A Delicate Balance: The Effects of Habitat Type on Frog Communities: A three-pronged study examining the effects of differing habitat characteristics on anuran diversity at el Centro de Investigación Sumak Kawsay in situ, Ecuador

Zane Libke
SIT Study Abroad

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A Delicate Balance: The Effects of Habitat Type on Frog Communities
A three-pronged study examining the effects of differing habitat characteristics on anuran diversity
at el Centro de Investigación Sumak Kawsay in situ, Ecuador

Zane Libke

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Davidson College
Biology and Latin American Studies
South America, Ecuador, Pastaza, Mera
Submitted in partial fulfillment of the requirements for Ecuador: Comparative Ecology and Conservation, SIT Study Abroad, Fall 2019
Goals of the Study:

1. To determine the effect of level of disturbance on anuran communities
2. To determine the effect of proximity to water on anuran communities
3. Compare results from two distinct surveying methods: Visual Encounter Surveys (VES), and Calling Surveys (CS)
4. To determine a potential mechanism for difference in anuran communities based on vegetation availability, habitat type, and use.
5. To establish an iNaturalist database of all Herpetofauna encountered to assist with future studies and citizen science in the region.

Abstract:

We conducted 60 hours of visual encounter surveys and 3 hours of calling surveys on 6 different habitat types near Sumak Kawsay in situ Reserva (SKIS) near Mera, Pastaza, Ecuador. We defined habitat types defined by two variables: type of forest and proximity to water. The aim of the study was to determine what effect each variable has on anuran community composition. We compared the effectiveness of the two survey methods as well. High anuran community dissimilarity was found between each habitat type surveyed, indicating that both forest type and proximity to water are important factors that shape species richness and relative abundance of anurans. We analyzed habitat characteristics and species life histories in order to determine the mechanisms for differences in frog communities among the habitats. We identify 3 habitat types as priorities for conservation based on high uniqueness: Primary dry, Primary wet, and Cultivated wet. As a supplement, vegetation preferences of frogs in the genera Dendropsophus were analyzed, and we found that they preferentially utilize broad leaf plants instead of cultivated grass as perching habitat. We also establish elevation records for 5 different species.

Acknowledgements:

I would like to extend my sincerest gratitude to my professors, Xavier Silva, Ana Maria Ortega, and Diana Serrano, for their incredible dedication and insightfulness throughout my program experience. Thank you to Alex Bentley for being my foremost advisor and research partner, and for sharing with me his fervent passion for these incredible creatures. Alex was not only a valuable knowledge base, but a great friend and supporter as well. Thank you to Theo Carr, Sydney Lewark, and Renee Heller for the constant companionship, support, and tolerance of my nocturnal sleep schedule. Thank you to the Herrera-Cueva family; Cesar, Rebeca, Juan, Camilo, and cats, for so graciously welcoming me into your family, and for your interest and passion for biology and nature that I feel so fortunate to have shared with you. Thank you to Mamita Gloria for taking care of us so well during our time at Sumak Kawsay, and to Henry Sanchez for protecting the precious forests of Sumak Kawsay and gifting us the opportunity to do research in such a special place. Lastly, thank you to my family, Susan, Mathew, and Megan Libke, for supporting my interest in herpetology for as long as I can remember, this piece of work is a direct product of your support.

Introduction:

Ecuador hosts one of the most diverse herpetofauna communities in the world, with over 1000 species (Tropical Herping 2019). Over half of these, 592, are frogs (bioweb.bio). Worldwide, herps are poorly studied, but Ecuador is reversing that trend. In 2018, Ecuador
became the world leader in herpetology-related publications, among those, 10 species newly described to science. This surge in interest in herpetology has arrived just in time, as herps are among the most threatened of all organisms. One in three of all amphibian species are listed on the IUCN red list, and at least half are currently experiencing declines. However, only 44% of amphibians have up to date extinction risk assessments (compared to 100% for birds and mammals), and it is estimated that at least half of these species are endangered (González-del-Pliego et al. 2019). Since 1970, roughly 200 amphibian species have gone extinct, with the largest concentration of extinctions occurring in Latin America. Current amphibian extinction rates are extremely high as well— even if all disturbance stops now, it is estimated that 6.9% of all amphibian species will be lost in the next century (Alroy 2015).

Frogs are especially vulnerable to habitat destruction because they express high species turnover, meaning that species can be unique to a small, specific geographic area, especially in mountainous regions (Dr. John Maerz, paraphrased from quote from Howstuffworks.com article “Are frogs on the brink of extinction?”)

Ecuador is one of the most anuran diverse countries in the world, yet at the moment it is experiencing the highest rate of deforestation in South America (Mosandl et al. 2008). As such, primary forest is being converted into pasture, and little is known about how this land use change effects amphibian communities. Prior research has found that in general, secondary forest has higher species richness and abundance than cultivated areas, but lower species richness and abundance than primary forest. However, effects on abundance were more variable than richness among studies (Thompson and Donnelly, 2018). This review also found the effect to be more pronounced on amphibians than in reptiles.

Vallan (2002) found that amphibian species richness of Madagascar secondary forest was 54% that of primary forest. Another study, by Wagner et al. (2010), found that amphibian species richness does decrease as forests are more impacted by anthropogenic disturbance. However, this study also found that reptile species richness and abundance was highest in cultivated areas. Other studies, such as Herrera-Montes & Brokaw (2010), show that relative abundance of herps is similar between stages of forest succession, but species dominance changes with succession.

In lieu of amphibians impending decline, many conservationists are opting to construct artificial ponds to provide critical breeding habitat for anurans. This habitat augmentation can prove successful, and recent studies suggest that constructed ponds are likely to support similar levels of frog diversity and abundance as natural ponds (Hazell et. al 2004). Pond character seems to be more important than origin; and this same study found that waterbodies with “high levels of emergent vegetation cover that lack fish” are likely to support high numbers of frog species.

