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Ryan Herman Herman
SIT Graduate Institute - Study Abroad

Alexandra Ulin
SIT Graduate Institute - Study Abroad

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Ryan Herman ’17, Natalie Kiley-Bergen ’17
Bowdoin College, Departments of Biochemistry and Biology
Alexandra Ulin ’17
Bates College, Department of Biology

Association Mitsinjo, Andasibe, Madagascar
School for International Training: Tanzania-Zanzibar
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Academic Director: Dr. Nat Quansah
Academic Advisers: Dr. Roger Daniels Randrianiaina & Devin Edmonds

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ABSTRACT

Facing immediate threats such as habitat loss, emerging infectious diseases, and climate change, the frog populations of the Madagascar rainforest, one of the richest amphibian diversity hotspots in the world, are an important focus for monitoring programs. This study focused on assessing the general diversity, key species population trends, and monitoring effectiveness of the Analamazaotra Forest Station's amphibian community, a population of over forty species within Andasibe, Madagascar. Building on a long-term monitoring program that began in 2012, visual encounter surveys were conducted over a two-week period in November 2015 along the edge and within the interior of the forest area managed by Association Mitsinjo. The current twenty-four transects were found to be an effective means of monitoring amphibian populations given species abundance curves and an individual assessment of each transect. Rank abundance curves, Simpson's Diversity Indexes, and a Jaccard's Index were calculated as diversity assessment values using three years of data collection. From species evenness and richness assessments, the interior of the forest appeared to be unchanging in terms of diversity (stabilizing around a SDI of 0.91) while the forest edge had a decrease in diversity since 2013 (2013 SDI, 0.98; 2015 SDI, 0.82). These trends did not support expected diversity increases given the recovering status of the Analamazaotra Forest Station, since its protection in 2003, suggesting that these results may have been influenced by the seasonality effect of edge preference, the forest's carrying capacity, and inconsistent surveys. For monitoring purposes, seasonality abundance baselines were calculated for the past four years of eleven key species. These eleven baseline species can be tracked in future years to assess population declines and judge habitat quality and forest health.

INTRODUCTION

Madagascar is one of a handful of amphibian diversity hot spots, ranked fourth in the world for endemic species and twelfth in the world for overall amphibians (Gehring et al., 2011). There are an estimated 500 species of amphibians, solely frogs, in Madagascar; 270 of which are both native and endemic to the island (Gehring et al., 2011; Perl et al., 2014). Although the extent of Madagascar's amphibian diversity is fairly well-understood, the specific frog species of Madagascar are only beginning to be described with emerging DNA-barcoding technology (Perl et al., 2014). Over 200 species are thought to still be undescribed and larval stages remain poorly documented (Gehring et al., 2011). With an estimated 32% of frogs in the world known to be threatened, endangered, or extinct, and a total of 42% showing declines in population, the health of frogs has become the focus of conservation efforts worldwide (IUCN, 2015-4).

In Madagascar, a study looking at 220 endemic species of frogs found 25% of them to be threatened; however, no frog species have yet to be declared extinct in Madagascar (Gehring et al., 2011). An emerging understanding of amphibian diversity in Madagascar makes conservation immediately important, in efforts to both conserve this pocket of diversity and assess its habitat quality. Frogs have proven to be extremely sensitive to environmental degradation seen in forest fragmentation studies, therefore acting as reliable indicator species for forest health (Hager, 1998). Frogs are especially vulnerable to forest cover lost and environmental pollutants because of their permeable skin. Additionally, due to the specificity of microhabitat location for frogs' developmental stages, damage to either terrestrial or aquatic areas can quickly reduce population counts. By monitoring frog populations, a decline in frog species richness or abundance function as a warning sign for forest degradation or additional species population declines (Hager, 1998).

Besides being a valuable indicator of forest health for conservation efforts, the frogs of Madagascar constitute a large and endemic diversity which must be conserved for future generations, as little is still known about the importance of these frogs and their potential uses. Socially, frogs have been the specimens for anatomical, physiological, and pharmacological education, in addition to serving various other medical purposes, such as pregnancy testing, anti-tumor agents, antibiotic peptides, and analgesics (Tyler, Wassersug, & Smith, 2007). Frog collection for the Madagascar food industry also serves as a supplement to local people's income (Jenkins, 2008). Environmentally, frogs act as generalized feeders, predating mostly on arthropods and small vertebrates. Conversely, frogs are the prey of birds, snakes, spiders, lizards, and mammals (F. a. V. Glaw, M., 2007). In addition to human disturbances, such as deforestation, other threats to frogs include emerging infectious diseases, climate change, overexploitation, and invasive species. With these threats in mind, conservation efforts should be attuned to population changes through regular monitoring.

Threat of Deforestation

As of 2004, Madagascar had a human population of 17.9 million and growing at an impressive rate of 2.8% annually (UNPF, 2004). This population relies heavily on shifting agricultural practices and wood for fuel, leading to widespread deforestation and habitat loss for frogs. Between 1950 and 2000, 43% of humid forest cover was lost, an essential habitat for frogs' growth (Harper, Steininger, Tucker, Juhn, & Hawkins, 2007). As of 2000, only 16% of Madagascar remained forested (Harper et al., 2007). This rapid loss of forest cover is compounded by forest fragmentation, affecting 80% of the remaining forests. Fragmentation creates negative edge-effects that decrease forest humidity which limits the area of suitable frog habitat in fragments (Harper et al., 2007). Increasing numbers of forest fragments have been shown to be detrimental to frog species richness (Vallan, 2000). Forest fragmentation does not only decrease population sizes, increasing vulnerability to local extinction, but also increases the homogeneity of surrounding vegetation, reducing the variability of accessible microhabitats (Vallan, 2000). While rapid and long-term deforestation has likely had a negative impact on poorly known frog communities, conservation efforts could still prevent the decline and extinction of many frog species.

Threat of Emerging Infectious Diseases

A second concerning threat to amphibians in Madagascar is emerging infectious diseases (EID), most notably the amphibian chytrid fungus. Amphibian chytrid is responsible for most of the severe amphibian extinctions over the past decade in various parts of the world (Fisher, Garner, & Walker, 2009). This disease is caused by the pathogenic fungus *Batrachochytrium dendrobatidis* which spreads through zoospores that reproduce into zoosporangia when in contact with amphibians during their aquatic life cycle (Voyles et al., 2009). Chytrid causes an epidermal infection and an osmoregulatory imbalance that negatively impacts the neurological system, eventually leading to cardiac arrest. With the emergence of *B. dendrobatidis*, there are risks of greater infection and increasing virulence of the strain, or potential strains, within Madagascar. The best approach to this threat is a combination of continued and intensive disease screening and population monitoring.

B. dendrobatidis was first detected in Madagascar in 2010, and low levels of the fungus have been found in five of twenty-three tested locations within Madagascar (Bletz et al., 2015). The other 18 locations tested negative for chytrid fungus for the years 2005-2014, suggesting no widespread breakout of the pathogen. Anthropogenic activity was most likely responsible for introducing the current strain of amphibian chytrid, given Madagascar's geographic isolation. Amphibian chytrid has previously been found in areas of mid to high-elevation. Unfortunately, these climates zones in Madagascar coincide with some of the areas with the highest amphibian diversity (Bletz et al., 2015).

The current strain of *B. dendrobatidis* is found to be hypovirulent (Bletz et al., 2015), and therefore has not proven destructive, but 40 out of 186 Madagascar frogs have a risk factor above 0.75 to this pathogen (Lötters, Rödder, Kielgast, & Glaw, 2011). Many of the at-risk species are endemic, found at higher altitudes, and with limited habitat ranges. Furthermore, since there have been no endemic strains of *B. dendrobatidis* detected, the endemic frog species are likely to be most vulnerable. Recent exposure trials and the resultant selective infection of captive frogs show that certain species are susceptible to *B. dendrobatidis*. This lineage has the potential to infect individuals of the species *Boophis madagascariensis, Boophis viridis, Heterixalus betsileo, Mantidactylus betsileanus,* and *Ptychadena mascareniensis*, among others, making monitoring health of these species a priority (Bletz et al., 2015).

Other Threats to Madagascar's Amphibians

Recent studies have also identified climate change as a present threat to frog populations in Madagascar. Evidence for rising temperatures in Madagascar have been shown by both coral core samples and widespread coastal coral bleaching (Raxworthy, 2008). In accordance with rise in global warming, researchers have modelled likely displacements of Malagasy amphibian populations into higher elevations. Such distribution shifts, termed upslope distribution displacement, are the direct result of increasing temperatures and increasing mist frequency, and reduce the availability of new habitats (Raxworthy, 2008). This trend causes great concern for the habitat loss and resultant endangerment of Malagasy montane frogs that exist within limited elevational zones. Although such threats are ever present, both the Global Amphibian Assessment and the most recent Intergovernmental Panel on Climate Change (IPCC) report have neglected to state the threat of climate change's effect on biological systems or the threat of distribution shifts for Malagasy amphibians (Raxworthy, 2008). Climate change has also proven to alter frog breeding patterns; increased temperatures cause premature emergence of frog species for breeding season, subjecting populations to the potential of snowmelt-induced flooding and early season freezes (Sayre, May 14, 2008). Another, less supported relationship claims that increases in frog extinction proneness as result of decreases in annual precipitation (Sayre, May 14, 2008).

Madagascar's frogs are also victims of overexploitation by humans, due to their popularity in the international pet trade and luxury food industry, particularly frogs' legs. Frogs, less notably, have also been harvested in mass amounts for medicine, research, and fish bait (AmphibiaWeb, September, 2003). Between 1981 and 2000, 26 million frogs were exported to Europe and the United States for food purposes alone (AmphibiaWeb, September, 2003). Additionally, between the years of 1994 and 2003, 230,000 individuals from the genus *Mantella* were harvested and exported from Madagascar (Gehring et al., 2011). Five species are eaten in Madagascar, four of which are endemic (Jenkins, 2008). In 2008, studies concluded that about 100-300 frogs were collected a day, even though collectors generally only profited 5 to 20 American cents per specimen (Rabemananjara et al., 2008). Malagasy frog collectors, interviewed around the same time, stated their concern for recent drops in edible frog species abundance in frequented forest sites (Jenkins, 2008). Such trade causes immediate danger to species experiencing pre-existing population declines or diminished habitats (Gehring et al., 2011).

