Herpetofaunal diversity and abundance across an anthropogenic disturbance gradient in the Peruvian Amazon

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Abstract

The department of Madre de Dios at the southwestern corner of the Amazon basin is a biodiversity hotspot with many species of reptiles and amphibians. These species, especially frogs and toads, are among the ones that suffer most from current land use changes. The recent increase of agriculture and population growth are transforming vast expanses of rainforest into grasslands. These transformations change the landscape into a mosaic of anthropogenic and natural habitats with varying degrees of contrast. To track the effects of these changes on herpetofauna composition and structure, a combination of drift fences and visual encounter surveys (VES) in the forest, edge, and grassland were employed. Each drift fence had three 5 m long arms separated by 120° that are buried 10 cm into the soil and were 50 cm high, with four buckets to catch specimens. In total 20 individuals of five species were caught by three drift fence arrays during 15 days and nights of trapping. This included two rare species of reptiles that inhabit the soil. While the sample size was too small to conduct statistical comparisons, the Shannon-Wiener diversity index anecdotally appeared to differ among the grassland, forest edge and forest interior habitats. The edge habitat had the highest number of effective species, likely because it can be used by both grassland and forest interior species, as it is a transition habitat. On the other hand, individuals in the edge may also increase their movement to reach more suitable habitat, increasing their likelihood of capture.

Introduction

The Amazon basin is the world's most diverse tropical evergreen forest, with many species still to be discovered. The department of Madre de Dios in southeastern Peru, on the western border of the Amazon, is considered a biodiversity hotspot (Myers et al., 2000) with a remarkably diverse herpetofauna (Warren-Thomas et al., 2013). This area of wilderness is threatened by gold mining (Warren-Thomas et al., 2013, Von May et al., 2009), agriculture (Oliveira et al., 2007) and other habitat changes such as anthropogenic fire (Barlow and Silveira, 2009). The expansion of agricultural areas, accompanied by pesticides, is especially dangerous for amphibians as they are highly susceptible to chemical toxicity. In fact, amphibians are among the most threatened groups of organisms worldwide (Stuart et al., 2004; Herrera-Montes and Brokaw, 2010). Despite these threats, herpetofaunal species richness is less well-known than that of other vertebrate groups such as mammals and birds (Garner et al., 2010), in part because many reptiles and amphibians inhabit the soil or leaf litter and are difficult to observe (Conant and Collins, 1998; Todd et al., 2007). Therefore it is of the utmost importance to monitor changes induced by habitat alterations and development by conducting studies on species richness over spatial and temporal scales to get a better overview of global biodiversity programs (Raven & Wilson, 1992; Sung, Karraker and Hau, 2011).

One way to investigate species richness is by conducting monitoring or survey projects. In fact, survey projects in new sites are key to inform the scientific community and land managers about species turnover among sites. This knowledge helps conservationists evaluate the effects of protected areas on species richness (von May et al., 2009). By focusing research on highly diverse regions, a high number of species and hence a huge amount of genetic, ecological and evolutionary diversity can be deciphered more thoroughly to improve scientific knowledge and thus enhance protection. Protection is especially valuable in tropical landscapes because most terrestrial species occur along the equator.

As humans change the environment substantially, ecotones—transitions between ecosystems—are created, with a distinct structure and community of plants (Murcia, 1995; Harper et al., 2005; Urbina-Cardona, Olivares-Pérez and Reynoso, 2006). Depending on the specific needs of different species, these new communities can act as barriers (Wilcove et al., 1986) or filters for a set of species (Gascon et al., 1999). For instance, while some species of anurans (frogs and toads) tolerate induced grasslands (Gascon et al., 1999; Laurance, 1999; Urbina-Cardona, Olivares-Pérez and Reynoso, 2006), others may not; hence it is important to find local differences in diversity between sites. While some studies have investigated the effects of disturbances like fire on herpetofaunal species richness by directly comparing disturbed and undisturbed areas (Warren-Thomas et al., 2013), there are few studies that compare changes in species composition and structure along a disturbance gradient.

