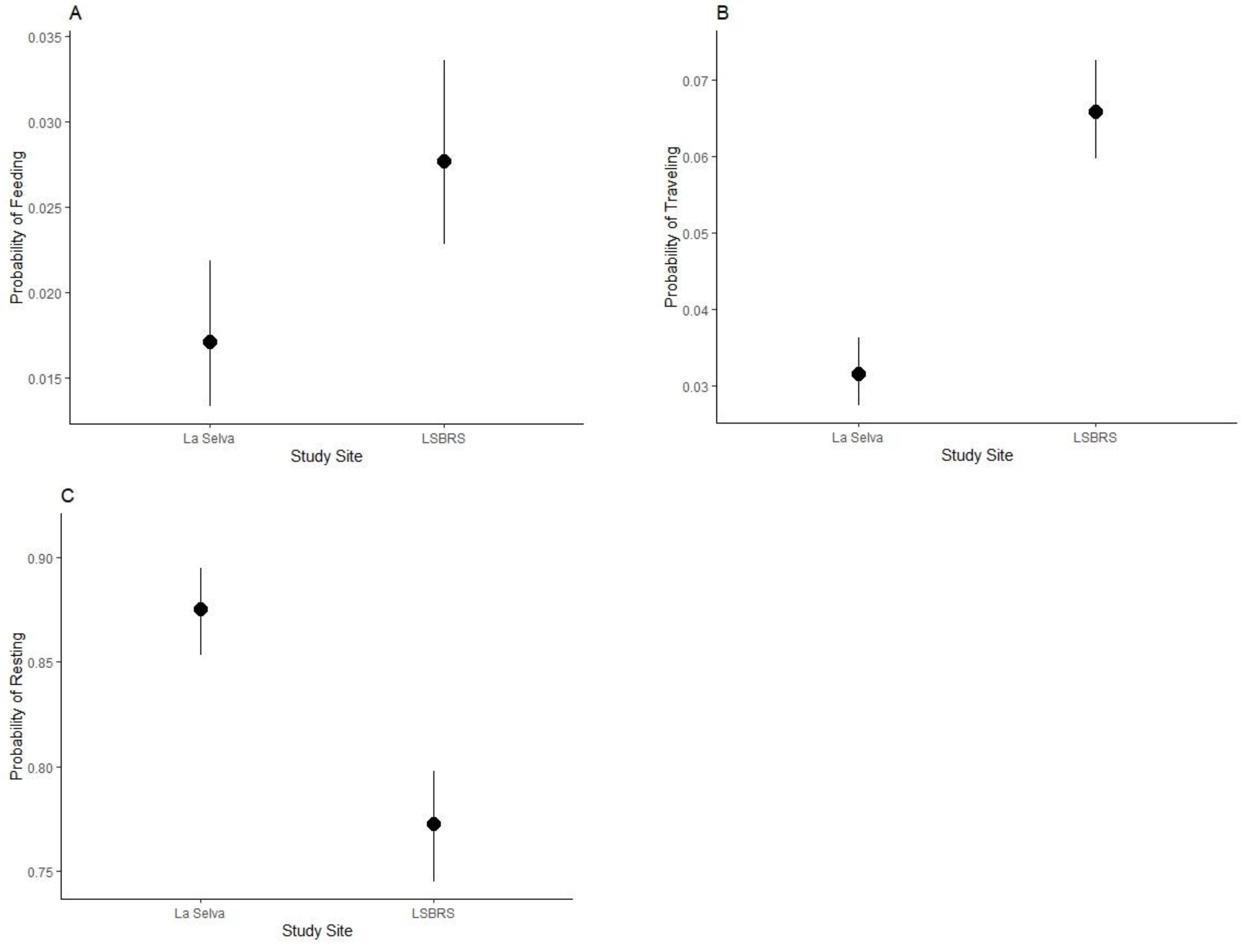

Figure 1. Mantled howler monkeys significantly increased time spent feeding (A) and traveling (B), and significantly decreased time spent resting (C) at LSBRS when compared to La Selva. Points represent the overall probability of participating in each activity, and lines represent the 95% confidence intervals.