Although there are over fifty known invasive species present in Madagascar, the most threatening to current frog populations is the common Asian toad, *Duttaphrynus melanostictus (ISSG, 2015).* While the species and its potential impact is not fully understood, conservationists fear the loss of amphibian diversity due to predation, competition for resources, the spread of disease, and the poisoning by toxins released from the toads (Pearson, 2015). Toad sightings have been contained to Madagascar's urban areas and their nearby degraded habitats so natural forests have yet to be exposed; however, further spreading of the species is expected (Andreone et al, 2014). Madagascar provides a suitable climate for the species and it is predicted that the toad will spread towards the eastern rainforests first (Pearson, 2015).

Amphibian Monitoring

Monitoring programs not only assist in the conservation of amphibian populations by tracking population declines and making it possible to prevent future extinctions, but can also use changes in abundance to assess habitat quality and forest health. With present threats, consistent monitoring of frog species in Madagascar is necessary to assess population health and trends. The initial goal of monitoring is to create baseline data about species diversity and evenness.

Once a baseline is established, frequent and long-term monitoring of the area is needed to detect reliable trends in population sizes. Declines in population can be skewed to appear more severe when only analyzing one to two years' worth of data. In one amphibian study, population declines appeared to be 28% after two years, but this estimate was corrected to 3% after five more years of data were collected (Skelly, Yurewicz, Werner, & Relyea, 2003). Conversely, disease outbreak can decimate a population within a few months making data collection over even a short-time period invaluable. If using visual encounter surveys, measurements need to be taken monthly to extrapolate long-term trends while also detecting sudden immediate changes. There are no statistical differences in the accuracy of detecting species richness when using auditory transects, visual transects, or general auditory monitoring, but these methods prove most effective when used in combination (Rödel & Ernst, 2004). These transect monitoring methods are more effective than alternative collection based techniques, like pitfall traps (Rödel & Ernst, 2004). Intensity, consistency, and time, are the best ways monitoring can proactively protect amphibian species in Madagascar.

Amphibian Conservation in Andasibe

Visual encounter surveys to monitor amphibian population trends have been conducted in the Analamazaotra Forest Station for the past four years. This initiative is run by the local amphibian conservation staff of Association Mitsinjo, a community-run conservation organization that manages about 700ha of mid-altitude, secondary rainforest near Andasibe in east-central Madagascar. This area supports an exceptional diversity of amphibians with more than 100 known species from Andasibe and surrounding forests, making it one of the most diverse amphibian regions worldwide (F. a. V. Glaw, Miguel, 2007). Through Association Mitsinjo's monitoring program, beginning in November 2012, 41 species of frogs have been identified. The organization uses 24, 100 meter long transects for visual surveying in both the Analamazaotra Forest Station and along the adjacent road bordering the forest (Appendix II). This monitoring program was initiated following a year-long intensive study in 2011 and 2012 that created data for amphibian species abundance comparisons (Heinermann et al., 2015).

J. Heinermann completed baseline amphibian research at Association Mitsinjo from August 2011 to August 2012 (Heinermann et al., 2015).Using visual encounter transects in the Analamazaotra Forest Station, he tracked sightings of specific species: *Blommersia blommersae, Paradoxophyla palmata, Aglyptodactylus madagascariensis, Boophis pyrrhus, Boophis viridis, Mantidactylus grandidieri, Heterixalus punctatus, and Heterixalus betsileo.* These species were suggested as monitoring species for Association Mitsinjo and are referred to as the *Heinermann Eight* throughout this study. The data previously published about these species provided a useful comparison for the more recent findings discussed in this paper.

This monitoring effort was continued throughout the month of November, 2015, A total of five surveys were conducted from November 8th, 2015 to November 18th 2015, four within the Analamazaotra Forest Station, termed the forest interior, and one along the Lalan'Andasibe roadway, termed the forest edge. Due to the nature of recovering secondary forests, amphibian species diversity, evenness, and abundance for the area are all expected to have increased over the past four years. Cumulative species sightings starting in November 2012 were compiled and used to determine a baseline of overarching amphibian diversity of the Analamazaotra Forest Station, contributing to ongoing population and diversity monitoring.

STUDY AREA

Madagascar, the fourth largest island in the world, is located 450km off the eastern coast of Mozambique. Eastern Madagascar has a humid tropical climate, receiving more rainfall than any other part of the country (Harper et al., 2007). Due to such high levels of rainfall, Eastern Madagascar is the geographical area that contains most of the island's remaining rainforest. These rainforests run in a narrow band of disconnected forest corridors along the eastern part of the island. This section of the island often suffers most from tropical cyclones, which typically occur between the months of December and March.

Analamazaotra forest, in east-central Madagascar, is located south of Andasibe village, a former logging center around 930-980m altitude (18˚56.288' S, 048˚24.851' E). Analamazaotra forest is one fragment of the eastern band of rainforests on the island (Figure 1). Today the ownership of the forest is divided between community groups and Madagascar National Parks, but the land collectively fell under national management as early as 1902. The forest has experienced extended periods of exploitation since that time; rubber was harvested for car tires during the 1920's and French timber companies selectively logged the area up until the late 1960's. In 1970, the eastern side of Analamazaotra forest was converted into a national reserve, while the western half remained as an experimental forest station. In 2003, 700ha of the western forest fell under the legal management of local communities around the Andasibe area, marking the end of selective logging among other harmful human activity. Analamazaotra Forest Station now refers to the 700ha of land managed by Association Mitsinjo, separate but bordering the Andasibe-Mantadia National Park (Figure 2) (Edmonds, Devin, Private communication, 2015).

The Analamazaotra Forest Station mainly consists of secondary growth of native Malagasy vegetation, as well as some patches of old growth (Figure 3). The forest contains three, slow-flowing streams and many temporary ponds that facilitate the aquatic stages of frog development (Heinermann et al., 2015). The surrounding area consists of low hills, covered by fragmented, degraded forests and a few smaller lakes. Additionally, this area is one of the 17 places in Madagascar that have tested negative for the chytrid fungus over the past decade (Bletz et al., 2015).

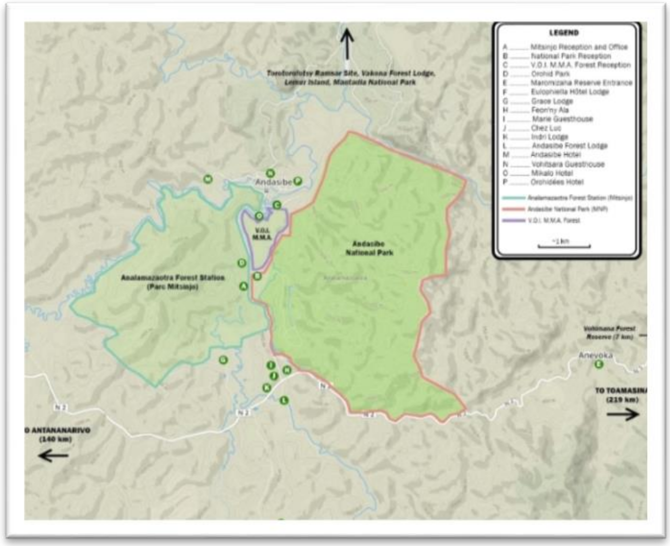

Figure 1. Map showing the location of the study site, Andasibe (shown as red dot), in relation to the rest of Madagascar. Andasibe is a mid-altitude region located in the central eastern part of Madagascar. Map courtesy of *Travel Madagascar* (www.travelmadagascar.org).

Figure 2. Aerial diagram of the study site, Association Mitsinjo, and the surrounding area including roads, Andasibe, and the adjacent National Park. Image courtesy of Association Mitsinjo, Andasibe, Madagascar.

Figure 3. Aerial photograph of the study site ca. 2012 showing dense forest cover compared to surrounding unprotected areas. Image courtesy of Google Earth, 2012.

MATERIALS & METHODS

Transect Sampling

Association Mitsinjo has established twenty-four transects for amphibian conservation purposes; twelve transects within the Analamazaotra Forest Station and another twelve along the adjacent road, Lalan'Andasibe. The transects within the forest represent species abundance data specific to the forest interior, whereas the transects along the road represent species abundance data specific to the forest edge. All transects were 100 meters in length. Visual encounter surveys were conducted after sundown to coincide with high amphibian activity, starting between 6:00PM and 6:30PM and, collectively, lasting 2-3 hours. To regulate observation time, transects in the forest were limited to a walking pace of five to ten minutes and transects along the road were limited to a pace of three to five minutes. On forest transects, described in Appendix I, frogs were detected on the ground and in vegetation one meter perpendicular to the transect on either side. A few of the transects were located in the Analamazaotra Forest Station main network of trails, but most existed on smaller, unmarked paths (Fig. 4). On the road transects, frog sightings were recorded for individuals identified on the pavement, generally 6m wide.

A total of five visual encounter surveys were conducted from November 8th 2015 – November 18th 2015. Transect walks were conducted by 3-4 observers, and were accompanied by 2-3 translators. Preference was given to rainy nights due to the frogs' increased presence during humid conditions. Frog species were identified *in situ* by a trained Association Mitsinjo field technician and voucher photographs of each individual were taken for later verification. During road transects, dead frogs, usually victims of passing traffic, were also identified, recorded, and photographed. Unknown species were captured by hand for analysis *ex situ*. Transects were led by the Association Mitsinjo guides, trained for a specific transect area, to avoid bias in search intensity. Each transect's search time, a value that depended largely on the amount of frog encounters, was also recorded to regulate search intensity.

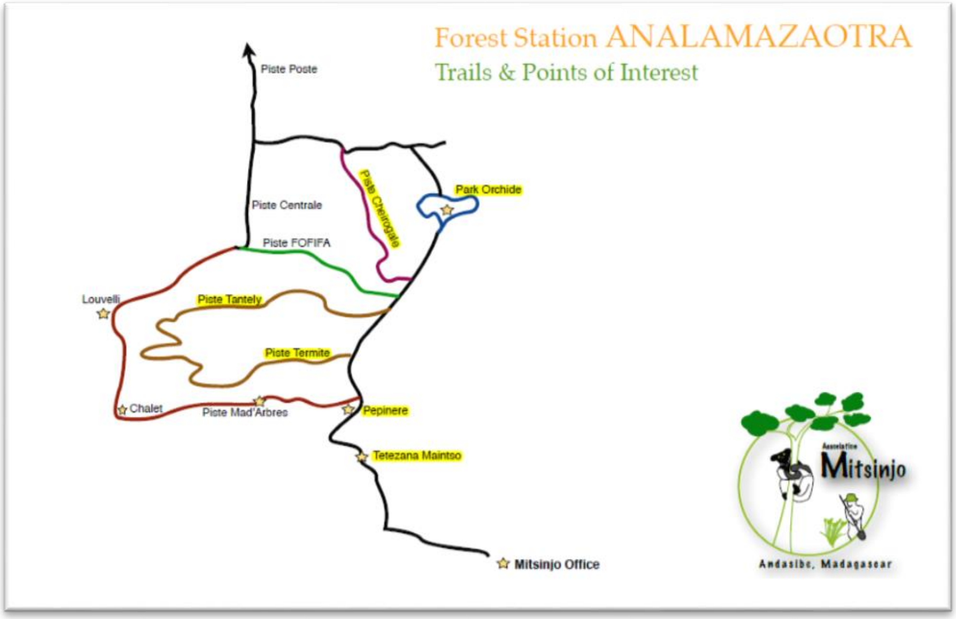

Figure 4. Map of the main trails at the study site, Association Mitsinjo. Trails with forest transects highlighted. Map courtesy of Association Mitsinjo, Andasibe, Madagascar.