As such, the goals of this study are to determine the effects of historical disturbance (secondary forest), current disturbance (cultivated areas), and lack of disturbance (primary forest) on amphibian communities. This study also aims to explore the mechanisms for these effects by analyzing pond and habitat characteristics that may influence frog community composition.

This study makes use of two traditional amphibian surveying techniques: Visual Encounter Surveys (VES), and Calling Surveys (CS).

Calling surveys in this study are defined as recording audio at designated sites and analyzing it for frog calls by playback. With calling surveys, calling activity can be greatly influenced by weather, time of year, and even time of night, leading to inaccurate detection
On top of this, many species, such as some small *Pristimantis*, have quiet to almost inaudible calls, that easily get lost in the deafening chorus of insects, frogs and other creatures at night. In the neotropics, species identification by call has its own hurdles: of the 592 frog species in Ecuador, only 197 have calls in the PUCE database (bioweb.bio, 2019), making it impossible to identify every call to species. Also, because of the difficulty of differentiating the number of individuals calling (and that for most species, only the male calls), CS is best used for species richness only. Despite many drawbacks, CS can be an effective way of surveying difficult to traverse areas and registering secretive species, that may be difficult to spot by sight. Also, calling surveys allow researchers to survey more than just the standard 3-meter height, potentially capturing species that spend most of their life in the canopy and thus out of reach of regular VES surveys.

So, if some calls can be identified, CS can still provide a simple and cost-effective way to monitor anuran community richness.

Visual encounter surveys consist of a search in a designated area for a prescribed amount of time (Guzy et. al 2014) and are a popular survey method among herpetologists. VES has some distinctive advantages over calling surveys: 1) Species can be identified by sight, and 2) species that call infrequently and/or have softer calls can be encountered. VES also allows information besides simple species richness to be collected: such as abundance, density, habitat preference, and behavior. However, VES does not account for differences in detectability among species, and researchers that incorrectly assume that all species have equal detection probabilities may miss or underrepresent more secretive species during VES.

For these reasons, Visual encounter surveys (VES), when used in combination with calling surveys (CS), generate a more complete picture of anuran communities when used together (Guzy et. al 2014). Given concerns with detectability’s influence on the accuracy of anuran population surveys, researchers are increasingly opting to use multiple techniques in order to generate a more accurate picture of anuran communities.

**Methods:**
This study was carried out in three distinctive parts:

1. 60 hours of visual encounter surveys across 6 different habitat types
2. Call recordings across all 6 habitat types
3. Habitat use and activity study in (3) small man-made ponds in cultivated areas.

**1. Visual encounter surveys**
60 hours of visual encounter surveys were completed. Surveys consisted of walking at a predetermined pace of 200m/hr and scanning the ground, water, and vegetation for individuals. When an individual was found, it was recorded on iNaturalist. If possible, an in-situ photo was taken. Vertical height above the ground was estimated, and type of vegetation on which the specimen was found was recorded (leaf lamina, grass, branch, ground, or water). Any other notable information was recorded. With difficult to identify individuals (Particularly of the family *Strabomontidae*), the individual was captured so ventral and lateral photos could be taken. If the specimen was of interest or unable to be identified in the field, it was collected and taken back to the station to photograph with high resolution cameras. Specimens were primarily identified using 4 different resources: La guía del campo de la Herpetofauna de Alto Rio Anzu, Ecuador, unpub. Alex Bentley et al., La guía dinámica de los Anfibios de Ecuador (Santiago Ron 2019), Alex Bentley, and Juan Pablo Reyes.
For nighttime surveys, a high-powered headlamp was used. Most surveys were performed by me, but when necessary Alex Bentley performed several surveys (for example, at sites where camping out was necessary, such as Anzu and Boana Pond, and thus only one trip could be made to survey the site).

10 hours of surveying were conducted in each habitat; 2 during the day between the hours of 9am-4pm, and 8 at nighttime, between the hours of 7pm-3:30am. Search hours were significantly biased towards nighttime based off of recommendations from prior SIT students, and the fact that the first 6 hours of daytime surveying revealed 0 individuals, while nighttime surveying was found to reveal significantly more individuals. Thus, because of time constraints, it was decided that community compositions would be better represented based off of nighttime surveying. Visual encounter surveys were conducted in 6 different habitat types, which are described below:

All survey areas were divided into two categories: forest type (Primary, Secondary, or Cultivated), and proximity to water (“wet” = close to a major body of water, such as a pond, creek, or wetland; “dry” = at least 15 meters away from a major body of water) *note that wet/dry has nothing to do with humidity or rainfall in each habitat*. Note that habitat types are abbreviated by their names in Spanish, so as to be more consistent with the SKIS transect names. The SKIS transects that were used in the sampling followed the specified parameters to define wet vs dry: wet transects were designed to follow a water source, such as the perimeter of a pond or along a stream. Dry transects were designed to be at least 15 meters away from a major water source. Every individual encountered was recorded on iNaturalist and was marked with a GPS point, and can be found under the following link:

https://www.inaturalist.org/observations?user_id=zalibke

**Primary forest wet (PM):**
Most sampling occurred on and around the TPM (Transecto Primario Mojado). Surveying occurred along the creek that flows through a valley in primary forest. The upper section of the creek flows quickly and has less canopy coverage because of its situation in the valley near frequent natural landslides and tree falls. The lower section of the creek, where the TPM itself runs through, is flatter, slower moving water with higher canopy coverage.

**Primary forest dry (PS):**
Old growth primary forest, all surveying was conducted on or around the TPS (Transecto Primario Seco) transect. This area is located on a ridge, and thus away from any large bodies of water. Characterized by large trees, low density of underbrush, and a thin cap of leaf litter, this forest has never experienced any type of large-scale anthropogenic disturbance.

