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LRH: H. C. Stevens et al.

RRH: Detection Methods for Birds in White-sand Forests

Use of autonomous audio recordings for the rapid inventory of birds in the white-sand forests of

the Peruvian Amazon

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ABSTRACT. White-sand forests are patchily distributed ecosystems covering just 5% of Amazonia that host many specialist species of birds not found elsewhere, and these forests are threatened due to their small size and human exploitation of sand for construction projects. As a result, many species of birds that area white-sand specialists are at risk of extinction, and immediate conservation action is paramount for their survival. Our objective was to evaluate current survey methods and determine the relative effect of the size of patches these forests on the presence or absence of white-sand specialist. Using point counts and autonomous recorders, we surveyed avian assemblages occupying patches of white-sand forest in the Peruvian Amazon in April 2018. Overall, we detected 126 species, including 21 white-sand forest specialists. We found that autonomous recorders detected significantly more species of birds per survey point than point counts. We also found a negative relationship between avian species richness and distance from the edge of patches of white-sand forest, but a significant, positive relationship when only counting white-sand specialists. While autonomous recorders detected more overall species, point counts were more effective for detecting canopy-dwelling passerines. Therefore, we recommend that inventories and surveys for rare and patchily distributed species in the tropics use a mixed-methods survey technique of bioacoustics and visual observations. Finally, conserving large, continuous patches of white-sand forest may increase survival likelihood for white-sand specialists, but future studies determining habitat occupancy should further investigate this relationship.

Key words: Crypturellus casiquiare, detection, point counts, *Polioptila clementsi*, remote audio recorders, tropical birds, white-sand forest

Avian monitoring is notoriously difficult in the tropics, mainly due to the sheer quantity of species as well as the structural complexity associated with tropical forests (Brandes 2008). Although effective monitoring tools for birds (Haselmayer and Quinn 2000), traditional pointcount methods are subject to human error and contingent on surveyor experience. Furthermore, the presence of a surveyor may affect detection, especially for cryptic species, species found at low densities, and species sensitive to human presence (Tegler et al. 2012). The combination of these factors and the density of vegetation in tropical rainforests highlights the need for alternative survey techniques.

Recent studies have identified autonomous audio recorders and acoustic analysis software as valuable tools in avian monitoring (Acevedo and Villanueva 2004, Brandes 2008, Tegler et al. 2012, Towsey et al. 2014, Alquezar and Machado 2015). Autonomous recorders can reduce error associated with species identification in the field by allowing surveyors to analyze audio files after the study period and accurately identify all species (Brandes 2008). Furthermore, they eliminate human disturbance by detecting avian species without the physical presence of a surveyor. This makes bioacoustic techniques particularly useful for detecting and monitoring rare and secretive species (Rognan et al. 2012), especially in the tropics where visual observations of these species occur sporadically and infrequently. Moreover, by deploying multiple recorders, multiple areas can be surveyed simultaneously rather than relying on several human surveyors. The combination of these benefits highlight the potential value of autonomous recorders for conducting species inventories while optimizing time and effort. Despite this, few investigators have used this method in tropical forests (Alvarez-Berríos et al. 2016, Ulloa et al. 2016, Ribeiro et al. 2017).

White-sand forests are restricted patches within the overall forest mosaic of Amazonia (Adeney et al. 2016). Although still debated, these patches are believed to have formed during dry, cooling periods of the Pleistocene, especially following tectonic reactivation events (Rossetti et al. 2012), when increased river erosion combined with the expansion of savannas and retraction of old-growth forest formed isolated pockets of nutrient-poor, sandy habitats (Daly et al. 2016). Subsequent warming facilitated the re-expansion of forests and deposition of glacial sediment, effectively forming 'islands' of white-sand forests (Daly et al. 2016). White-sand forests are globally renowned for their unique species assemblages, with many regional endemic species found in these forests across the tropics (van Rensburg et al. 2000, Shany et al. 2007, Mora 2014, Adeney et al. 2016). These white-sand-forest specialists have adaptations that allow them to succeed in a nutrient-poor environment characterized by acidic, sandy soils and resulting in stunted vegetative growth and lower overall species richness compared to broadleaf terra firme forests. Patches of white-sand forest effectively act as islands for many of these specialists (Capurucho et al. 2013) that, given their degree of habitat specialization and isolated evolutionary history, cannot compete in surrounding broadleaf terra firme forest. In Amazonia, white-sand forests represent 5% of forests present in the Amazonian basin, and have been heavily exploited by humans for their sand (Adeney et al. 2016). As a result, these forests have received increasing conservation attention over the past decade, focused mainly on protecting their specialized flora and fauna (Shany et al. 2007, Daly et al. 2016, Fine et al. 2016).