Data Analysis

Prism Graphpad Version 6.05 software was used for data analysis in this study. Visual encounter survey results from November of 2012, previously collected by Association Mitsinjo amphibian conservation field technicians, were compiled with current November 2015 data for analysis. Species counts (N), transect success rates, and annual and November species-specific sightings were used for comparison. These values were used to create species accumulation and rank abundance curves.

Transect success rates were calculated by determining the rate of finding one or more frogs on a transect out of the total number of surveys conducted on that transect. Transect success rates were calculated individually for all twenty-four transects.

Simpson's Diversity Index (SDI) was calculated, by year, for transect data for annual and November comparisons. This value was calculated for the road transect data and forest transect data separately. SDI is a relative value that calculates the probability that if two individuals were randomly selected from a population, those two individuals would be of different species. SDI, therefore, represents a community's species richness and species evenness. SDI was calculated using the following equation; ƛ represents SDI, and P_i represents the proportion of species i over the total community population:

$$ƛ = 1 - \sum_{i=1}^{s} P_i^2$$

When calculating SDI, P_i was calculated using species sightings per survey for both November and annual values.

The Jaccard Index was also calculated using combined transect data for the past four years. The index calculates the similarity of species among two sample sets. The Jaccard Index was calculated using the following equation; $J(A,B)$ represents the Jaccard Index for habitats A and B (in this case, forest interior and forest edge), S_c represents the number of shared species between habitats A and B, S_n represents the number of unique species to habitat n.

$$J(A,B) = \frac{S_c}{S_a + S_b + S_c}$$

Species-Specific Population Tracking

Certain species were tracked specifically for long-term population trends, as selected from previous research conducted by Heinermann and other Association Mitsinjo personnel. The focus species for population tracking were *Aglyptodactylus madagascariensis, Blommersia blommersae, Boophis pyrrhus, Boophis viridis, Heterixalus betsileo, Heterixalus punctatus, Mantidactylus grandidieri,* and *Paradoxophyla palmata.* Three additional species (*Boophis madagascariensis, Gephyromantis boulengeri,* and *Mantidactylus betsileanus)* were also tracked for population trends due to their relative abundance. Sightings per survey (*i/s*) of each species were calculated for monthly baselines by calculating mean *i/s* data. Due to species preferences between forest interior and edge environments, population trends were graphed using transect location data that best reduced error (either forest transect data, road transect data, or combined transect data).

RESULTS

November 2015 Transect Surveys

From the five surveys completed in November 2015, one on the road and four in the forest, 22 species of frogs were encountered (Table 1). All species were common to the Andasibe area and have been previously encountered within the Analamazaotra Forest Station. Although a majority of these species were only encountered once on the visual encounter surveys, some appeared more frequently. *Aglyptodactylus madagascariensis* was seen most often (25 sightings), followed by *Mantidactylus betsileanus* (21 sightings), and *Blommersia blommersae* (20 sightings). Of the 22 species, 73% were seen only on forest transects, 14% only on road transects, and 14% seen on both.

Compared to results from previous November visual encounter surveys, combined November 2015 transects encountered an average number of species (2015, 22 species; 2012-2014AVG, 21.667 species). Three species, *Blommersae grandisonae, Boophis goudoti,* and *Boophis guibei,* were first observed during the month of November, this year. Conversely, there were three species that have been continuously encountered in the past three years of November transects that were not sighted in 2015: *Boophis pyrrhus* (2012, 2 sightings; 2013, 1 sighting; 2014, 3 sightings), *Boophis rappiodes* (2012, 4 sightings; 2013, 1 sighting; 2014, 2 sightings), and *Scaphiophryne marmorata* (2012, 1 sighting; 2013, 5 sightings; 2014 2 sightings).

Table 1. Recordings of species-specific sightings on visual encounter surveys for November 2015 on both forest and road transects in the Analamazaotra Forest Station, Andasibe, Madagascar. Number of species sightings, vertical distribution (T=terrestrial, A=arboreal, SA=semi-arboreal) and location (F=forest, R=road, F/R=both).

Species	Sightings	Vertical Distribution	Location
Aglyptodactylus madagascariensis	25	T	F
Blommersia blommersae	20	T	F/R
Blommersia grandisonae	1	T	F
Boophis bottae	4	T	R
Boophis goudoti	1	T	F
Boophis guibei	1	T	R
Boophis madagascariensis	10	A	F
Boophis viridis	2	T	R
Gephyromantis boulengeri	5	A	F
Guibemantis liber	1	A	F
Guibemantis pulcher	1	A	F
Guibemantis sp. aff. Albolineatus	1	A	F
Mantidactylus betsileanus	21	T	F/R
Mantidactylus femoralis	3	SA	F
Mantidactylus grandidieri	8	T	F
Mantidactylus melanopleura	2	SA	F
Mantidactylus mocquardi	1	A	F
Paradoxophyla palmate	14	SA	F/R
Platypelis barbouri	12	A	F
Plethodontohyla mihanika	4	A	F
Plethodontohyla notosticta	4	A	F
Ptychadena mascareniensis	1	T	F

Amphibian Diversity Assessment

Simpson's Diversity Index (SDI) calculations indicated lower amphibian diversity on road transects than forest transects (Table 2). Average amphibian species diversity from 2012 to 2015 was lower for road transects (AVG SDI, 0.85) than forest transects (AVG SDI, 0.92). Similar values are seen for average November species diversity, where road transects (AVG SDI, 0.79) had a much lower amphibian diversity than forest transects (AVG SDI, 0.88). Similarly, the average species richness (N) for road transects was considerably lower than species richness for forest transects, seen in both annual and November assessments (road AVG ann. N, 26; forest

AVG ann. N, 27; road AVG Nov. N, 13; forest AVG Nov. N, 16). Road and forest transects shared 61.9% of all amphibian species discovered from 2012 to 2015 (Jaccard's Index).

The annual SDI for forest transects appeared to be unchanging for the past four years (forest AVG. ann. 2012-2015 SDI, 0.92). Conversely, there was an increase in species richness for forest transects in 2015 (forest AVG ann. 2012-2014 N, 25; forest ann. 2015 N, 34). November forest amphibian diversity remained relatively stable over the last four years (forest AVG Nov. 2012-2015 SDI, 0.88) except for a drop in November 2013 (forest Nov. 2013 SDI, 0.81). Unlike the forest, the annual species diversity for the road has been dropping continuously since 2013 (road ann. 2013 SDI, 0.92; road ann. 2014 SDI, 0.84; road ann. 2015 SDI, 0.82). There was no evident trend for November amphibian diversity for the road transects; however, amphibian diversity did drop considerably below average in Nov 2015 compared to past Novembers (road AVG Nov 2012-2014 SDI, 0.84; road Nov. 2015 SDI, 0.63). Similarly, species richness for November 2015 on the road was much smaller than past years (road AVG Nov 2012-2014 N, 15; road Nov 2015 N, 6).

Table 2. Calculations of *amphibian diversity assessment values* (Simpson's Diversity Index, SDI, and species richness, N) for annual and November transect data in the Analamazaotra Forest Station, Andasibe, Madagascar. Values were calculated for both road and forest data, by year since 2012.

		Annual Simpsons Diversity Index	*Annual N*	*November Simpsons Diversity Index*	*November N*
2012*					
	Road	*0.74*	*19*	*0.82*	*15*
	Forest	*0.92*	*25*	*0.91*	*21*
2013					
	Road	*0.98*	*30*	*0.82*	*14*
	Forest	*0.92*	*25*	*0.81*	*7*
2014					
	Road	*0.84*	*28*	*0.89*	*16*
	Forest	*0.92*	*25*	*0.92*	*17*
2015					
	Road	*0.82*	*27*	*0.63*	*6*
	Forest	*0.91*	*34*	*0.88*	*19*

*Only November and December data was available for 2012

Species evenness was assessed by creating rank abundance curves for both road and forest transects for annual and November surveying data (Fig. 5). Annual curves showed that one to three species represented the largest proportion of the total community population (> 0.10 proportional abundance) and between 15-20 species constituted only very small percentages of the rest of the community (< 0.03 proportional abundance) (Fig. 5A & 5B). There was little change in annual species evenness between 2012 and 2015 for both road and forest transects. The forest consistently showed a higher level of annual species evenness than the road over the past four years.

Neither forest nor road November rank abundance curves showed a discernable trend in evenness from 2012 to 2015 but the forest amphibian species richness remained more even than that of the road for all four years (Fig. 5C & 5D). In general, for both the forest and road surveys, a few species dominated the community makeup with the rest of the species only accounting for a small proportion of the community population. On road transects, the dominating species varied greatly in proportional abundance from year to year. For road transects in November 2015, one species accounted for 59% of the total community population, a 37% increase from the average dominating species for the last three years (species rank 1 Nov 2015, 0.59; species rank 1 Nov AVG 2012-2014, 0.23). The most dominant species for road transects in November 2012 only made up 19% of the overall community population (species rank 1 2012, 0.19). The most dominant species for November road transects was always *Blommersia blommersae* (2013-2015) except for 2012, in which *Paradoxophyla palmata* was the most common species sighted. Forest November populations showed less variation with the dominating species generally making up about 30% of the overall population. The most dominant species for November forest transects were *Heterixalus punctatus* (2012), *Mantidactylus melanopleura* (2013), and *Aglyptodactylus madagascariensis* (2014-2015).