**Secondary forest wet (SM):**
Several different sites were sampled:
- the TSM transect (Transecto Secundario Mojado) (2 hours), a transect on a creek that runs through secondary forest near the research station. This area was once under cultivation for pasture.
- “Anzu Pond” (3 hours) A small pond created by the road to the Caves in the Rio Anzu Ecominga reserve. Located a couple hundred meters down the road from the camping shelter, this pond is medium size, approximately 50m^2, and surrounded by secondary forest. The vegetation around the pond is a mix of grass and early successionary shrubs.
This pond was surveyed by wading around its edge and bushwacking through the dense forest immediately surrounding it. Elevation 1240m

- “Boana Pond” (3 hours) (so named based on our findings there) A rather large pond, created by the via Anzu. This pond is at 1360m above sea level. Coordinates -1.4322896, -78.0804003.

**Secondary forest dry (SS):**
6 hours were conducted on and around the TSS (Transecto Secundario Seco) transect nearby the research station. As secondary forest, this habitat is characterized by lower vegetation diversity, thick leaf litter cap, and very dense underbrush. This forest was once pasture but has been allowed to regenerate naturally. 2 hours were conducted in a similar secondary forest away from bodies of water near “Boana Pond”.

**Cultivated area wet (CM):**
All surveying was conducted on the TCM (Transecto Cultivado Mojado). This transect passes through the pasture used to feed horses at SKIS. This area is a wetland, with a small section of creek and several small ponds. The vegetation is largely dominated by introduced grass, but there are several small stands of small trees and bushes.

**Cultivated area dry (CS):**
All surveying was conducted on the TCS (Transecto Cultivado Seco). The transect passes through an area of pasture used to feed the horses at SKIS. The vegetation is largely dominated by introduced grass, but there is also a significant amount of small early successionary vegetation and bushes.

Map of established transects at SKIS

*For an in-depth study of coverage and forest character along the transects, see Carr (2019)

2. **Call Recordings**
3 separate 10-minute recordings were made in each habitat, for a total of 30 minutes of recordings in each habitat. Each 10-minute recording was made in a distinct location. For PM, PS, SS, CS, and CM, a recording was made at the beginning, middle, and end of the corresponding 200m transect on the same night. Distances were judged using stakes placed for Carr (2019). For SM, because of the variety of sites sampled, one 10-minute recording was
analyzed from each of the 3 sites. Recordings were made using a Sony digital sound recorder with a Rhode VideoMic GO Light Weight on-camera microphone connected.

Once recorded, each recording was analyzed by playback for frog calls. Calls were identified to species with the help of the PUCE call database (bioweb.bio), and a total species richness count was established for each recording. If a call was heard but could not be identified, it was given a morphospecies designation.

3. Habitat use in small man-made ponds in cultivated areas.

For this section of the study, 3 small man-made ponds were selected. Each pond was surveyed on the same night, for 20 minutes each. Pond #1 was surveyed 8 times, #2 was surveyed 7 times, and #3 was surveyed 6 times. We intended to survey all ponds an equal amount of times, but we missed several surveys because of abrupt rain. During this every 20 minute survey, we marked positions of individual frogs using the iPhone’s “markup” feature on pictures of the pond, and vegetation type was noted. We identified species, and for Dendropsophus sarayacensis, we identified individuals using photos to identify unique back and leg patterns. During the surveying, we also watched to see which individuals were calling. We also marked all egg masses, and nightly maps of frog activity and individual presence was created for each 3 ponds.

In order to determine vegetation preferences of Dendropsophus, the availability of different vegetation types was quantified. To measure this, we overlayed each pond picture with a grid using Adobe Lightroom. Once this grid was overlayed, we followed normal procedures used to measure a densiometer reading: it was imagined that there were 4 equidistant dots in each square, and thus each vegetation type was assigned a value from 0-4 in each square. Any vegetation hanging over the water or within a ~0.5m radius of the shoreline was evaluated. Note* low groundcover or areas of the shoreline that were mostly mud were not included in this analysis, only areas of dominant vegetation were included.

Vegetation types were divided up as such:
- Leaf: any non-grass type plant with broad leaves
- Grass: any monocot with long thin leaves, typically characterized as a grass and grown for cultivation
- Bromeliad*: Any plant belonging to the family Bromeliaceae (Pond #1 only)

The 3 ponds are described below:

Pond 1:
A small ~4m^2 man-made, decorative pond directly in front of the Sumak Kawsay in situ research station. The back half of this pond has planted vegetation around it, consisting of several bromeliads, orchids, and broad leaf Araceae. The front half of the pond has little vegetation, mostly short grass, with several decorative rocks placed in front.
Results:
Part 1: Visual Encounter Surveys (VES)

Table 1: Anuran species presence and occurrence across all 6 different habitat types, results from visual encounter surveys. Total individual and species counts are included. IUCN red list status included for each species in the far-right column. *note IUCN status was not designated for undescribed species and morphospecies*

Pond 2:
A larger, rectangular pond, with an area of 18.9m^2 (3.5mx4.2m) that was originally constructed to cultivate tilapia. Located directly next to the Sumak Kawsay in situ research station. At the moment of surveying, it was not under use, so fish were not present in the pond. The walls of the pond consist of vertical bamboo poles ~.5m in height. No vegetation was planted, so all plants are successionary. One side of the pond is largely dominated by 4 broad leaf *Melastomataceae* plants. Another smaller broad leaf shrub is dispersed throughout along with grass. Several tall, small leaf begonia plants also exist throughout.

Pond 3:
A small, ~ 8m^2 man made pond in the middle of the cultivated area. This pond is dominated by grass cultivated for pasture, but several broad leaf plants are present. The front half does not have tall growing grass, as it is part of the trail to get to the transects. The back half and sides consist of dense grass.
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<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td>5</td>
<td>5</td>
<td>DD</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL INDIVIDUALS</strong></td>
<td>30</td>
<td>49</td>
<td>127</td>
<td>29</td>
<td>98</td>
<td>19</td>
<td>352</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL SPECIES</strong></td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>8</td>
<td>11</td>
<td>7</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>
A total of 352 individuals pertaining to 31 species were encountered. Individuals that could not be identified because they were juveniles were not included (ex. Juvenile *Pristimantis*). During visual encounter surveys, 99% of individuals were encountered during the 48 hours of nighttime surveying. The 12 hours of daytime surveying revealed only 3 individuals (*Lithodytes lineatus*, all of which were encountered during the same survey period: Cultivated Wet AM).