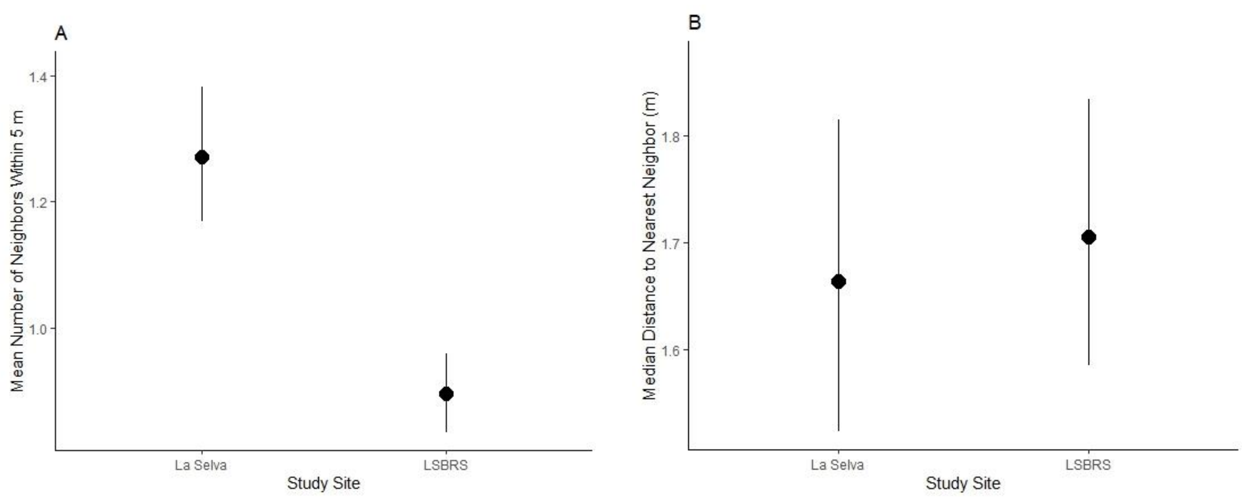

Figure 2. Howler monkeys significantly decreased the number of conspecifics within 5 meters (A), but did not significantly alter the distance to nearest neighbor (B). Points represent the median distance to neighbors and mean number of neighbors, and lines represent the 95% confidence intervals.

Discussion

In this study, we assessed differences in mantled howler monkey activity and spatial cohesion across a small forest fragment (LSBRS) and a large continuous forest (La Selva). While prior studies on the LSBRS mantled howler monkey population evaluated variation in activity, diet, and spatial cohesion across edge and interior forest zones (Bolt et al. 2018; Schreier et al. 2021, 2022), the differences in habitat quality, size, and population density between LSBRS and La Selva allowed us to assess the overall effects of habitat fragmentation and feeding competition on these two howler monkey populations in northeastern Costa Rica. Our results indicate that, as predicted, howler monkeys spent more time feeding and reduced their spatial cohesion in the forest fragment compared to the continuous forest, consistent with the fact that LSBRS has smaller and less diverse trees than La Selva (Schreier et al. 2024). However, differences in time spent resting and traveling did not conform to expectations. The combination of reduced food availability and extreme population density may have driven the differences we observed between the two sites.

Optimal foraging theory dictates that monkeys would need to feed more in areas with lower food quality to meet energy requirements and achieve a balanced diet (Altmann 2006; Dunn et al. 2010). This aligns with our hypothesis that lower food availability in fragments (Arroyo-Rodríguez and Dias 2010) would require monkeys to spend more time feeding. Our findings coincide with many other observations of howler monkeys increasing their feeding effort in fragmented landscapes. For example, red howler monkeys in Colombia (Stevenson et al. 2015) and mantled howler monkeys in both Mexico (Dunn et al. 2009) and La Pacifica Costa Rica (Clarke et al. 2002) increased the amount of time spent feeding and foraging in forest fragments compared to larger forests. The tendency for primates to increase time spent feeding in fragments is also consistent across many other primate species. Central American spider monkeys in Mexico (Chaves et al. 2011) as well as black and white ruffed lemurs (*Varecia variegata*) in Madagascar (Peterson et al. 2023) also increased time spent feeding in smaller fragments compared to larger ones. While prior studies at LSBRS did not find differences in feeding activity across forest edge and interior (Schreier et al. 2021, 2022), there were shifts in diet and a reduction of feeding tree size in the forest edge (Bolt et al. 2021).