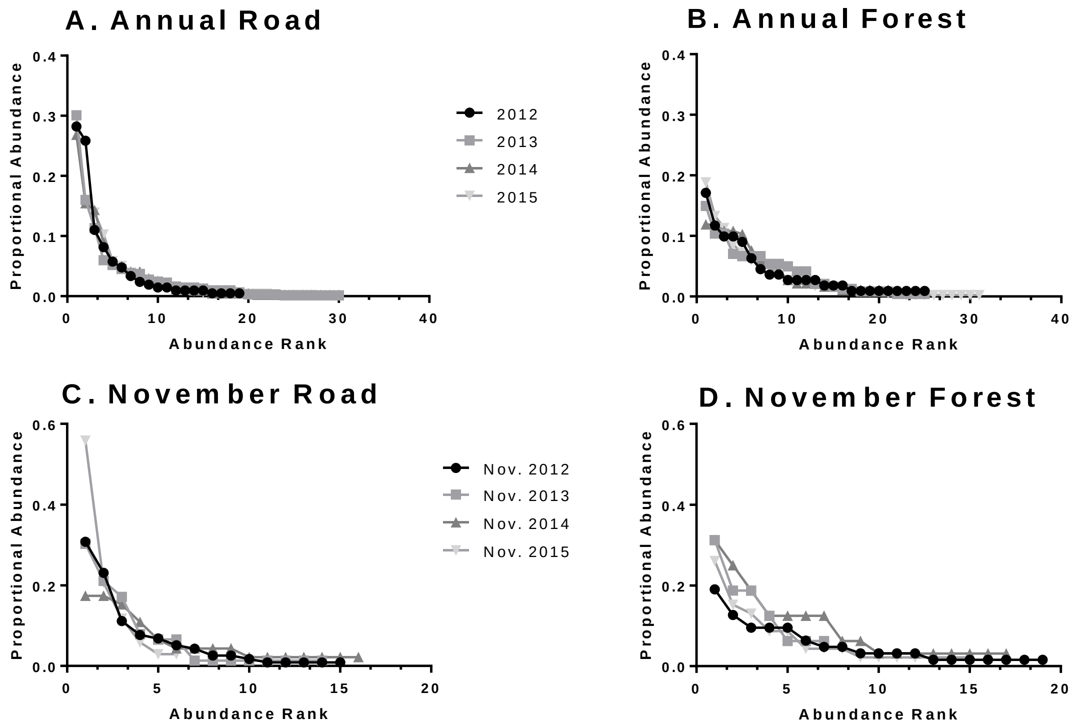

Figure 5. Rank abundance curves of frog species annually for 12 road (A) and forest (B) transects and for Novemeber road (C) and forest (D) surveys in the Analamazaotra Forest Station, Andasibe, Madagascar. Species were ranked by their proportion of overall frog sightings. No difference was seen in evenness annually from 2012 to 2015 on the road or in the forest. Evenness appears greater in the forest than the road. Little change is seen during November surveys on the road. November forest surveys show initial evenness in 2012 and again in 2014 with lower evenness in 2013 and 2015. Number of individuals and species for each year and November are presented in table 2. Number of surveys completed each year and each November reported in Appendix III.

Species-Specific Population Tracking

Baselines of monthly sightings per survey were created for the *Heinermann Eight*, using data starting in 2012, in order to provide reference points for future research and ongoing population trends (Fig. 6). Sightings per survey differ greatly per month between the eight species, although there was a general observation of higher species abundance during the summer months (October-March) and lower species abundance during the winter months (April-September). Some species were observed more during different months, such as *Blommersia* *blommersae* in September (Fig. 6A), *Paradoxophyla palmata* in May (Fig. 6B), *Aglyptodactylus madagascariensis* in May (Fig. 6C), *Boophis viridis* in September (Fig. 6D), and *Mantidactylus grandidieri* in May (Fig. 6F). Sightings per survey also differed greatly in magnitude between species; whereas an average of ten *B. blommersae* were sighted per survey per month (Fig. 6A), there were less than 0.5 sightings per survey for *Heterixalus betsileo* per month (Fig. 6G).

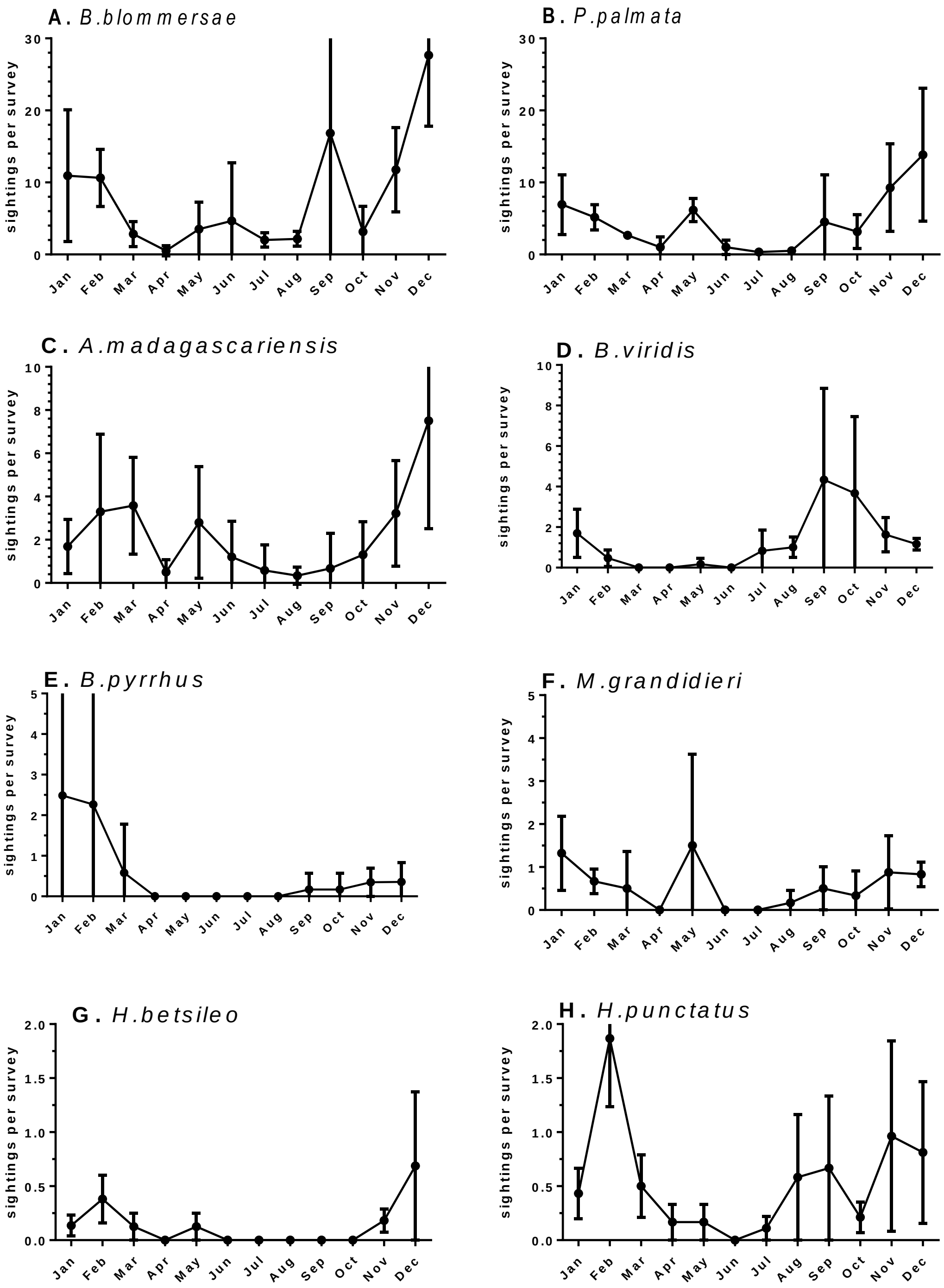

Figure 6. Mean sightings per survey (± SEM) of 8 species- *B. bloomersae* (A), *P. palmata* (B), *A. madagascariensis* (C), *B. virids* (D), *B. pyrrhus* (E), *M. grandidieri* (F), *H. besileo* (G), *H punctatus* (H)- from November 2012 to November 2015 in the Analamazaotra Forest Station, Andasibe, Madagascar. Surveys were done along 12 100m road and forest transects. A, B, & D represent road transect data, F represent forest transect data, and C, E, G, & H represent road and forest transect data. These graphs are baseline seasonal patterns for species specific sightings. These 8 species were also surveyed in Heinerman, 2015. Number of surveys completed each month reported in Appendix III.

Due to the greater abundance of the eight selected species between the months of November and February, sightings per survey for these four months were also compared annually (Fig. 7). Generally, *B. blommersae*, *P. palmata, A. madagascariensis,* and *B. viridis* all showed greater numbers of sightings per survey in the months of November and December than January and February (Fig. 7A, 7B, 7C, & 7D), *Boophis pyrrhus* and *Heterixalus punctatus* were more frequently seen in the months of January and February than November and December (Fig. 7E & 7H), and *M. grandidieri* was seen consistently throughout the four months (Fig. 7F). *H. betsileo* was not sighted frequently enough in the past four years to visualize dominance between the four months (Fig. 7G).

B. blommersae, P. palmata, and *M. grandidieri* showed decreases in sightings per survey for the years 2012-2014, but showed increases in November 2015 transects. A similar trend was seen in *B. pyrrhus;* however the species was not sighted at all in November 2015. *A. madagascariensis* showed increases in sightings per survey for all four months for the past four years. There were no clear trends for increased or decreased sighting frequency for *B. viridis.* Sightings per survey for both *H. betsileo* and *H. punctatus* dropped considerably since 2012.