**Figure 1-6:** Pie graphs representing percentage of species encountered in each habitat.
Figure 7-14: Species Accumulation Curves and Sample Coverage Curves for all individuals encountered during standardized sampling (TOTAL), followed by curves for each habitat type organized by forest type (Wet and Dry Curves are included in the same graph).

Table 2. Biodiversity values calculated for each habitat type

<table>
<thead>
<tr>
<th>Measures of Biodiversity</th>
<th>PM</th>
<th>PS</th>
<th>SM</th>
<th>SS</th>
<th>CM</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformed Shannon Entropy</td>
<td>6.78</td>
<td>9.43</td>
<td>3.987</td>
<td>3.58</td>
<td>7.1</td>
<td>5.2</td>
</tr>
<tr>
<td>Transformed Gini-Simpson</td>
<td>5.17</td>
<td>7.92</td>
<td>2.611</td>
<td>2.22</td>
<td>5.66</td>
<td>4.06</td>
</tr>
</tbody>
</table>
Results Part 2: Calling Surveys

Table 4. Presence/absence results from Calling surveys (CS). x’s denote that a species was detected in that habitat type in at least one of the 3 recordings. Yellow denotes a species that was never encountered during VES, and green denotes a species that was never encountered in that habitat type during VES. All habitat types are abbreviated in Spanish. See Appendix for results from each recording site.

<table>
<thead>
<tr>
<th>Color Key</th>
<th>Species never registered by VES</th>
<th>Species not registered in habitat type by VES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS#12</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>MS#13</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>MS#14</td>
<td>X</td>
</tr>
</tbody>
</table>

Table 3. Jaccard Index

Using species richness from VES, the Jaccard index compares two different communities and gives a measure of the similarity between 0-1, with 0 being no similarity, and 1 being completely similar (expressed as a percentage from 0-100 from here on out). This index is calculated by taking the total number of shared species between a community divided by the total number of species in both communities. Table 3 shows the calculated Jaccard index for 5 different lines of comparison.
Figures 15-17: Activity maps compiled from all survey maps. Symbols for species and individuals are denoted in the key for each pond.
Discussion Part 1: Visual Encounter Surveys

Wet vs Dry within the same forest type: Effects of wet and dry habitats

Both Primary forest and Secondary forest showed a low degree of similarity between their wet and dry habitats, with 15% and 10%, respectively. This reflects the high species turnover seen in frog species; similar but slightly different habitats can have very different frog richness. This highlights the necessity to conserve both wet and dry types of habitats; it is not enough to simply conserve one habitat or the other, because both have very different species compositions. Thus, they should be viewed as distinct conservation targets.

This difference can be partly explained by the abundance of species of the family Strabomantidae in Primary and Secondary dry areas (9 and 5 species, respectively), which have direct development, and thus do not need bodies of water to reproduce.

Frogs of the family Hylidae on the other hand, are biphasic – meaning they have an aquatic stage - and thus require water to reproduce successfully. This could explain the abundance of Hylids in wet areas; in fact, every Hylid species recorded was present in at least one wet area. Primary wet habitats had 5 Hylid species vs. 1 species in Primary Dry habitats, Secondary habitats had 7 species in wet habitats vs. 1 in dry habitats, and cultivated areas had 6 vs. 3.

The patterns of reproduction in these two families explains the low similarity between wet and dry Primary and Secondary species richness, and also explains why cultivated areas had a much higher level of similarity – almost 40%. In the Cultivated dry habitat, Strabomantidae species richness was remarkably low, with only 2 species, and there was slightly more overlap in Hylid inhabitance. This finding shows that as a dry habitat, cultivated areas are very poor at fostering Strabomantidae diversity. That being said, Cultivated wet habitat does show a marked increase in total richness over cultivated dry, as all species found in the Cultivated dry habitat, except for one (Osteocephalus fusciacies) were also found in the Cultivated wet habitat. This finding provides support for habitat augmentation by creation of artificial ponds, showing that this action can bolster amphibian species richness in areas under Current disturbance. However this only bolsters populations of biphasic amphibians (such as Hylidae) which use the bodies of water for development.

### Tables 5-7

Vegetation availability in each pond is compared to *Dendropsophus* vegetation use observations in each pond. (*Dendropsophus minutus, sarayacuensis, and bifurcua were all included in this analysis). Differences between availability and use of leaves/grass were significant in every pond, showing that *Dendropsophus* actively selected to use leaves over grass in every pond. For pond #1, leaves and bromeliad use was compared. No preference was uncovered for leaves or bromeliads, as usage rates were roughly the same as availability.

<table>
<thead>
<tr>
<th>Pond #1</th>
<th>Availability</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>33%</td>
<td>13/26 50%</td>
</tr>
<tr>
<td>Grass</td>
<td>35%</td>
<td>0/26 0%</td>
</tr>
<tr>
<td>Bromeliad</td>
<td>32%</td>
<td>13/26 50%</td>
</tr>
<tr>
<td>Significant: p=0.019697</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pond #2</th>
<th>Availability</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>44%</td>
<td>26/28 92%</td>
</tr>
<tr>
<td>Grass</td>
<td>56%</td>
<td>2/26 8%</td>
</tr>
<tr>
<td>Significant: p=0.000062</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pond #3</th>
<th>Availability</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>6%</td>
<td>31/71 44%</td>
</tr>
<tr>
<td>Grass</td>
<td>94%</td>
<td>40/71 56%</td>
</tr>
<tr>
<td>Significant: p&lt;0.00001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
water to reproduce. This finding is corroborated by Goldspiel et. al (2019), who states that “larval habitat augmentation can boost populations of amphibians with complex life cycles”.