In order to keep track of changes and to detect potential critical thresholds of species loss, I compared a recently burned area which is now a grass-dominated community, a tropical forest edge, and a selectively-logged terra firme forest, in Madre de Dios, Peru. The purpose of this preliminary study was to get a broad picture of which species of herpetofauna occur in forest-agriculture transition landscapes, as well potentially detect rare or secretive species that can be captured with drift fence and pitfall trap arrays (Gibbons and Semlitsch, 1982; Crosswhite, Fox and Thill, 1999; Sung, Karraker and Hau, 2011). I also compared species richness, abundance, and composition among these sites. Composition analyses are informative because measures of species richness and diversity cannot illustrate the species turnover among sites (Kurz et al., 2014). I predicted that the anthropogenic grassland would have the lowest species richness and an increased abundance of common or generalist species (as per Wanger et al., 2010; Kurz et al., 2014). I predicted that forest edge would have the highest species richness; this may be caused by a delay of edge effects, as some animals with annual cycles return to sites of their birth even though habitat is severely degraded. Another reason that species richness may be highest in the edge is that it is an ecotone; it may harbor species from both forest and open habitats.

Methods

Amphibians vary in life histories, habitat requirements and behavioral traits. Because herpetofauna also includes reptiles that differ significantly from frogs and toads, researchers are forced to include multiple sampling methods when investigating species richness of herpetofauna (Corn and Bury, 1990). In this study I included two widespread methods: drift fence arrays with pitfall traps and visual encounter surveys. Drift fences with pitfall traps are commonly used to catch herpetofauna and other vertebrates (Bury and Corn, 1987; Enge, 1997). Budget, time constraints and study-specific aims should guide material choice and trap configuration (Wilson and Gibbons, 2009). Practicability and efficiency should also be considered for methods (Rödel and Ernst, 2004), because these may vary among study sites.

Study site

The study took place at Finca Las Piedras, the field site of the Alliance for a Sustainable Amazon, near Puerto Maldonado, Madre de Dios, Peru (S 12°13.570'; W 069°06.850'). The approximately 54 ha-site includes habitats such as terra firme forest, palm swamp (aguajal) and grassland. The terra firme forest, an elevated forest lacking seasonal flooding around 20-40 m above floodplain areas (Pitman et al., 1999; von May et al., 2010), is a relatively-intact natural habitat, while the grassland occupies abandoned agricultural fields. However, the terra firme forest was subjected to selective logging over the last 30-40 years, removing all mature big-leaf mahogany (*Swietenia macrophylla*), Spanish cedar (*Cedrela odorata*) and most of the ironwood (*Dipteryx* *micrantha*). The grassland at Finca las Piedras recently increased in size due to a fire in August 2016, which may also have changed community structure and composition. Data collection spanned from 7/22/2017 to 9/06/2017 (in the Southern Amazonian dry season).

Site selection & habitat assessment

Drift fence sites were randomly chosen with the software BaseCamp (Garmin, 2016). All arrays were separated by at least 100 m from each other, at least 50 m from an unpaved road, and at least 5 m from a trail to avoid human disturbance and edge effects that may bias the outcomes (Von May et al., 2010). The forest-edge drift fence was located within 20 m of the edge (Urbina-Cardona, Olivares-Pérez and Reynoso, 2006) and the forest drift fence at least 80 m away from the edge habitat to avoid overlap. I examined each site to pick the best spot within 12 m of the randomly-chosen location in order to avoid big trees and thick roots in the forest sites and big shrubs in the grassland sites. In the forest and edge habitat drift fences, tree density, canopy cover, and leaf litter were estimated to detect possible differences between habitats. I counted all big trees (> 10 cm DBH) and small trees or shrubs (< 10 cm DBH; with a stem > 10 cm above the ground) within 3 m at both sides of each arm. I took canopy coverage at all four bucket spots by looking upward through a toilet paper roll and estimating cover to the nearest 10%. Leaf litter cover was estimated around each bucket of all arrays with a quadrat measuring 0.5 x 0.5 m intersected by four strings into four sub-quadrats (Urbina-Cardona, Olivares-Pérez and Reynoso, 2006).