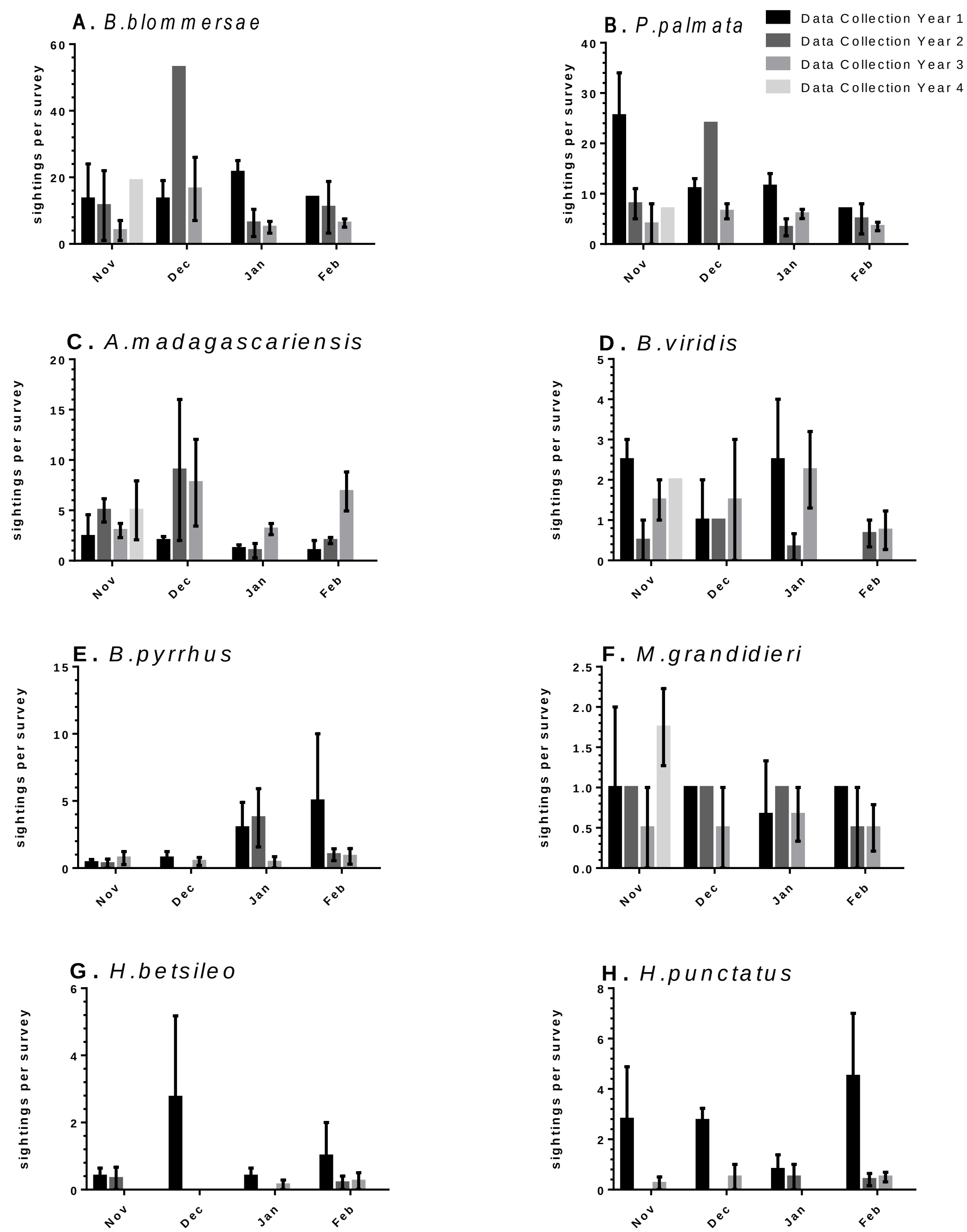

Figure 7. Mean number of sightings (± SEM) of 8 species- *B. blommersae* (A), *P. palmata* (B), *A. madagascariensis* (C), *B. viridis* (D), *B. pyrrhus* (E), *M. grandidieri* (F), *H. betsileo* (G), and *H. punctatus* (H)- during the 4 months of the rainy season (Nov-Feb) from 2012 to 2015 in the Analamazaotra Forest Station, Andasibe, Madagascar. Surveys done along 12 100m road and forest transects. A, B, & D represent road transect data, F represents forest transect data, and C, E, G, & H represent road and forest transect data. General population declines are seen for species A, B, E, F, G, and H. General population increases are seen for species C and D. Exceptions to this trend exist for species A, B, E, and F. These 8 species were also surveyed in Heinerman, 2015. Number of surveys completed each month reported in Appendix III.

Due to their abundance, three additional species were tracked in order to create reliable trends for analysis. Similar to the *Heinermann Eight*, these three species also showed seasonality for frequency of sightings on forest and road transects (Fig. 8). *Boophis madagascariensis* was most common in the months of January, February, November, and December (Fig. 8A), *Mantidactylus betsileanus* in the months of February, March, August, and November (Fig. 8B), and *Gephyromantis boulengeri* in the months of January, October, and November (Fig. 8C). In accordance with trends also seen in the *Heinermann Eight*, the months from April to August showed noticeably lower than average sightings per survey for all three species.

A slight decline in sightings per survey was seen for *B. madagascariensis* from November to February over the past four years (Fig. 9A). Conversely, *M. betsileanus* and *G. boulengeri* showed an increase in sightings per survey during the same time periods over the past four years (Fig. 9B & 9C).

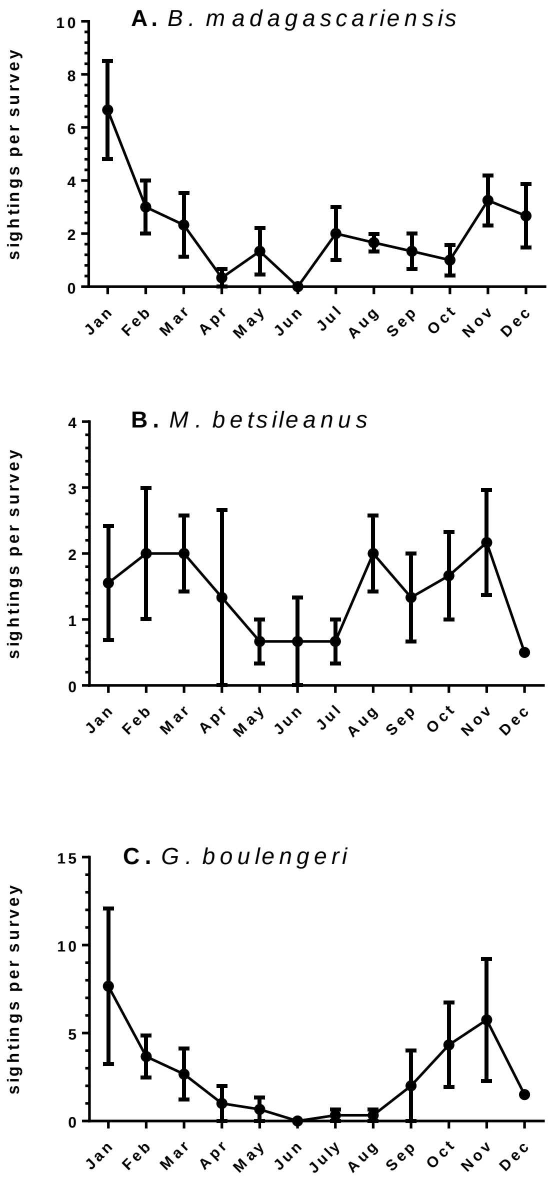

Figure 8. Mean sightings per survey (± SEM) of 3 species- *B. madagascariensis* (A), *M. betsileanus* (B), and *G. boulengeri* (C)- along 12 100m forest transects from Novemebr 2012 to November 2015 in the Analamazaotra Forest Station, Andasibe, Madagascar. Frogs sighted during visual night forest transects. These graphs provide a baseline of seasonal patterns for species specific sightings. Number of surveys per month reported in Appendix III.

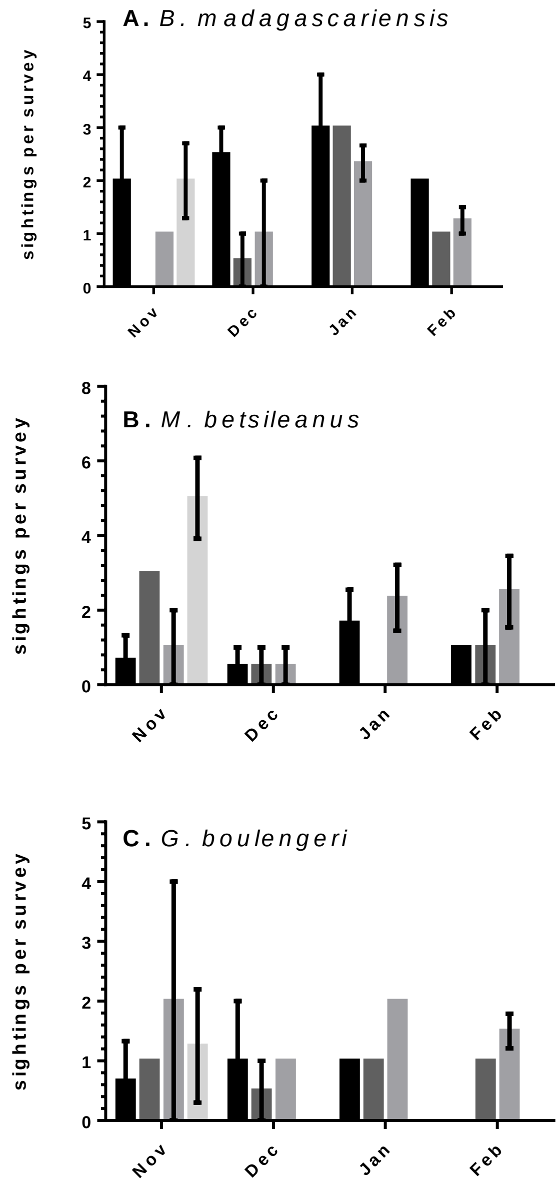

Figure 9. Mean number of sightings (± SEM) of 3 species- *B. madagascariensis* (A). *M. betsileanus* (B), *G. boulengeri* (C)- along 12 100m forest transects during the 4 months of the rainy season (Nov-Feb) from 2012 to 2015 in the Analamazaotra Forest Station, Andasibe, Madagascar. A slight population decline is seen in species A for each of the 4 months. Species B and C both show an increasing population trend each of the four months. N=1 (Jan-2014), N=2 (Nov-2014; Dec-2012, 2013, 2014; Feb-2014), N=3 (Jan-2015) N=4 (Feb-2015; Nov-2015).

Effectiveness of Visual Encounter Surveys

Species accumulation curves were calculated, using November specific data from 2012 to 2015, to determine the efficiency of the current visual encounter survey methods employed at Association Mitsinjo. The curves detailed the collective amount of species observed with increasing amount of transects conducted in a singular survey. For both road and forest transects, the number of sighted species increased proportionally over the first 8 of 12 transects as the number of surveys completed increased (Fig. 10; Appendix III). The number of species sighted, on average for the past four years, did not increase after 8 transects in the forest but continued to increase slightly on the last 4 road transects (Fig. 3A & 3B). The only year that did not follow this trend was 2015, in which unique species sightings plateaued after 8 transects for the road. On average, after the completion of twelve transects, between 5 and 13 species were encountered on road transects, and between 6 and 11 species were encountered on forest transects.