**Comparing wet habitats: Effect of forest type on wet habitats:**

There are several notable presence and absence differences here. Frogs of the genera *Dendropsophus* were only present in Secondary and Cultivated wet habitats, and were absent from Primary wet habitats. We hypothesized that this distinction has something to do with pond character, and this point is explored further in part 3. It is also important to note here that because a no pond was available to survey in primary forest, a fast moving, rocky creek was surveyed in primary forest. Thus, this difference in Dendropsophus inhabitance could instead be due to water source character (ie. pond vs creek), and not forest type.

It is worth mentioning that of the wet habitats, Cultivated and Secondary had the highest level of similarity. Again, this could simply be because the most of the cultivated and secondary sites had ponds while the primary had a creek, or it could signify the uniqueness of primary wet areas, as comparing the primary wet habitat to both the secondary wet and cultivated wet habitats reveals a low level of similarity (~20% for both comparisons).

Two species, *Osteocephalus mutabor* and *Hylocirus phyllognathus*, were only encountered in the Primary wet habitat. Both of these species were also the only threatened Hylid species encountered, with an IUCN status of vulnerable. Thus, while preserving and/or creating wet areas bolsters Hylid species richness, only preservation of primary wet areas is beneficial for certain vulnerable Hylids.

**Comparing Dry Habitats: Effect of forest type on dry habitats:**

Per the Jaccard’s index, the Primary and Secondary dry habitats showed a high degree of similarity (43%) compared to the Primary/Cultivated and Cultivated/Secondary comparisons, which showed similarities of 11% and 15%, respectively. This is in part due to the high *Strabomantidae* diversity in the Primary and Secondary habitats, 9 and 5 respectively. Interestingly, Cultivated and Secondary Dry habitats are more different from each other than their respective wet habitats are, indicating that the forest type effects direct developers (*Strabomantidae*) more than proximity to water.

Amid all of the discussion about differences in similarity between habitat types surveyed, it is important to note that despite differences between similarities being unique, all Jaccard’s indexes were low – indicating that each habitat is significantly unique, and thus that every habitat variable tested (forest type and proximity to water) has a significant effect on frog species richness and community composition.

**Discussion Part 1 Continued: Biodiversity and Designation of Priorities for Conservation**

**Primary wet (PM)**

78% of individuals from this habitat belonged to the family Hylidae (*Figure 1*), and these four Hylid species (*Hylocirus phyllognathus, Osteocephalus mutabor, Boana almendarizae, Boana cinerasens*) were also the top 4 most abundant species. As mentioned above, *Hylocirus phyllognathus* and *Osteocephalus mutabor* were only encountered in the Primary wet habitat (where they were encountered in abundance), and may represent habitat specialist that can only survive in this habitat type.
This habitat had a very low percentage of individuals from *Strabomantidae*, with only 6% of observed individuals belonging to the family. That being said, the two species observed in the family (*Pristimantis rubicundis* and *Pristimantis sp. 5*) are of conservation priority, with *P. rubicundis* being endangered and *Pristimantis sp. 5* being a species that is rarely encountered, recently discovered, and thus yet to be described, with no information existing about its conservation status in the wild.

Another important finding from the Primary wet habitat was that frogs from the family *Centrolenidae* constituted an astonishing 13% of individuals encountered, and all 3 species encountered during the study were encountered in this habitat. Frogs of the family *Centrolenidae* (known colloquially as glass frogs for their translucent skin) are of priority conservation concern because they show extreme levels of geographic restrictedness, high levels of rarity, and high segregation among species (Mendoza and Arita 2014). As such, having 3 species of Centrolenids at one site is fairly remarkable, and further research may reveal more (6 species have been registered in the area). Glass frogs are also bio-indicators of clean, well oxygenated water (Yañez-Muñoz y Reyes Puig 2008). Thus, not only is their presence of conservation concern, but their presence indicates the health of the habitat surveyed, highlighting it as a quality choice for conservation effort.

Based on these findings, we designate Primary wet habitat types as priorities for conservation for the following reasons:

1) Represents the only viable habitat for two vulnerable species, *Osteocephalus mutabor* and *Hylocirtus phyllognathus*.

2) High *Centrolenidae* diversity (which also signals high habitat health)

3) Presence of 2 *Pristimantis* species of special conservation concern.

**Primary Dry (PS)**

84% of individuals encountered in the Primary Dry habitat belonged to the family *Strabomantidae*. This dominance is likely explained by their direct development strategy of reproduction, releasing them from the necessity to be near bodies of water to reproduce. However, part of this has to do with Primary forest habitat, as Secondary and Cultivated dry habitats have lower percentages of *Strabomantidae* individuals. Within *Strabomantidae*, Primary Dry habitat had the highest species richness (and likely diversity?) of any habitat type, with 9 species (the next closest is Secondary Dry, with 5 species). Thus, Primary dry habitat should be of utmost conservation priority because of its high *Strabomantidae* richness, diversity, and abundance.

*Pristimantis quaquaversus* was only found in primary dry habitat, where it was found in abundance (10 individuals). Since this species was found in abundance in this habitat, but not found at all in any other habitat, this species likely represents another “habitat specialist” that is only found in this habitat type. While it’s conservation status is currently listed as least concern, if it is unable to colonize secondary or cultivated habitats, and is experiencing rapid habitat conversion throughout its range, it’s conservation status should be reevaluated.

1 unknown species, *Pristimantis sp. “casque head”* was only found in the Primary dry habitat. Individuals grouped into this morphospecies were relatively patternless, with markings on their neck resembling a casque, and characteristic red coloration at the base of the legs (see picture in appendix 1). Alex Bentley and Juan Pablo Reyes believe these individuals to be either: *Pristimantis albujai* (Brito et al. 2017), a morphological variant of *Pristimantis sp. 2* (a
newly discovered species in the Anzu region awaiting description), or a new species altogether. Either 3 of these possibilities are of conservation priority.