Drift fences

Because many studies are missing specific details such as distance to roads and tree stands or depth and height of the drift fence, proper standardization is hard to achieve. Nevertheless, some form of standardization is critical to compare among studies. Often, 18.9 L buckets are used as pitfall traps (McKnight, Dean and Ligon, 2013). The material of the drift fence varies, ranging from chicken wire or silt to galvanized metal or aluminum (Bury and Corn, 1987; Enge, 1997). I built drift fences with black plastic mesh in a Y-shape with three arms, each extending for 5 m. An arm consisted of two wooden stakes with a fence stretched between them. Each array had four pitfall traps (diameter = 30 cm, height = 39.5 cm), one in the middle where all arms meet, and one at the end of each arm (Enge, 1997; Gardner et al., 2006; Fisher et al., 2008). To prevent animals from digging underneath or climbing over the fence, the fence was buried 10 cm deep and extended 50 cm high (Ribeiro-Júnior, Gardner and Ávila-Pires, 2008). Because of time constraints I used only one drift fence in each habitat (abandoned agricultural land, tropical forest edge and terra firme forest) although at least three replicates in each habitat are recommended. An additional replicate drift fence in each site was under construction at press time, for future use.

I dug a 10 cm deep trench for each fence and 40 cm and 80 cm deep holes with an excavator for the pitfall traps and the wooden stakes, respectively, at the end of each arm and in the center of the complete array. Wooden stakes were cut into 1.55 m long pieces and then put into all six 80-cm holes at each array. Black mesh was cut into pieces with 65 cm width and 550 cm length for the fence and then stapled tight to the two stakes of each arm. If there was a root or stem (diameter > 4 cm) crossing the trench I cut the black plastic mesh as far as needed (between 4 and 8 cm, depending on the size of the obstacle) and attached it to and around the obstacle. After fence was in place, dirt was added on both sides of the fence to partially bury the fence and further stabilize it.

Because some drift fences developed holes, I repaired these with mesh and duct tape. I drilled five 1.5 mm holes into each bucket, so that rainwater could leave the buckets to avoid drowning specimens. To provide an escape route for animals when buckets were not being regularly checked, bark (width > 4 cm, length > 50 cm) and big leaves (width > 6 cm, length > 50 cm) were put into the buckets to act as escape ladders. I opened drift fence arrays during each week and closed them from Friday night until Monday morning.

Visual encounter surveys

To get a more accurate picture of species composition and structure, I included visual encounter surveys (VES). Because of the high temperature and radiation during daytime in the tropics, which is more pronounced during the dry season, many species are nocturnal (Von May et al., 2010). Therefore I conducted the surveys in the morning (before 7 am) or at night (after 6 pm). Another advantage of nocturnal surveys is the avoidance of desiccation and overheating of captured specimens. I conducted time-constrained (25 min) and distance-constrained (50 m) visual encounter surveys. On trails within the forest or on a road that runs through the field I thoroughly searched the vegetation within 2m of either side of the trail/rod and up to 3m height (von May et al., 2010; Kurz et al., 2014).

Data analysis

All herpetofauna species encountered by VES and caught by pitfall traps were identified to species level or, if this was not possible, to genus or family level. Specimens were photographed, and then released at the capture point. For proper identification I took different shots of each specimen; one focusing on the color of the eyes, one of the ventral pattern and shape, one of the thighs and the hind legs, and one of the dorsal pattern and shape. The Shannon-Wiener index was used to assess diversity at each site. The proposed ANOVA could not be applied because not enough data was collected to conduct this form of test.

Results

Habitat measurements

Whereas the edge drift fence had 12 big trees and 81 small trees, the forest drift fence had 13 big trees and 519 small trees. The mean canopy coverage in the edge drift fence was smaller (40%) than in the forest drift fence (65%). The leaf litter coverage was almost identical in the edge (80%) and forest drift fence (85%).

Drift fences

A total of 20 individuals were discovered in the pitfall traps within 15 days and 14 nights of survey, belonging to three species of frogs and to two species of lizards. The most abundant species were *Adenomera* spp. (45 %, n = 9), nearly equally distributed between forest interior (n = 5) and forest edge (n = 4), and *Ameiva ameiva* (40 %, n = 8), that were all captured in the grassland. All other species only occurred once (singletons) in the forest edge.