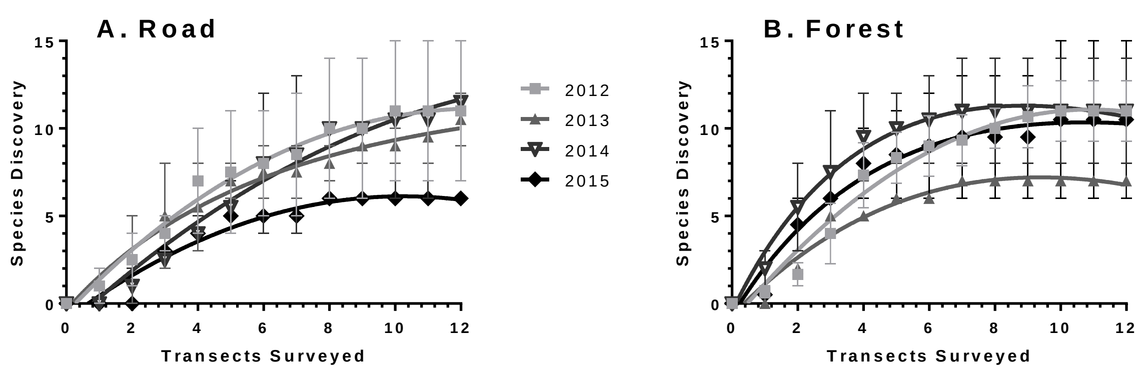

Figure 10. Species abundance curves for 12 road (A) and forest (B) transects in the Analamazaotra Forest Station Andasibe, Madagascar. Mean (± SEM) unique frog species sightings accumulated after each transect for November surveys from 2012 to 2015. Species accumulation curves show a plateau in new species discovery around transect 10 for all years in the forest and on the road. The road generally shows slightly less of a plateau than the forest. The 2015 road survey and 2013 forest survey had the fewest species discovered. GPS quadrants and visual descriptions of the transects are available in Appendix II. N=1 (road-2015, forest-2013), N=2 (road-2012, 2013, 2014; forest-2014), N=3 (forest-2013), N=4 (forest-2015).

Each transect was inconsistently successful at encountering frog species over the past four years of visual encounter surveys, none perfectly successful or complete failures (Fig. 11).

Overall, the road transects had a larger average success rate (61.77%) than the forest transects (49.57%). Out of the road transects, the least successful transects were R01 (22.22%), R02 (39.68%), and R11 (49.21%) (Fig. 11A). All other road transects had a success rate over 60%. Out of the forest transects, the least successful transects were TF12 (10.34%), TF11 (18.97%), and TF12 (18.97%) (Fig. 11B). Transects TF01-TF05 and TF08 all had success rates over 50% and transects TF06, TF07, and TF09 had success rates between 30-40%.

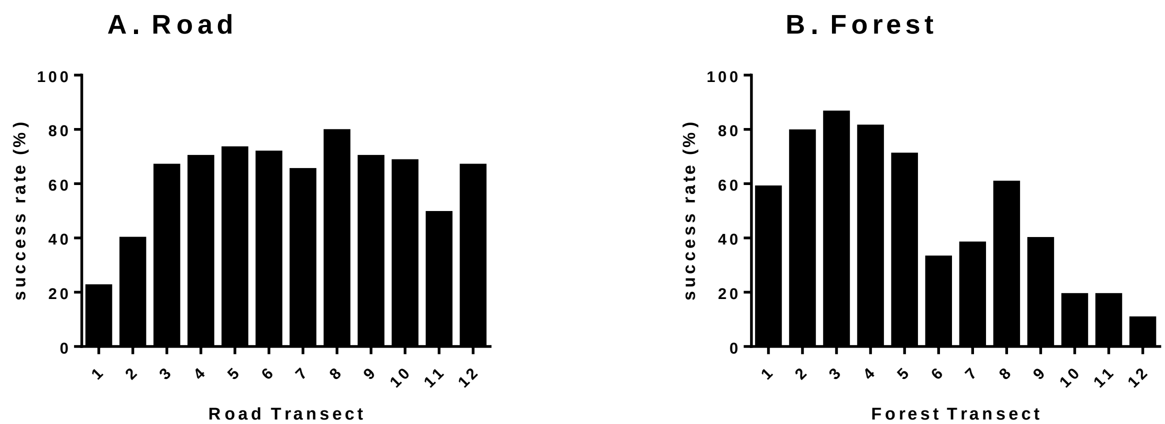

Figure 11. Mean success rate (± SEM) in finding at least one frog along each transect on the road (A) and in the forest (B) in the Analamazaotra Forest Station, Andasibe, Madagascar from Nov 2012 to Nov 2015. Transects were surveyed in the evening and preference was given to rainy nights in order to increase the number of frogs spotted. Road N=64, Forest N=59.

DISCUSSION

The high diversity of frogs in Andasibe, Madagascar makes it an important location for long-term monitoring and diversity analysis. This study compiled three years of amphibian diversity data from night-time transect surveys completed each month in Analamazaotra Forest Station, Andasibe, Madagascar. Data was available from November 2012 to November 2015 with additional data for comparison collected during the previous year by another researcher, J. Heinermann. In this report, particular attention was given to November data because of the four available years of data and the high abundance of frogs seen during November. This study expected to see increases in amphibian diversity but results did not support this prediction

The species richness for November was consistently high (ann. range, 19-34; Nov. range, 7-21). Higher observations of species abundance during November may be due to seasonal increasing humidity and the start of the amphibian breeding season. Unlike species richness, actual species present was highly variable. The most recent surveys (November 2015) found three species never sighted before in November, and three species which had been seen for the last three Novembers, were not detected. These fluctuations in community species composition could be due to a limited number of surveys, particularly for road transects, or could be an indication of shifting populations. Additionally, the evenness of species was poor as a few species generally made up the majority of frogs seen on the transects.

Diversity Assessment

It was believed that the Analamazaotra Forest Station, being a secondary rainforest, was still recovering through succession and many populations had yet to reach their full carrying capacity (Heinermann et al., 2015). Diversity measures, including Simpson's Diversity Index (SDI) and species counts, showed that the amphibian community was unchanging, though, in relative terms of species richness and evenness. The unchanging yearly annual forest SDI values and combined-transect-location rank abundance curves suggest that the amphibian community of Analamazaotra is actually plateauing in its level of diversity instead of continuing to grow with the recovering forest habitat. Assuming the entire forest community is recovering, recovery of predatory species, such as birds, snakes, and mammals, may slow the growth of frog populations. It is possible that increases in diversity will still be seen over a greater time scale given the lag time often associated with population sizes. Frog species abundance have also shown to have a logarithmic relationship with forest area, making it possible that the Analamazaotra Forest Station has reached its carry capacity for amphibian species ((Vallan, 2000) . Other amphibian research suggests a minimum of five years to extrapolate trends in community change (Skelly, 2003). Research is also needed on the forest composition's succession to truly determine whether the forest habitat is changing enough to affect amphibian populations and whether it is returning to the same composition as a native primary forest or developing a new composition.

Despite the relative stability seen in amphibian diversity from 2012 to 2015, there were noticeable differences in diversity between the two sampling areas. The forest interior (forest transects) seemed to have a more even and diverse amphibian community than the forest edge (road transects) according to SDI values and rank abundance curves. Calculations of low species diversity and evenness for the road may have been a result of a disproportionate number of edge-avoiding to interior-avoiding frog species present in the Analamazaotra Forest Station area. Edge-avoiders are species that reside within the core of fragmented habitats (over 30m from the edge of the forest), whereas interior-avoiders are species that prefer residing along the edge of fragmented habitats (Lehtinen, Ramanamanjato, & Raveloarison, 2003). Because Analamazaotra Forest Station is a large forest fragment with only one clear 'edge' (the Lalan'Andasibe roadway), there may be a larger population of edge-avoiding species than interior-avoiding species in the area. Thus the forest edge may support a relatively small community given the limited area of suitable habitat for interior-avoiding species. This justification is supported by the low Jaccard Index between the road and forest findings, showing almost 40% of species observed are not seen in both habitats. Lower diversity along the forest edge could also be caused by the seasonality effect of edge preference. Edges of rainforest tend to have higher air and dew temperatures, and lower humidity levels, which increases the threat of desiccation during dry seasons (Lehtinen *et al*., 2003). This means that only certain frog species are interior-avoiders year-round (termed *omnipresent* species), whereas many other species will switch location preference depending on the season, remaining more on the edge during wet seasons and in the interior during the dry seasons (Lehtinen et al., 2003). Seasonal migration due to edge presence would make annual SDI and evenness values drop due to lower species counts and abundances during dry seasons.

These baselines highlight the seasonality of amphibians caused by changes in humidity throughout the year. During dry seasons, frogs may not be found because they are hidden under leaf litter or in marshy areas. Because seasonality is variable from year to year, averages across many years become even more important. Unusually dry or wet seasons could easily skew surveying data and long-term changes in climate could affect amphibian populations.

Across sampling years, only the most recent November data showed a change in diversity. The November forest edge SDI value and species count for 2015 showed a large drop compared to mean 2012-2014 data, which may be a cause for concern for the road habitat quality and the resultant effect on the frog populations; however, such values could be heavily biased because only one road survey conducted in November, 2015, and therefore diversity assessment values do not benefit from a large sample size as past November values. An assessment of increased road traffic and changes in habitat could help determine whether there is truly cause for concern.

Heinermann Eight Population Tracking

The monthly population baselines for the *Heinermann Eight* species in this study were averages based on three years of surveying (Fig. 6). The *Heinermann Eight* baselines support a strong seasonality of amphibian abundance, observing more frogs in the rainy season, during frog breeding season, than in the dry season, when no important frog activity has ever been observed (F. a. V. Glaw, M., 2007). Such accordance with frog activity patterns lead this study to believe in the accuracy of the seasonal trends, with less consistency regarding magnitude of sightings, for the *Heinermann Eight* baselines. Only one of the eight baselines supported Heinermann's 2011 data in both seasonality trends and magnitude of sightings. *Aglyptodactylus madagascariensis* (IUCN status: Least Concern) was consistently most active during the rainy season with sightings peaking in the middle of this period (Fig. 6C) (IUCN, 2015-4). This suggests that the baseline for *A. madagascariensis* is the most reliable of the seven species, according to the previous Heinermann baseline.

Three of the other eight species followed the same trend that Heinermann found of greater activity during the rainy season (Nov to Apr) and lower activity during the drier season (May to Oct), but differed in the magnitude at which they were found. *Blommersia blommersae* (IUCN status: Least Concern) were generally detected in greater numbers than the 2011 study (Fig. 6A). *Boophis pyrrhus* (IUCN status: Least Concern) and *Boophis viridis* (IUCN status: Least Concern) also showed the highest amount of activity during the rainy season but both of these species were seen in fewer numbers than in 2011. For these three species, while seasonality trends supported Heinermann's findings, baseline population sizes were revised.