Primary Dry habitat had the highest biodiversity out of all sites surveyed (see Table 2). Summarized, Primary dry habitat is of conservation priority because of the following 4 findings:

1) High Strabomantidae diversity
2) Represents the only habitat for Pristimantis quaquaversus
3) Potential inhabitance of unregistered or undescribed Pristimantis
4) Highest biodiversity of all habitat types surveyed

Secondary Wet (SM)

In Secondary wet habitat, Boana almendarizae was extremely dominant, representing 56% of individuals encountered. In fact, at one of the sites surveyed, “Boana Pond”, Boana almendarizae represented 86% (25/29) of individuals encountered. While Boana almendarizae is Near Threatened, these results show that it benefits from conversion of wet areas to secondary forests to an excessive degree, becoming extremely abundant and dominant, where it seems to outcompete other species. In fact, Boana almendarizae was by far the most abundant species encountered, accounting for 29% of all individuals encountered. While this is a positive for the species in question, its dominance seems to overtake other species.

Secondary wet habitat had the highest number of individuals and highest species richness of all habitats sampled, with 127 individuals belonging to 13 species. 5 of these species, Boana boans, Lithodytes lineatus, Scinax garbei, Dendropsophus bokermanni, and Pristimantis prolatus were only encountered in this habitat type. Of these 5 species, we believe 4 of these to be due unforeseen elevation differences in sampling sites: the two ponds surveyed, Anzu Pond and Boana pond, were located at 1250m and 1360m, respectively. 3 of the species, Boana boans, Lithodytes lineatus, and Dendropsophus bokermanni, were found above their published elevation ranges, and represent new elevation records for these species. (Although the Scinax garbei found during surveying at Anzu pond was just within it’s altitude range, we found an individual outside of standardized sampling near the SKIS research station at 1430m, which is a new altitude record for the species). Thus, these 3 species were not likely to have been encountered if sampling had been conducted at the higher elevation (1430m), where the other sample sites and transects are located. Because of this discrepancy in elevation between sites surveyed, we are tentative to say that Secondary wet habitats actually contain the highest amphibian species richness.

Secondary Dry (SS)

Secondary Dry habitat, like Secondary wet habitat, was dominated by one species. In this case, Pristimantis altamnis was extremely dominant, accounting for 66% (19/29) of individuals encountered. Interestingly, Pristimantis altamnis is also of conservation concern per the IUCN, being listed as Vulnerable. Pristimantis altamnis was the second most commonly encountered species across all sampling and was encountered in 5 out of the 6 habitat types. P. altamnis was also relatively commonly encountered in the Primary dry habitat, where it represented 19% of individuals, indicating that P. altamnis may naturally be one of the more abundant species in the area. Its hyperabundance and dominance in the Secondary dry habitat could be due to two possibilities: 1) P. altamnis may be a pioneer species, much like Cecropia are pioneer trees in forest succession. Thus, P. altamnis may be one of the first species able to colonize newly regenerating secondary forest, and as succession continues it may become less and less dominant
as other species are able to colonize the area. 2) *P. altamnis* may be outcompeting and replacing other species that for whatever reason cannot survive as well in the Secondary dry habitat.

Of these two possibilities, #2 is concerning from a conservation standpoint, and despite *P. altamnis* being a vulnerable species, we do not emphasize Secondary dry habitat as a conservation priority. While Secondary dry habitat can be inhabited by other species of concern, its biodiversity values were the lowest of any habitat surveyed (see Table 2). For these reasons, we do not designate Secondary dry habitat as a conservation priority.

**Cultivated Wet (CM)**

Cultivated wet habitat registered the highest levels of biodiversity of any non-Primary habitat surveyed (see Table 2). This is clear when looking at the Pie graph, as some species represent a large portion of the population, but no species was overwhelmingly dominant. While most of the species encountered in this habitat were of Least Concern for conservation, several species were encountered that are of conservation concern: *Pristimantis sp. 5, Pristimantis nigrogriseus*, and *Chimerella mariaelenae*. This, combined with the fact that the Cultivated wet habitat is far more diverse than the Cultivated Dry habitat and harbors several species that were not found in Primary forests, provides support for habitat augmentation by pond construction in cultivated areas. As cultivated areas are necessary for human survival, this method of habitat augmentation provides a reasonable, easy, and inexpensive way to foster human-amphibian coexistence while compromising little. As such, we designate Cultivated areas as targets for the creation of Cultivated wet habitats, which have a unique conservation interest as a area of human-amphibian coexistence.

**Cultivated Dry (CS)**

In Cultivated Dry habitat, we encountered the least number of individuals (19) and species (7). Despite this, CS registered a higher biodiversity than either of the secondary habitats (see Table 2) and was only moderately dominated by *Pristimantis altamnis* (42%). Because of the low abundance, we would initially not designate CS as a habitat of conservation concern, but the surprisingly high prevalence of *Pristimantis sp. 5* may be worth the designation. This taxon, first discovered and listed as *Pristimantis sp. grupo conspicillatus* by Yáñez-Muñoz y Reyes Puig 2008, resembles *Pristimantis conspicillatus* but is believed to be a new species based on several differing morphological characteristics, including a white lip and orange blotching on the inside of the back legs. The 2008 study registered 4 individuals, and Alex Bentley has registered 1 more. This study effectively doubled the number of individuals ever registered, and 3 of those 5 individuals were encountered in the Cultivated dry habitat. This very surprising finding shows that new species can be potentially be found in any habitat type, and perhaps researchers should begin to invert more time in surveying disturbed habitats when searching for undiscovered or undescribed species. Because of this finding, we recommend that more surveying is inverted into this habitat type to help describe and better understand *Pristimantis sp. 5*, and to determine the proper conservation designation for Cultivated dry habitat.