We predicted that because howler monkey diets include more lower-quality foods compared to other species (Windley et al. 2022) and require them to center their activity budgets around resting (Milton, 1980), reduction of food quality in fragments would require more energy conservation in activity budgets. Primates have been observed conserving energy in fragments by resting more and traveling less (e.g. Chaves et al. 2011; Gabriel 2013), including howler monkey populations (Dunn et al. 2009; Stevenson et al. 2015). Additionally, the mantled howler monkey population at LSBRS rested more and traveled less in edge zones compared to interior forest (Schreier et al. 2022), presumably to conserve energy. However, when compared to La Selva, mantled howler monkeys at LSBRS traveled significantly more. Mantled howler monkeys in smaller fragments may need to vary their food selection more than in larger forests (Dunn et al. 2009), which may require them to travel more in order to find sufficient food and fulfill their dietary needs. The need to feed more and spend more time searching for food to reach dietary requirements at LSBRS may have precluded the possibility of increasing time spent resting. Similarly, Colombian night monkeys (*Aotus lemurinus*) substantially decreased resting and increased traveling behavior in a smaller fragment compared to a larger one (Bustamante-Manrique et al. 2021), and mantled howler monkeys at La Pacifica, Costa Rica also increased time spent feeding without increasing resting and reducing traveling (Clarke et al. 2002).

These results imply that smaller fragment size influenced mantled howler monkey activity. However, meta-analyses of mantled howler monkeys in Mexico (Cristóbal-Azkarate and Arroyo-Rodríguez 2007) and *Alouatta* species throughout Central and South America (Bicca-Marques 2003) determined that patch size alone does not have significant impact on activity patterns. Instead, populations pressures appear to have the greatest effect on howler monkey activity throughout their range (Arroyo-Rodríguez and Dias 2010; Cristóbal-Azkarate and Arroyo-Rodríguez 2007). This effect may be due to increased intraspecific competition within populations as a result of higher population densities in fragments (Arroyo-Rodríguez and Dias 2010). Our results follow these conclusions in that LSBRS has an extremely high howler monkey population density (109.5 individuals/km^2), about five times larger than at La Selva (23.4 individuals/km^2), suggesting much higher competition at LSBRS (Bolt et al. 2022; Schreier et al. 2024). Therefore, mantled howler monkeys may increase time spent feeding to reduce feeding competition and increase time spent traveling in order to avoid other individuals, subsequently reducing time spent resting. However, LSBRS contains fewer and smaller trees than La selva (Schreier et al. 2024), and both population density (Cristóbal-Azkarate and Arroyo-Rodríguez 2007) and food availability (Bolt et al. 2021; Dunn et al. 2009; Gould and Gabriel 2015; Rimbach et al. 2014) drive diet selection in many primates Therefore, we maintain that the combination of heavy population pressures and reduction of tree size and diversity in the small fragment contributed to the behaviors we observed.

While there was no observable difference across the forest fragment and continuous forest with respect to distance between neighbors, howler monkeys at LSBRS reduced their spatial cohesion by having fewer conspecifics within 5 meters, despite the high population density (Bolt et al. 2022; Schreier et al. 2024). These results are not consistent with prior work at

LSBRS that showed spatial cohesion increased in edge zones compared to forest interior (Schreier et al. 2022). However, on a broader scale, lower spatial cohesion in forest fragments may allow primates to reduce competition in areas with reduced food abundance and increased population pressures.

Many measures of howler monkey spatial cohesion are related to group size, which is generally lower in forest fragments (e.g. Clarke et al. 2002; Dunn et al. 2009; Juan et al. 2000; Stevenson et al. 2015) despite increasing population density in fragments (Arroyo-Rodríguez and Dias, 2010), perhaps to decrease competition. However, the population at LSBRS did not decrease their group size compared to La Selva (Schreier et al. 2024), possibly due to very high population density, or the ability to mitigate competition through their behavioral and spatial adaptations. Distancing between individuals has been seldom studied in regards to comparing fragments and continuous forests. However, one study of diademed sifakas (*Propithecus diadema*) in Madagascar showed increased distances to neighbors and a lower probability of having a neighbor within 5 m in smaller forest fragments compared to larger ones (Irwin 2007). Our study adds to the limited information on spatial cohesion in fragments and implies that mantled howler monkeys can limit resource competition by reducing the number of conspecifics with whom they are in proximity. This adaptation may be driven by varying spatial cohesion while participating in certain behaviors. For example, sifakas reduced spatial cohesion in fragments while in feeding, resting, and traveling behaviors, with the most marked reduction occurring during feeding (Irwin 2007). Colobus monkeys (*Colobus angolensis ruwenzorii*) in Uganda also significantly reduced spatial cohesion when feeding (Teichroeb et al. 2022). Therefore, our study quantifies the overall differences in spatial cohesion across LSBRS and La Selva, but future studies should examine whether differences in mantled howler monkey cohesion are influenced by specific behaviors and may provide more information about what drives spatial cohesion patterns in fragments.