Mantidactylus grandidieri (IUCN status: Least Concern) showed a steady population for all four rainy months. *Heterixalus betsileo* (IUCN status: Least Concern) and *Heterixalus punctatus* (IUCN status: Least Concern) were both detected in such low numbers over the past 3 years, it was difficult to extract any reliable baseline seasonality trends (Fig. 6G & H). These two species need close monitoring for the next few years to determine if this declining trend reflects anomaly or a disappearing species. Differences in the magnitude of species sightings between this paper and Heinermann's reflect the importance of having multiple surveying years before creating reliable baselines. Given the importance of seasonality to species sightings shown in this study, anomalies in weather from year to year could easily have skewed data. Changes in weather can only be controlled for by using averages from multiple years of data.

One species showed a considerably different seasonal trend than in Heinermann's study. In 2011, *Paradoxophyla palmata* (IUCN status: Least Concern) was only found in February presumably because of its explosive breeding (Heinermann et al., 2015); however, this study's baseline showed sizeable numbers of *P. palmata* throughout the rainy season (Fig. 6B). Such erratically seen species are poor for monitoring although perhaps 2011 was a more erratic year than usual. Surveying of this species might be more effective as a number of sightings per year.

Because of the increased number of frogs during the rainy season, looking at these months annually could provide a means of tracking population growths and declines. Signs of decline were seen across years for at least two of the months during the rainy season for *B. blommersae, P. palmata, B. pyrrhus, M. Grandidieri, H. betsileo,* and *H. punctatus* (Fig. 7). *B. viridis* actually showed increases across all four months from 2012 to 2015. Species declines in the Analamazaotra Forest Station could be caused by interspecies competition, increasing air and water temperatures, or the introduction of environmental pollutants from nearby communities (Gehring et al., 2011). Given the relatively few years of data collection, these trends should be watched for several more years to confirm actual population declines or growth.

Given the large amount of error in these baselines despite being three year averages, monthly averages should be understood to vary each year without necessarily raising concern. The number of surveys completed each month was inconsistent, contributing to this uncertainty. From November 2012 to November 2015, the number of surveys completed ranged from 0 to 8 each month (Appendix III). A consistent and high number of monthly surveys would make measurements more reliable; however, these values still provide a more reliable baselines than

Heinermann was originally able to report with only a single year of data collection. Additionally, the *Heinermann Eight* may not be the best indicator species to track because of their inconsistent appearance in the last three years. Given some of the larger differences in baselines for a few of the species between this study and Heinermann's, it seems possible that weather conditions during Heinermann's surveying year may have been unusual and skewed his findings.

Proposed Species for Population Monitoring

During November 2015 surveying and data analysis from the past 3 years, three species were identified for potential monitoring; *Boophis madagascariensis, Mantidactylus betsileanus,* and *Gephyromantis boulengeri.* These species consistently showed up in large numbers on forest transects minimizing error for analysis of their population sizes over time. While these three species are not in critical need of conservation given their large population sizes, they could serve as an indicator of overall frog community and habitat health because of the reliability in their collected baseline data (Fig. 8).

B. madagascariensis (IUCN status: Least Concern) is a tree frog endemic to Madagascar and found on the eastern side of the island (Narins, Lewis, & McClelland, 2000). It is most easily spotted after sunset in shallow water or perched on foliage making it ideal for the current forest transects which often pass over streams (F. Glaw, Vallan, & Vences, 2010). These frogs rely on brooks for breeding in November although this may be more dependent on rains which sometimes do not occur until December (F. Glaw et al., 2010). In addition, these frogs are not easily disturbed by movement and therefore are reliably spotted during surveys (F. Glaw et al., 2010). For the first two data collection years, a decline is seen in *B. madagascariensis* for all four months. For November, December, and February a recovery is seen in population size during the 2014-2015 year. The most recent November 2015 survey also shows a considerable increase in the population. Because all fluctuations were relatively small and too few surveys were completed to run any statistical analysis, it is not possible to determine whether these differences are due to actual declines and recovery of the population or anomaly in frog activity or surveying.

M. betsileanus (IUCN status: Least Concern) is a forest frog endemic to Madagascar (F. a. V. Glaw, M., 2007). It is most commonly seen near and in streams and marshes (F. a. V. Glaw, M., 2007) making it ideal for sighting along the current forest transects. The baseline for *M. betsileanus* created from the last three years of data showed sightings of this frog every month making fluctuations in its population identifiable year-round unlike many species which are only reliably spotted from November to February. Data from the last three years showed fluctuation of the species population during November surveys and then a large increase in the population in November 2015. Populations also increased for the months of January and February from 2013 to 2015. Populations remained constant across December surveys. In general, *M. betsileanus* populations appeared to be increasing which could be a result of the forest habitat continuing to recover since its protection by Association Mitsinjo in 2003 or this species' competitive success. While the number of sightings was extrapolated to population sizes, number of sightings could also be affected by frog activity and surveying anomaly.

G. boulengeri (IUCN status: Least Concern) is a forest frog endemic to Madagascar (M. V. a. F. Glaw, 2008). It is most commonly seen on the forest floor and perched on low foliage (M. V. a. F. Glaw, 2008) making it easy for surveyors to sight. *G. boulengeri* showed an increased population for the first three data collection months of November, January, and February. The population then dropped again during the most recent survey, November 2015. December surveys showed a steady population size. While these findings were not statistically strong enough to claim a definitive increase in the *G. boulengeri* population size, the general trend toward a larger population size could point to increased habitat health or the species' competitive success. Again, increased sightings were not only dependent on population size but factors including amphibian activity.

Transect Methods Effectiveness Assessment

According to species accumulation curves, Association Mitsinjo has been conducting enough forest transects during each survey to reliably monitor amphibian diversity. Additional forest transects would not result in more effective monitoring; however, there is potential for finding a higher species count along the forest edge, and therefore more effective monitoring, with the addition of more road transects. According to the persistent upward trend of the species accumulation curve for road transects, there is potential for more comprehensive and accurate transect data with an increased number of transects.

Transect success rates show that a relationship likely exists between the success of road transect species sightings and the transect's proximity to human disturbance. The least successful road transects, R01, R02, and R11 all lay in close proximity to manmade structures. R01, the least successful transect, was the closest to the Andasibe village, R02, the second least successful, started very near a restaurant and hotel complex, and R11, the third least successful, crossed a manmade concrete bridge. All these structures disrupt the natural amphibian environment and may explain why frogs were seen less frequently in these areas. This assumption could benefit from further research.

Transect success rates for the interior habitat suggest that forest transect success was dependent upon the existence of stream crossings. The six most successful forest transects, TF01-TF05 and TF08-TF09, all had one stream crossing. This relationship suggests that reconfiguring forest transects so that they all include stream crossings could increase the abundance of species seen, giving possibly more accurate species diversity estimates. The Analamazaotra Forest Station could also be surveyed to determine whether forest transects are misrepresenting the abundance of streams, and therefore stream frog species, in the area. Additionally, it is possible that transect success rate could be influenced by time of surveying; the three final transects, TF10-TF12, showed the least successful rates of species sightings and were consistently surveyed later in the evening. Lower success rates on these three transects may be caused by a slightly earlier time of amphibian activity than surveying rather than differences in the physical transects.

RECOMMENDATIONS

Data from the last three years has produced a baseline of population sizes for several species and shown some trends of population growth and decline for specific species. In order to continue monitoring trends of population growth and decline, increased surveying on the 24 road and forest transects is recommended. Furthermore, consistent surveying of transects four times each month is recommended to enable statistical analysis of future data. *Gephyromantis boulengeri, Mantidactylus betsileanus,* and *Boophis madagascariensis* should also be added to the list of species monitored closely because of their reliable sighting frequency. In the upcoming months, special attention should be paid to the three species which are expected to be seen during November according to past surveying but were not seen during this November, 2015's surveys (*Boophis pyrrhus, Boophis rappiodes,* and *Scaphiophryne marmorata).* Special attention should also be given to the *Heinermann Eight* species with the most noticeable declining trends (*Mantidactylus grandidieri, Heterixalus punctatus,* and *Heterixalus betsileo*).

Generally, transect surveying seems effective for surveying the area's amphibian community health. Species abundance curves suggest no more forest transects are needed; however, adding three more road transects could potentially increase the reliability of surveying. In addition, surveying success drops off during forest transects TF10 through TF12. To ensure that this drop off is not due to the later timing of surveying, it is suggested that forest transects sometimes be surveyed in the opposite direction, from transect TF12 to transect TF01.

While weather conditions were not included in this study, factors such as temperature, rain, humidity, and wind, are known to affect frog behavior and thus sightings during surveying (Heinermann *et al*., 2015). Future research is recommended to better understand this relationship and how it could potentially be skewing monitoring data. A qualitative assessment of rain and wind as well as stream, road, and air temperature already exist for surveys from November 2012 to November 2015, but has yet to be analyzed. Recording of humidity is recommended for future studies because of its known effect on frog abundance (Heinermann *et al*., 2015).

Understanding Association Mitsinjo's limited funding, transects are still the most cost-effective way of monitoring frog communities. In the future, if Association Mitsinjo were to receive increased funding for amphibian surveying, further surveying could be carried out to obtain a more comprehensive understanding of frog populations. Audio surveying would be recommended as the first addition to Mitsinjo's amphibian surveying because of its success in monitoring for other locations (Rödel & Ernst, 2004; Vences *et al*., 2008). Further funding would be recommended to implement annual tadpole collection for DNA analysis. This would allow Mitsinjo to analyze individuals that are more difficult to identify or find during transect surveying and may lead to the discovery of new and possibly undescribed species (Rödel & Ernst, 2004).

CONCLUSION

Madagascar is a hotspot for amphibian diversity supporting an estimated 500 species. With declining frog populations due primarily to habitat loss and infectious disease, conservation efforts accompanied by monitoring is imminently needed. In this study, frog census data is analyzed from surveys conducted along 24 road and forest transects at Analamazaotra forest station, Andasibe, Madagascar from November 2012 to November 2015. Data compilation provides a monthly population baseline for eleven key species, including the eight species observed by Heinermann from August 2011 to August 2012 and three additional species found to be appropriate monitoring species by this study. Over the past three years, minor population growths and declines were seen among these species; however, populations have remained relatively constant. Generally diversity has also stayed constant and is higher within the forest interior than along the forest edge according to Simpson's Diversity Index. Minimal changes in amphibian diversity over the last three years suggest a healthy amphibian community and overall healthy forest habitat. Due to a lack of existent data, the statistical significance of population changes could not be determined, entailing that this study's results will be most useful as a baseline to compare future monitoring data at Association Mitsinjo.