**Visual Encounter Survey Results, comparison with prior studies and total registers.**

During this study, we encountered 352 individuals of 31 different amphibian species after 60 hours of standardized sampling. Yáñez-Muñoz and Reyes Puig (2008) established a baseline for the Rio Anzu area, registering 233 individuals pertaining to 30 species after 240 hours of search effort. Of the species registered by the 2008 survey, 19 were registered by this study. In
total, 65 anuran species have been registered in the Rio Anzu area (Bentley et al. unpublished). This study registered a 47% of the total species richness in the area. Because the species accumulation curves generated for the habitat types surveyed in this study do not have extremely steep final slopes (Figures 7-14), it is likely that species will accumulate more quickly by sampling more sites, as opposed to repeatedly sampling the same sites. Thus, based on the species accumulation curves from this study, and the fact that anurans have high species turnover, we suggest that future studies in the area should aim to sample more sites in order to generate the most complete picture of anuran diversity in the Rio Anzu area as possible.

**Discussion Part 2: Calling Surveys**

The Calling Surveys carried out in this study proved very effective at rapidly registering species richness of frogs of the *Hylidae* family. For the Cultivated wet habitat type, all Hylids registered during VES were registered, and 3 Hylids that were never registered during VES were registered. As far as quickly assessing Hylid species richness in this habitat type, CS proved to be a much more effective and less time-intensive manner of doing so. An 150% increase in Hylid species richness (6 species VES vs. 9 species CS) occurred over 1/16th (30 minutes versus 8 hours) (see Table 4) the amount of sample effort. Therefore, for detecting Hylid species richness in Cultivated wet habitat types, CS is a much more effective method.

Aside from this finding, CS surveys revealed the following insights:

1) **They detected almost no Strabomantidae species.** 0% of the species in this family detected by VES were registered using CS. This is likely due to a number of reasons: Firstly, most *Strabomantidae* have a very faint call that is easily drowned out by the loud cacophony of the night. Secondly, the majority of *Strabomantidae* do not have calls that are known to science, or at least in the bioweb.bio database. Because of this, we would not have been able to ID most of the species, even if we had heard them. Thus, many of the calls that were identified as morphospecies could belong to members of *Strabomantidae*, but as of the time of this writing we were unable to identify the majority of the call morphospecies.

2) **Recording site matters.** The table showing species richness registered at each individual recording site can be found in the appendix. This table shows that within a given habitat type, there was significant variation between species richness registered at each recording site. Take Cultivated Wet (CM), for example. Only three species were registered at the first recording site, versus ten at the second recording site. This finding has insights for CS methods, and about anuran reproductive behavior. As calling typically represents reproductive activity (or at least reproductive intentions by males), this shows that for many frog species, reproduction tends to be concentrated geographically. This makes sense for biphasic amphibians such as *Hylidae*, which need water to reproduce successfully and thus congregate around bodies of water. As far as methods go, this points to the need for researchers performing call surveys to select their recording sites carefully, or to survey as many sites as possible within a habitat type.

3) **Call identification presents a steep learning curve for researchers.** Due to the lack of available resources concerning anuran calls in Ecuador, the high variability of calls within a species, and the high similarity of some calls between species, it can be very difficult for an inexperienced researcher to identify frog calls correctly. Because we have little experience identifying Ecuadorian frog calls, we are using the information gained from these call surveys as insights into future methods only. We believe that most of the
species encountered during VES and during CS were identified correctly, as we often observed and heard these species calling during VES, where we could associate the call with the species to 100% accuracy. For individuals that were never observed during VES however, we are reluctant to confirm their presence solely off of CS, because of the variability of calls within a species. However, if a strong database could be assembled with call ID’s of Ecuadorian frog species, or if an expert was consulted/involved in identification, we believe that Calling Surveys could be an extremely valuable, inefficient, and low-cost method for rapidly assessing anuran species richness in a given habitat.

Discussion Part 3: Pond study

This study was designed to investigate whether or not vegetation preferences were partly responsible for *Dendropsophus*’ inhabitation of ponds in cultivated areas. Particularly, it was hypothesized that *Dendropsophus* may show an affinity for cultivated grass that is so dominant in many of the ponds, giving them an advantage over other groups that may not have the same affinity for cultivated grass. This experiment was carried out in small ponds so that we could characterize the availability of vegetation in the habitat, which would have been unreasonable and extremely difficult to do in the larger transects or habitat types. Results from the vegetation preference section of this experiment are discussed below.

Tables 5-7 show that frogs of the genera *Dendropsophus* actively selected broad leaf plants as perching habitat over cultivated grasses. These results are contrary to what we hypothesized, showing that *Dendropsophus* do not inhabit cultivated wet areas more effectively than other frog species because they have a preference for cultivated grass. Instead, these results show that although *Dendropsophus* are inhabiting grassy areas in high abundance, they are relying heavily on the presence of broad leaf plants. Looking at Figure 18, this is apparent, as individual sightings are heavily grouped on and around the few broad-leaved plants surrounding the pond. This finding represents the elimination of one possibility for high *Dendropsophus* abundance in cultivated wet areas and opens up the door for the testing of another theory. One possibility, suggested by Alex Bentley, is that *Dendropsophus* need still water, such as that found in the ponds, to reproduce. This could be possible, as *Dendropsophus* were found in several of the still water (pond) sites analyzed, and not in the sites with fast moving water (such as the primary wet transect). A standardized future study could focus in on this variable and attempt to uncover the mechanisms for high *Dendropsophus* inhabitance in cultivated areas.