Due to edge effects, forest fragments typically provide fewer resources for primates than larger, continuous forests (Arroyo-Rodríguez and Mandujano 2006; Bolt et al. 2018), presumably increasing feeding competition and requiring primates to alter their behaviors. Many species, including mantled howler monkeys, appear to reduce feeding competition by having flexible activity budgets and spatial cohesion patterns, but the effects of reduced food resources may be compounded by high population density in fragments. There is a commonality among many primate species of increasing time spent feeding in areas with fewer resources (Brugiere et al. 2002; Chaves et al. 2011), sometimes at the expense of increasing energy expenditure (Clarke et al. 2002; Dunn et al. 2009; Stevenson et al. 2015). More must be done to reduce feeding pressures on primate species by expanding and connecting fragments to decrease edge effects and population densities. Costa Rica has been a leader in conservation and reforestation over the past decades (Cunningham et al. 2020; Kleinn et al. 2002), and the Sistema Nacional de Áreas de Conservación (SINAC) is implementing a plan to connect large forests through connectivity corridors (Plan Estratégico 2018-2025). However, corridors proposed by SINAC connect large, protected forests to one another and ignore some small fragments such as LSBRS. High biodiversity in LSBRS and similar fragments may only increase the future success of connectivity corridors and primate species such as mantled howler monkeys with their inclusion. Our study highlights differences in mantled howler monkey populations across different forest sizes and may influence conservation practices and future research on vulnerable primate species in fragments.

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CHAPTER 4. STAKEHOLDER ANALYSIS

Introduction

Deforestation and fragmentation reduce forest cover and biodiversity on a global scale (Haddad et al., 2015). Forest fragments supply wildlife with fewer resources, reduce genetic diversity, increase competition among wildlife species, and compound effects of other anthropogenic impacts compared to continuous forests. Human impacts causing deforestation include urban expansion and use of natural resources, but the explosion of agriculture is the primary cause of deforestation throughout the tropics, contributing to over 90% of global rainforest loss (Estrada, 2015; Pendrill et al., 2022). In recent decades, neotropical rainforests have experienced the highest rates of annual agricultural expansion and deforestation (Estrada & Garber, 2022), and in 2011, Central American rainforests were losing the highest proportion of rainforest cover annually (Marsh et al., 2013). The Central American country of Costa Rica is home to highly biodiverse plant and wildlife species and is at the forefront of conservation (Cunningham et al., 2020; Kull et al., 2007; Sánchez-Azofeifa et al., 2001). While Costa Rican rainforests are impacted by global agriculture needs, primarily large-scale beef, pineapple, palm oil, coffee, and banana operations, it is one of just a few countries that have actually increased rainforest cover over the past decades (FOA, 2015). However, there is much work to be done to connect forest fragments in order to mitigate the negative impacts of agriculture on wildlife. La Suerte Biological Research Station (LSBRS) is a small forest fragment in northeastern Costa Rica that is surrounded by agriculture and is disconnected from larger protected areas in the country. Costa Rica should address the stress placed on the diverse wildlife species at LSBRS by connecting it to larger forests to create a continuous forest that is more representative of historical forest regimes.