In the grassland, 0.6 herpetofauna specie per array-day were captured, almost three times as much as in the forest interior drift fence (0.28 species/array-day). The edge drift fence captured two secretive and rare fossorial species, *Thyphlops reticulatus* (Giant Blindsnake) and *Bachia dorbigny* (a "legless" lizard). While the forest drift fence revealed at least two distinct species of the *Adenomera* group, differentiated by their eye colors (silver vs. golden), I only caught one specimen of an unidentified frog (in the grassland drift fence). Beside the captures in the drift fences and the encounters, I made several anecdotal herpetofauna records during the study period. I discovered a Fer-de-Lance (*Bothrops atrox*) near a small stream while it was crossing over tree stumps toward the water. An *Ameerega trivittata* (three-striped poison frog crossed my way while off-trail in the forest interior. We caught two Rainbow Boas (*Epicrates cenchria*) two days before a major cold front entered the area. One was lying on the trail, ca. 300 m into the forest, while the other was spotted around 6 m away from the forest edge where it moved toward the grassland.

Visual encounter surveys

Two individuals were seen during 9 visual encounters along the road and along the trails. One individual was the robber frog *Pristimantis reichlei* and the other the false coral snake *Oxyrhopus petolarius*. All VES combined resulted in an encounter rate of 0.5 specimens per survey hour.

Herpetofaunal diversity

Shannon-Wiener diversity indices of 0.349, 1.06, and 0.673 were calculated for the grassland, edge, and forest, respectively. The Shannon-Weiner index is a discontinuous quantitative value that includes evenness and relative abundances. These values translate into 1.4, 2.9 , and 2 effective species, respectively. Visual encounter surveys detected species not seen in pitfall traps, which may be because they are diurnal species that sleep in low vegetation or semi-arboreal species that were not captured with the pitfall traps, or because snakes such as *Oxyrophus*, can easily escape when falling into pitfall traps.

One frog, the Lowland Neotropical Bullfrog (*Adenomera andreae*), was the most common at the forest (n = 5) and edge drift fence sites (n = 5), but was almost not present at the grassland sites (n = 1). The most common species in the field, according to the captures in the drift fence, was the Giant Racerunner (*Ameiva ameiva,* n = 8). Comparing the occurrence of herpetofauna to rodents (which were common bycatch), herps were only about half as common in the field (n = 10) as rodents (n = 22) but of similar abundance in the forest (n = 4, n = 5) and the edge (n = 6, n = 5).

Discussion

Habitat measurements

Edge and forest interior habitats only differed by the number of small trees and canopy coverage and surprisingly not by the number of big trees. Also, leaf litter covered a similar percentage of the substrate around the drift fence arrays. The similar number of big trees could be an artifact of selection at the site to avoid the largest trees. Given that canopy coverage was higher in the forest, the big trees may be more abundant in the forest interior but further away from the actual drift fence. Furthermore, there were bigger roots and stems around the forest drift fence that were lacking at the edge.

Species diversity

Effective species richness was similar among all sites, but the field had a relatively smaller value (1.4) than both forest (2) and edge (2.9). These values are certainly underestimating the real species richness, even when considering only leaf-litter and fossorial herpetofauna, and are used for preliminary comparisons. As predicted, the edge habitat seemed to have the highest herpetofauna species richness. Edge habitat may be suitable for species that are more adapted to light gaps and grassland as well as forest dwelling species. Furthermore, although it is a hard edge, meaning that it is a sharp transition between two fairly distinct habitats, it is relatively young when considering the life cycles of reptiles and amphibians. These species may need a longer time to adjust their behavior and habitat preferences. On the other hand, individuals within the edge may be forced to move more extensively to find suitable habitat, and thus may have a higher chance of falling into the buckets while dispersing. This is a major limitation of the drift fences: they assume all leaf-litter species have the same chance to fall into the buckets, even though some species or 'transient' individuals within one species may move more than others (Thompson and Withers, 2003; Thompson et al., 2003). Therefore the type of method could have influenced the results of this study (Dixo and Martins, 2008).