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APPENDIXES

Appendix I

Table 1.Total Species Sightings by Association Mitsinjo, 2012-2015

Species	2012 Sightings	**2012 November Sightings**	2013 Sightings	**2013 November Sightings**	2014 Sightings	**2014 November Sightings**	2015 Sightings	**2015 November Sightings**
	9 surveys	**5 surveys**	31 surveys	**3 surveys**	40 surveys	**4 surveys**	43 surveys	**5 surveys**
Aglyptodactylus madagascariensis	15	**8**	87	**16**	90	**12**	122	**25**
Anodonthyla pollicaris	0	**0**	0	**0**	0	**0**	1	**0**
Blommersia blommersae	64	**35**	202	**25**	128	**10**	173	**20**
Blommersia grandisonae	0	**0**	0	**0**	1	**0**	3	**1**
Boophis albilabris	0	**0**	1	**0**	0	**0**	0	**0**
Boophis bottae	24	**9**	37	**5**	18	**5**	51	**4**
Boophis goudoti	0	**0**	1	**0**	0	**0**	1	**1**
Boophis guibei	1	**0**	10	**0**	39	**0**	16	**1**
Boophis idae	3	**1**	3	**0**	0	**0**	2	**0**
Boophis luteus	1	**0**	6	**0**	4	**1**	3	**0**
Boophis madagascariensis	11	**6**	26	**0**	21	**2**	29	**10**
Boophis pauliani	0	**0**	15	**1**	11	**0**	5	**0**
Boophis pyrrhus	5	**2**	34	**1**	27	**3**	13	**0**
Boophis rappiodes	7	**4**	15	**1**	5	**2**	7	**0**
Boophis sibilans	0	**0**	0	**0**	1	**0**	2	**0**
Boophis tasymena	1	**1**	2	**0**	4	**1**	1	**0**
Boophis viridis	8	**5**	28	**1**	18	**3**	30	**2**
Gephyromantis boulengeri	4	**2**	16	**1**	20	**4**	45	**5**
Gephyromantis ventrimaculatus	0	**0**	1	**0**	2	**1**	1	**0**
Guibemantis depressiceps	1	**0**	0	**0**	0	**0**	1	**0**
Guibemantis liber	3	**1**	60	**1**	25	**0**	25	**1**
Guibemantis pulcher	4	**4**	0	**0**	1	**1**	1	**1**
Guibemantis sp. aff. albolineatus	0	**0**	2	**1**	2	**0**	6	**1**
Guibemantis tornieri	1	**0**	10	**0**	2	**0**	3	**0**
Heterixalus betsileo	13	**1**	7	**1**	3	**0**	3	**0**

Heterixalus punctatus	29	**18**	22	**0**	15	**1**	7	**0**
Mantidactylus betsileanus	7	**4**	48	**4**	28	**4**	100	**21**
Mantidactylus femoralis	2	**2**	5	**0**	16	**3**	5	**3**
Mantidactylus grandidieri	5	**3**	12	**0**	4	**1**	16	**8**
Mantidactylus melanopleura	7	**6**	13	**5**	22	**3**	22	**2**
Mantidactylus mocquardi	3	**3**	12	**1**	12	**2**	19	**1**
Mantidactylus opiparis	0	**0**	0	**0**	5	**0**	7	**0**
Mantidactylus zipperi	0	**0**	0	**0**	0	**0**	1	**0**
Paradoxophyla palmata	61	**38**	101	**16**	66	**9**	87	**14**
Platypelis barbouri	13	**6**	16	**0**	10	**3**	35	**12**
Plethodontohyla mihanika	1	**1**	7	**0**	3	**1**	6	**4**
Plethodontohyla notosticta	1	**1**	3	**0**	4	**1**	11	**4**
Ptychadena mascareniensis	3	**1**	14	**1**	2	**0**	4	**1**
Scaphiophryne marmorata	1	**1**	10	**5**	5	**2**	6	**0**
Spinomantis algavei	0	**0**	4	**0**	4	**0**	7	**0**
Stumppffia kibomena	0	**0**	0	**0**	1	**0**	4	**0**

Appendix II

Table 2. Forest transect GPS and qualitative information for the forest interior of the Analamazaotra Forest Station, Andasibe, Madagascar. Transects were all 100 m in length. Frog species were recorded 1 m in either direction from the transect (width = 2m).

FOREST	Starting Coordinate	Ending Coordinate	Number of Stream Crossings	Path	Notes
Transect 1	39K 0227566 UTM7904245	39K 0227516 UTM790433	1-cement bridge	Stone w/stone steps	Some uphill, mostly flat
Transect 2	39K 0227341 UTM7904298	39K 0227301 UTM7904362	1-wooden bridge	Natural w/log steps	Steep up & downhill
Transect 3	39K 0227299 UTM7904366	39K 0227271 UTM7904323	1-wooden bridge	Natural	Mild downhill
Transect 4	39K 0227271 UTM7904323	39K 0227322 UTM7904295	1-wooden bridge	Natural	Steep uphill & flat
Transect 5	39K 0227516 UTM790433	39K 0227481 UTM7904383	1-cement bridge	Natural & Stone	Uphill w/ steps & railings
Transect 6	39K 0227481 UTM7904383	39K 0227500 UTM7904493	0	Natural w/log steps	Mild up & downhill
Transect 7	39K 0227500 UTM7904493	39K 0227554 UTM7904567	0	Natural w/log steps	Downhill
Transect 8	39K 0227554 UTM7904567	39K 0227540 UTM7904635	1-cement bridge	Natural	Beside pond
Transect 9	39K 0227540 UTM7904635	39K 0227599 UTM7904633	1-wooden bridge	Natural, some stone	Beside pond
Transect 10	39K 0227599 UTM7904633	39K 0227491 UTM7904700	0	Natural	Flat
Transect 11	39K 0227491 UTM7904700	39K 0227385 UTM7904660	0	Natural	Flat
Transect 12	39K 0227385 UTM7904660	39K 0227350 UTM7904594	0	Natural	Flat

*Water temperature was taken on transect 2. Air temperature was taken at the end of transect TF09.

Table 3. GPS information for road transects conducted along the Lalan'Andasibe roadway along the forest edge of the Analamazaotra Forest Station, Andasibe, Madagascar. Transects were all 100m in length. The width of the road was 6 m. Road surface temperature, air temperature, and water temperature are all taken at the beginning of transect R11.

ROAD	Starting Coordinate	Ending Coordinate
Transect 1	39K 0227863 UTM7905391	39K 0227854 UTM7905290
Transect 2	39K 0337699 UTM7905248	39K 0227634 UTM7905311
Transect 3	39K 0227507 UTM7905155	39K 0227516 UTM7905062
Transect 4	39K 0227516 UTM7905062	39K 0227546 UTM7904967
Transect 5	39K 0227546 UTM7904967	39K 0227577 UTM7904874
Transect 6	39K 0227577 UTM7904874	39K 0227615 UTM7904783
Transect 7	39K 0227615 UTM7904783	39K 0227645 UTM7904695
Transect 8	39K 0227645 UTM7904695	39K 227674 UTM7904594
Transect 9	39K 227686 UTM7904235	39K 227689 UTM7904136
Transect 10	39K 227691 UTM7904003	39K 227803 UTM7903861
Transect 11	39K 227803 UTM7903861	39K 227899 UTM7903833
Transect 12	39K 227991 UTM7903627	39K 0228006 UTM7903542

Appendix III

Table 3. Number of Surveys Completed Monthly by Association Mitsinjo for road and forest transects within the interior and along the edge of Analamazaotra Forest Station, Andasibe, Madagascar.

Month	**2012**			**2013**			**2014**			**2015**		
	Forest	Road	**Total**	Forest	Road	**Total**	Forest	Road	**Total**	Forest	Road	Total
January	-	-	**-**	3	2	**5**	1	3	**4**	3	4	**7**
February	-	-	**-**	1	1	**2**	2	3	**5**	4	4	**8**
March	-	-	**-**	2	2	**4**	2	2	**4**	1	2	**3**
April	-	-	**-**	1	1	**2**	1	1	**2**	0	0	**0**
May	-	-	**-**	1	1	**2**	1	1	**2**	0	2	**2**
June	-	-	**-**	1	1	**2**	1	1	**2**	0	2	**2**
July	-	-	**-**	1	1	**2**	1	2	**3**	1	2	**3**
August	-	-	**-**	1	1	**2**	1	2	**3**	2	2	**4**
September	-	-	**-**	1	1	**2**	1	1	**2**	2	2	**4**
October	-	-	**-**	1	1	**2**	3	2	**5**	3	2	**5**
November	3	2	**5**	1	2	**3**	2	2	**4**	4	1	**5**
December	2	2	**4**	2	1	**3**	2	2	**4**	-	-	**-**
Total	**5**	**4**	**9**	**16**	**15**	**31**	**18**	**22**	**40**	**20**	**23**	**43**

ISP Review Sheet

Completed this review sheet and bind along with the original of your ISP paper as the final page. It is for the use of future SIT students interested in your topic and is intended to give them nuts and bolts information about the types of problems they can run up against in the field, as well as the suitability of both the topic and the ISP site. These reviews have proven to be very helpful, as you may have perhaps already learned, so be sure to include it.

1. Your topic - suitability, development, accessibility

We worked with amphibian conservation in Andasibe, Madagascar in the Analamazaotra Forest Station, tracking seasonal and annual population trends of key species, calculating values of species diversity and evenness, and analyzing the effectiveness of Association Mitsinjo's monitoring methods

2. Location of field study - where you conducted your field study, who helped set it up (who was helpful and who was not; include names, addresses, and phone numbers if possible), strengths and weaknesses of the site

Andasibe, Madagascar, worked in the Analamazaotra Forest Station for Association Mitsinjo.
Director of Amphibian Conservation: Devon Edmonds (0346914438) – super helpful, extremely knowledgeable in his field, future best friend
Field Technician for Forest Interior: Justin Claude Rakotoarisoa (0346914473/261325040810): great English, wonderful man with great sense of humor, also attempt to befriend
Field Technician for Forest Edge: Jeanne Soamiarimampionona (0349249434): also great English, wonderful woman with great sense of humor, also attempt to befriend

3. Nuts and bolts - where to get water & food, costs, where to stay, medical resources, other problems

Vohitsara Guest House, set up by Academic Director, centered in village, about 1.5km walk to Mitsinjo, water provided by Guest House, meals available if requested in advance

4. Other noteworthy comments

This was a very special circumstance.

List your secondary sources and contacts, where they were found, and which were most helpful here:-

Dr. Roger Daniels Randrianiaina – co-adviser, helpful in preliminary lecture about amphibian conservation, not able to give edits to final paper due to communication difficulties.