La Suerte Biological Research Station

LSBRS is almost completely surrounded by cattle ranches, coconut plantations, and pineapple farms (Baltensperger & Brown, 2015; Pruetz & Leasor, 2002). LSBRS is approximately 400 ha in area and contains high levels of biodiversity including three primate species: mantled howler monkeys (*Alouatta palliata*), white-faced capuchins (*Cebus imitator*), and Central American spider monkeys (*Ateles geoffroyi*). Large-bodied mammals such as primates are especially vulnerable to extinction and habitat fragmentation (Cardillo et al., 2005; Tomiya, 2013; Turner 1996), and are among the most important seed-dispersers in tropical forests (Andresen et al., 2018; Bufalo et al., 2016). Globally, primate populations are declining (Estrada et al., 2017) and the limited resources provided by small forest fragments cause primates to alter their behavioral and social patterns (Arroyo-Rodriguez & Dias, 2010; Boyle & Smith, 2010). The inability to disperse across fragments creates extreme population pressure resulting in competition, loss of genetic diversity, and smaller home ranges (Teichroeb et al., 2022). LSBRS holds one of the highest recorded population densities of mantled howler monkeys (Bolt et al., 2022; Schreier et al., 2024), increasing feeding competition and compounding the negative effects of fragmentation.

LSBRS suffers from population pressure, lack of resources, and declining biodiversity as a result of fragmentation (Baltensperger & Brown, 2015). Therefore, the need to address the possibility of restoring connectivity corridors between LSBRS and surrounding forests in a country that depends on ecotourism, research, and a reputation for successful conservation practices is paramount (Embassy of Costa Rica, n.d.; Global Economy, 2024; Sánchez-Azofeifa et al., 2001). LSBRS should be included in Costa Rica's connectivity plan to connect it to protected forests in northeastern Costa Rica such as Tortuguero National Park to the east, Braulio

Carrillo National Park to the west, or the Barra del Colorado wildlife refuge to the north-northeast. These large, protected forests are just ~10-25 km from LSBRS, but are separated by both rangeland and crop plantations.

Connectivity Corridor Plan

Costa Rica's Sistema Nacional de Áreas de Conservación (National system of conservation areas, SINAC) has identified protected areas such as national parks and wildlife refuges that account for 26.5% of Costa Rica's land mass (Fig. 1; SINAC, 2019). They have also established 43 biological corridor areas through the Programa Nacional de Corredores Biológicos de Costa Rica connecting protected forests that account for 33.1% of Costa Rica's land (Fig. 1; SINAC, 2019). These areas are not necessarily connected currently, but the Costa Rican government has planned connectivity operations through the year 2025. Corridors connecting protected areas can be a patchwork of reforested areas that can be used as steppingstones between fragments, one cohesive strip, or a complex of smaller strips of revegetated forest (Morera-Beita et al, 2021). Some propose a strip of forest that is 1 km wide at minimum to promote wildlife dispersal (Moran et al., 2019). There

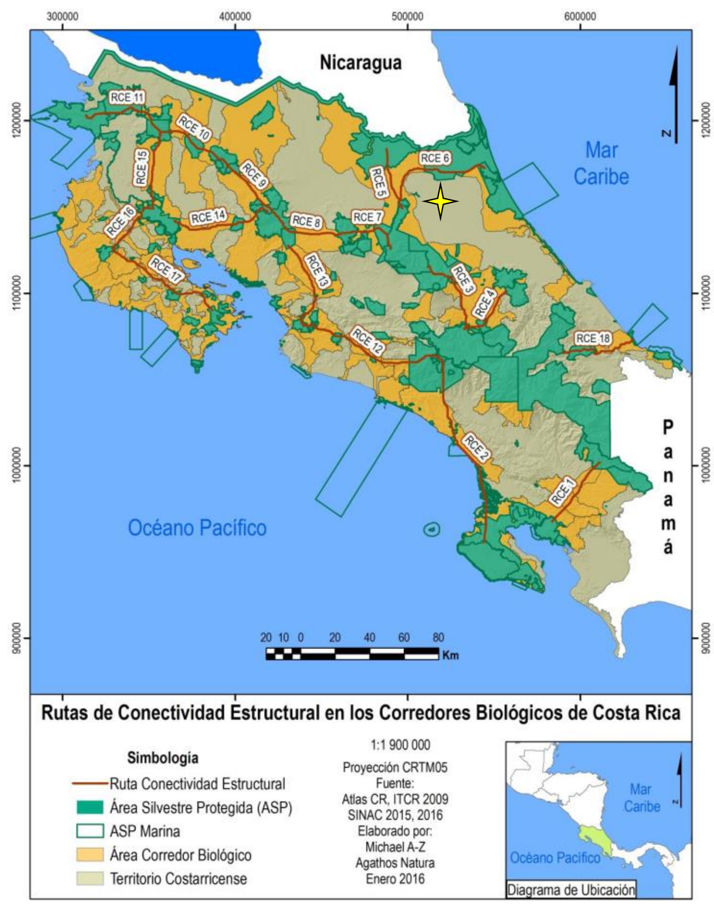

Figure 1. Protected areas (green), biological corridors (orange), and proposed connectivity routes (red) in Costa Rica. The yellow star indicates the approximate position of LSBRS in the center of the gray, unprotected area in the northeastern portion. Source: SINAC