In the edge drift fence I found two distinct species of the *Adenomera* genus, the Lowland Neotropical Bullfrog *(Adenomera andreae)* and the Sapo Neotropical Bullfrog (*Adenomera hylaedactyla*). In the forest interior drift fence traps I found two distinct morphs of *Adenomera andreae*, which potentially are different species or at least subspecies (Von May et al., 2009). Phenotypic plasticity may have lead to the differences, but as all morphs were found at one array that likely covers a relatively small area, they are adapted to the same environment and thus likely are at least different subspecies. Nonetheless, the number of *Adenomera* species as well as the proper nomenclature are both heavily debated

(Angulo, Cocroft and Reichle, 2003; von May et al., 2009).

As predicted, rare and secretive species were detected in the drift fence arrays, such as the Giant Blindsnake *(Thyphlops reticulatus*) and the semi-fossorial lizard *Bachia dorbigny*. While I caught two species of reptiles in the edge drift fence, no reptiles were caught in the forest interior. The same pattern arose in a study conducted in Equador, where no reptiles were caught in the interior of a lowland tropical rainforest but only within the edge of the forest (Maynard et al., 2016).

Trap efficiency

The grassland drift fence had the highest capture rate of my study (0.6 specimen per array-day), almost twice as high as the edge drift fence (0.32 specimen per array-day) and the forest drift fence (0.28 specimen per array-day). One reason for this difference may be that the Giant Racerunner (*Ameiva ameiva*), which I commonly caught in the grassland, is a generalist species that is quite abundant. Reptiles are less prone to desiccation, and as this study was conducted during the dry season, amphibians that might have been caught more frequently in the forest and edge may have been less abundant or more stationary than during the wet season.

In a study conducted in Florida in which researchers caught 0.22 specimens per array-day (Greenberg et al., 1994), a similar amount of specimens were caught in the edge habitat and the forest interior habitat. They concluded that there are biases against larger species and snakes that easily can get out of the pitfall traps. The only snake I caught, a blind snake that was small compared to other snake species in the region, might demonstrate this point. A study conducted in Oklahoma specifically focusing on the efficiency of pitfall traps with drift fences (McKnight et al., 2013) caught three times as many specimens per array-day (1 specimen per array-day). However, the drift fences used in this study had six traps per array and each fence was six times longer (30 m). Drift fences in other studies were mostly built with aluminum or other stable material that did not need external force to be upright. The overall capture rate in a study conducted in Orellana, Ecuador was similar: 0.42 specimen per array-day (Maynard et al., 2016). Because of the low capture rate of fences it is important to have replicates, and an additional set of arrays was under construction at the time of this study. Small drift fences with only one large arm (10 m) including two or three buckets could also be distributed within the different habitat types to cope with time and material constraints.

As I saw no signs of burrowing activity (and because I was able to catch fossorial species) I assume that the fences were buried deep enough. On the other hand, I cannot firmly assume that amphibians were not able to climb the fence. The edge drift fence was overrun by leaf-cutter ants that damaged some fence material and fell into the pitfall buckets in great numbers. The increased abundance of these ants may have some repulsive or attractive effects on amphibians and reptiles, depending on their avoidance behavior or feeding habits toward certain species. It is hard to tell if spiders eat frogs that fall into the bucket, but I did not find carcasses of frogs that indicated predation by spiders. On the other hand, mice and lizards may cause frog predation without leaving a trace, thus leading to a decreased number of caught frogs, especially in the grassland drift fence with its many specimens of lizards.

My observations were all within three weeks during the dry season, when many amphibians do not breed and thus move less. Humidity and precipitation may increase the movement of amphibians and therefore more specimens might fall into the pitfall traps in the wet season. It is important to track the changes in composition that may accompany the onset of the wet season to make proper inferences. Other parameters like temperature and moisture should be included in statistical models, because they affect the distribution and diversity of reptiles and amphibians (Fauth et al., 1989; Folt and Reider, 2013). Meta-analyses could contribute to interpreting my results in a broader context.

Conclusion

Although the sample size was small, some preliminary comparisons can be made among the habitats that should guide ongoing data collection. Early indications from investigating fossorial and leaf-litter herpetofauna for 15 days in the dry season may show that the field habitat has a decreased diversity of species as a result of different abundances when comparing among habitats. Regardless of the fact that arboreal species might be absent due to the absence of trees in the field, the species richness of soil dwelling amphibians and reptiles is likely decreased.

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