The design of this study also allowed for several other findings that are tangentially related to this paper, which are discussed here:

1) **Segregation by species.** In ponds #2 and #3 (Figures 17 and 18). Species are clearly grouped together, in distinct patches. In pond #2, *Dendropsophus sarayacuensis* was only found on the upper end of the pond, and *Dendropsophus bifurcus* was only found on the lower end of the pond. In pond #3, species segregation is extremely apparent. *Dendropsophus minutus* completely dominates the center of the habitat, where it exists in high densities and exhibits clear reproductive behavior (see amplexing pairs). *Dendropsophus bifurcus* is mostly relegated to the left periphery, and *Dendropsophus sarayacuensis* solely exists on the right periphery.

2) **Segregation of individuals/territoriality?** By identifying individuals of Dendropsophus sarayacuensis, we had the unique opportunity of tracking individuals of small hylid frogs
in a relatively non-invasive manner. Pond #2 (Figure 17) had the highest number of individuals tracked (8), and several individuals seemed to exhibit preferences for certain areas. For example, DS-9 was found 3 times on the same exact leaf, and nowhere else. DS-2 was always found in the same corner, and DS-7 was found in the adjacent corner. These individuals only overlapped in range once. Of course, the small sample size gathered from this study prevent us from making any conclusions, but these findings could warrant future studies on territoriality in *Dendropsopus*.

3) **Low Detectability.** By calculating the amount of times an individual was observed vs. amount of potential observations (#individuals x #visits), we discovered that an individual *Dendropsopus sarayacuensis* had a 29% chance (31/106) of being detected on a visit. This percentage is representative of all individuals and observations, but it likely varies per individual and per abiotic factors, as some individuals were only observed once while others were observed up to 5 times, indicating that individuals may have differing detection probabilities. Some nights no individuals were observed at a given pond, indicating that weather, time, or other temporal factors may also play a role in detection. Either way, 29% detection is astonishingly low, especially for small habitats that were surveyed intensively for 20 minutes each. This finding corroborates a fact well known by herpetologists: detection is low, and all individuals are never detected in a single survey. Further sampling using this methodology could reveal the true population size of *Dendropsopus sarayacuensis* in each pond, using an individual accumulation curve (just like a species accumulation curve, but species are replaced with individuals).

**Additional Notes on the Photographic Identification technique used on *Dendropsopus sarayacuensis***

The format of this study represents a marked improvement over other frog microhabitat studies, such as Gondim et al. (2013), which utilized collecting and euthanization to identify sexes and individuals. By using unique patterns to identify individuals, we were able to track the habitat use of individuals, and observe the same individuals over and over again, with little disturbance (as taking out individuals likely alters habitat use of other individuals). Also, through simple observation, we were able to determine if individuals were males (observed calling) and at times, females (observed amplexing). This photographic identification method also exemplifies an improvement over other, more invasive yet common mark-recapture techniques, such as toe clipping. Toe clipping is considered unethical by many, but is one of the only effective mark-recapture techniques currently existing for amphibians. In many cases however, toe clipping shows serious negative health effects on individuals: Golay and Durrer (1994) found that 12 out of 66 recaptured toads (*Bufo calamita*) had infections as a result of toe clipping. In recent years however, the photographic identification has been on the rise, and has been used successfully on spotted salamanders, *Ambystoma maculatum* (Loafman 1991). Our search of surrounding literature revealed no study to date detailing the successful use of photographic identification on Hybrids, so this study may very well be the first (although many studies do already exist which use the technique on other families). Kurashina et al. (2003) establish photographic identification as a useful, inexpensive and non-invasive method on endangered amphibian species. Due to the plight of amphibians worldwide, and particularly in Ecuador, this method could be particularly useful on frogs of conservation priority in Ecuador.

Much of this information is beyond the scope of this study in particular and thus are not displayed in this paper (and low sample sizes would likely not reveal significant results).
However, this study provides a preliminary test and exposition of methods that could be applied extremely effectively to other studies in the future.

**Conclusion**

In conclusion, this study analyzed the effect of two different habitat variables, forest type and proximity to water, on frog species richness and diversity. All habitats expressed a low similarity between themselves, indicating that both habitat variables tested have a significant impact on frog communities. We discovered that Primary dry habitats had the highest biodiversity, as well as the highest *Strabomantidae* species richness. *Strabomantidae* was in general more diverse and commonly encountered in dry habitats, likely due to its direct-development strategy of reproduction. All *Hylidae* species encountered were encountered in at least one wet habitat. Wet habitats had the highest species diversity of Hylids. Secondary and Cultivated habitats had extremely high relative abundances of Hylids, and in Secondary wet habitats, *Boana almendarizae* became extremely dominant. Primary wet and dry habitats are designated as priorities for conservation, along with Cultivated wet areas, which are highlighted as a priority for creation by habitat augmentation with man-made ponds. Combined, these three habitats show low levels of similarity, and represent the best combination to conserve maximum anuran biodiversity in the area.

We explored the use of Calling Surveys (CS) as a rapid detection method for anurans. We discovered that the method was extremely effective for *Hylidae*, but almost completely ineffective for *Strabomantidae*. More knowledge is needed about Ecuadorian frog calls to overcome the steep investigator learning curve in call identification. If this hurdle can be overcome, careful recording site selection or monitoring of multiple sites is an efficient way to survey for Hylid species richness.

We investigated vegetation use in 3 small ponds and discovered that frogs of the genera *Dendropsophus* preferentially use broad leaf plants as perching sites. This study also revealed small-scale geographical segregation by species and hinted at territoriality by individuals through identification and repeated observation of *D. sarayacuensis* individuals.

These findings highlight potential guidelines and methods for future studies regarding the effects of habitat type on anuran communities, and the potential mechanisms for those effects.

**Bibliography:**


Marc J. Mazerolle "Estimating Detectability and Biological Parameters of Interest with the Use of the R Environment," *Journal of Herpetology*, **49**(4), 541-559, (1 December 2015)


Appendix 1. Reptile species presence and occurrence across all 6 different habitat types, results from visual encounter surveys.

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<th>SM</th>
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Appendix 2. Raw data registered from redording surveys, showing species registered at each individual recording site.

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