are 18 such routes proposed in the plan that will ultimately connect all protected areas in Costa Rica (Fig. 1). However, LSBRS is neither in a protected area, nor a corridor zone (Fig 1.), and the species of LSBRS will not benefit from this conservation plan.

Stakeholders

Farmers/Large-scale Agriculture

The agricultural sector occupies 36% of Costa Rica's land mass and employs 14-17% of the population (FOA, 2024; Global Economy, 2024). The primary agricultural products from Costa Rica are coffee, pineapple, and bananas. There is also a large amount of cattle ranching that occurs throughout the country. While coffee plantations occupy highland areas (El Benni & Reviron, 2009), pineapple, banana, and cattle operations occupy much of the lowland areas around LSBRS and nearby prospective connectivity areas. There is also a patchwork of small coconut plantations immediately surrounding LSBRS, and a nearby school called "Cocotales" (coconut trees) and township called "Las Palmitas" (The Palms) may indicate a local economy that depends on coconut palms and their products. Although some land owners may see monkeys and other wildlife from nearby fragments feeding on their crops, this may be out of necessity due to lack of resources within fragments (Batensperger & Brown, 2015; Personal communication May 2023)

Large, corporate producers of pineapple and banana in this region include Dole, Chiquita, and Del Monte. While both pineapple and bananas are among Costa Rica's top exports (Fagan et al., 2013; Embassy of Costa Rica, n.d.), agriculture only contributes to 4-7% of their overall economy (Global Economy, 2024; Lambin & Meyfroidt, 2011). However, large-scale production provides a significant proportion of these products around the world and companies have an obligation to provide for the needs of consumers as well as continue to make money for their shareholders and employees. Also, with 1 in every 6 or 7 Costa Ricans working in agriculture, there is major economic value for people at the local level to continue to be employed by crop production.

Connectivity corridors are narrow strips or small patches of reforested areas between large forests and forest fragments. In large-scale crop operations, these corridors may not result in noticeable loss of production and therefore employment or revenue. However, small-scale and/or family-owned farms such as some ranchers or coconut farmers around LSBRS may suffer relatively large losses to production by converting farmland to forest. These losses can be offset by avoiding properties where the risk may be higher for specific landowners, or by Cost Rica's payment for environmental services (PES) program. PES services offer landowners hundreds of dollars per hectare of land that is reforested and maintained (Centre for Public Impact, 2016; Malavasi & Kellenberg, 2002).

Policy Makers

The Cost Rican government has prioritized conservation and rainforest regeneration (Cunningham et al., 2020; Sánchez-Azofeifa et al., 2007) but must maintain an economy that relies partially on agricultural exports and employment in the agriculture sector. They have implemented clear-cutting bans throughout the country, PES services, and biological corridor protections as well as the connectivity corridor plan (Cunningham et al., 2020; SINAC, 2019). These policies protect and enhance ecological services, tourism, recreation, and aesthetics in a country that clearly values them. While economically, the country must maintain a certain level of crop production to support its citizens, ecotourism also provides a large portion of national revenue and increases the quality of life for the population, further incentivizing the role of government in conservation.

The Public of Costa Rica

The general public, including farmers and ranchers as well as other land owners and citizens, may be cautious about converting land to forest if it results in loss of income due to conversion of profitable land into forest, or requires higher tax payments. However, the involvement of NGOs in contributing to PES services assured land owners that they would still own their land, profit off of ecosystem services, and some of the funding would be provided from outside sources (Centre for Public Impact, 2016). Additionally, a recent survey of over 1,000 Costa Ricans showed that over 90% of the population would support stricter environmental policy (Froimovici, 2023) demonstrating that they may be willing to support further implementation of the connectivity plan. Finally, growth in ecosystem services may improve other aspects of the local economy and regional aesthetics, supporting increased health, ecotourism, and recreation activities.

Scientists/Researchers

Costa Rica is home to over 5% of the world's biodiversity, including many threatened and endangered species (Criado-Hernández & Marín-Cabrera, 2008) and is therefore a hub for scientific research. For example, a simple academic journal search (EBSCO, 2024) using the keywords "Costa Rica" resulted in over 400,000 journal publications in the past 50 years. For comparison, an identical search for neighboring "Nicaragua" resulted in about one third as many academic journal publications. Scientific research in Costa Rica, including in LSBRS, has improved our knowledge of tropical ecosystems, the effects of forest fragmentation, and allowed for collaboration between local knowledge, scientists, and policy makers (Bolt & Schreier, 2023). LSBRS is a research station that hosts students and researchers from around the world, and the implementation of connectivity corridors will allow for novel research on the effects of corridors on a small forest fragment. Influxes of researchers and students studying corridors may also benefit local ecotourism and guide economies as well as other local businesses.

Ecotourism

Even after the 2020 collapse in the hospitality sector due to COVID-19, 150,000 Costa Ricans are employed in the tourism industry (OECD, 2024). Prior to the decline, over 10% of net GDP was supported by tourism (Statista, 2024) and 80% of visitors came for ecotourism related activities (Embassy of Costa Rica, n.d.). By 2021, the tourism industry was showing modest recovery, and accounted for 6% of GDP (Statista, 2024) even while the global pandemic was still a factor. Prioritizing natural areas and connectivity corridors may only increase the number of tourists to a nation whose economy depends in large part on ecotourism. The subsequent increase in hospitality employment would also help to improve local and regional economies.

Recommendation

Costa Rica is currently implementing a plan to connect protected forests and forest fragments through connectivity corridors (SINAC); however, LSBRS is not included in this plan, and its inclusion would benefit not only LSBRS and the species therein, but also the scientific community, the ecotourism economy, and the general public. Although LSBRS could be connected to either Tortuguero National Park to the east, or Braulio Carrillo National Park to the west, the most straightforward route to a protected area is directly north to the southwestern portion of the Barra del Colorado Wildlife Refuge (see Fig. 1). Barra del Colorado is only 10 km to the north and a corridor would not cross any major highways or pass through any large towns. Therefore, I recommend a corridor of forested areas up to one km in width (Moran et al., 2019) that should be restored, connecting LSBRS and Barra del Colorado in order to reduce competition among primate populations, increase resource abundance, and provide ecosystem services to the surrounding areas that are currently overtaken by agricultural production. Small-scale farmers' and landowners' properties should be avoided to a conceivable extent, potentially creating a patchwork of connected fragments or smaller corridors less than one km in width (Morera-Beita et al, 2021).

Primary conflicts related to my recommendation are largely economic concerns. For example, farmers and landowners may be fearful of losing cropland that they depend on for their livelihoods, and the Costa Rican government must be concerned with GDP that is supported by both agriculture and ecotourism. However, with the relatively short distance and location of the corridor proposed here, many detrimental effects can be mitigated and the ecological improvements would be disproportionately beneficial to the natural communities, research community, and ecotourism industry. Furthermore, the government must work with landowners to explain the benefits of ecosystem services and provide adequate compensation for the conversion of cropland or pasture back to forest.

Conclusion

Costa Rica is a global leader in conservation and reforestation and is home to a large proportion of the world's biodiversity (Criado-Hernández & Marín-Cabrera, 2008; FOA, 2015; Sánchez-Azofeifa et al., 2007). While much progress has been made in restoring Costa Rica's tropical forest systems, many unprotected areas contain small fragments such as LSBRS. Although they still support high biodiversity, these fragments contain fewer resources and high primate population densities, placing undue pressures via competition and limited range size on these populations (Arroyo-Rodríguez & Dias, 2010, Teichroeb et al., 2020). Connectivity corridors linking small fragments to large, continuous forests may release many wildlife species along with vulnerable primates such as mantled howler monkeys from competition and improve their conservation status going forward. My recommendation of a small connectivity corridor extending from LSBRS to Barra del Colorado Wildlife Refuge would not only have tremendous, positive impacts on wildlife in the region, but also on local economies due to the influx of ecotourism, researchers, and PES payments with little effect on the agricultural sector. This initiative would also align with Cost Rica’s goals of reforestation and protection of natural resources into the future.

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