These patchily distributed forests host multiple avian specialists of conservation concern, but little is known about these species (Shany et al. 2007, Zarate et al. 2015, Borges et al. 2016). Given the limited connectivity between patches of white-sand forest, assemblages of bird species occupying these forests can vary over even a regional scale (Capurucho et al. 2013, Matos et al. 2016). A study of the intraspecific and interspecific landscape genetic variation for two species of white-sand manakins revealed that gene flow between birds occupying different patches of white-sand forest was significantly lower than that for birds inhabiting broadleaf *terra firme* forest (Capurucho et al. 2013). This low connectivity between patches renders many of these avian specialists particularly vulnerable to extinction (Borges et al. 2016), especially because patches of white-sand forest are small and cannot support viable source populations.

We defined white-sand specialists using the same criteria as Alonso et al. (2013), i.e., facultative, local, and strict. Facultative specialists like Rufous-tailed Flatbills (*Ramphotrigon ruficauda*) primarily inhabit white-sand forests, but may also use other forest types for foraging or acquiring nest materials (Alonso et al. 2013). Conversely, local specialists like Yellow-throated Flycatchers (*Conopias parvus*) are associated with white-sand forests in some regions, but may not be specialists elsewhere in their overall range (Alonso et al. 2013). Finally, strict specialists like Iquitos Gnatcatchers (*Polioptila clementsi*) exclusively occupy white-sand forests, rarely or never visiting other forest types (Alonso et al. 2013). Based on these classifications, there are currently 26 white-sand specialists historically recorded in Reserva Nacional Allpahuayo-Mishana, Peru. Many of these species are Peruvian endemics and notoriously difficult to detect, such as Allpahuayo Antbirds (*Percnostola arenarum*), Mishana Tyrannulets (*Zimmerius villarejoi*), and Iquitos Gnatcatchers (*Polioptila clementsi*) (Shany et al. 2007). Several of these species are of immediate conservation concern, including the Iquitos Gnatcatcher, one of the rarest birds in the world (Table 1; BirdLife International 2016).

Using species-detection data collected from both point counts and autonomous recorders, our objective was to evaluate the efficacy of each survey method by comparing their respective total detected species, and determining the effect of surrounding broadleaf *terra firme* habitat on detected species richness in the Reserva Nacional Allpahuayo-Mishana. We predicted that autonomous recorders would record greater species richness than traditional point-counts, given that the use of recording devices reduces human disturbance, gathers more data, and allows for the ability to re-listen to complicated dawn choruses, call notes, and other sounds that may not be easily identifiable in the field. Additionally, we expected species richness of white-sand specialists to be highest in core areas of large white-sand forest patches because these isolated areas experience low levels of human disturbance and are relatively unperturbed by the edge effects of surrounding broadleaf *terra firme* forest. Evaluating current survey methods and determining the relative effect of patch size on white-sand specialist presence serve as vital preliminary steps for monitoring tropical birds across habitat types, and especially those that mirror the patchy distribution of white-sand forests. This information can, in turn, guide future research and conservation efforts aimed at protecting avian white-sand forest specialists throughout Amazonia.

METHODS

The Reserva Nacional Allpahuayo-Mishana (RNAM) is located 25 km (S -3.95219, W -73.4321) from Iquitos, Peru, and protects a mosaic of unflooded and flooded forest types, hosting 580 km² of broadleaf *terra firme* and the highest concentration of white-sand forest habitat in Peru (Shany et al. 2007). The climate in the Iquitos area is less seasonal than the rest of Amazonia, with a mean annual precipitation of ~3100 mm (Alonso and Whitney 2003). The reserve was established in 1999 specifically to preserve the flora and fauna of white-sand forests, and is currently administered by the Regional Administration Government of Loreto (CTAR), the Peruvian Amazonia Research Institute (IIAP), and the National Institute of Agricultural Research (INIEA) (Alonso and Whitney 2003). We visited five different white-sand patches within 5 km of the Iquitos-Nauta road. These patches differed in size and composition, with patches ranging from 1.05 ha to 175.7 ha and covering three white-sand forest types, including *varillal humedo*, *varillal seco*, and *chamizal*. *Varillales* are distinguished from *chamizales* by overall larger tree size and a lower water table. *Varillales* can be further divided by relative tree height into tall (*alto*) and short (*bajo*) categories, and by relative moisture availability into moist (*húmedo*) and dry (*seco*) categories, both of which are indicated by plant composition (Adeney et al. 2016). All patches were surrounded by broadleaf *terra firme* forests (Fig. 1).

Experimental procedure. We affixed 12 recording devices (Model SM4, Wildlife Acoustics, Maynard, MA) to trees and selected 16 points to conduct point-counts along previously established trails in patches of white-sand forest in both IIAP and INIEA sites within the RNAM. Points for each method were separated by at least 300 m to ensure independence of sampling points (Ralph et al. 1995). We alternated point-counts and recording devices every 150 m, and did not use both methods at each point in a direct side-by-side comparison to eliminate the effects of observer disturbance near the audio recorders, which would have negated one of the advantages of autonomous recording. This survey design reflect how the respective methods would be used in practice, rather than a direct comparison at each point; the different methods inevitably result in different sampling regimes. The autonomous recorders used dual channel acoustics with two microphones directed horizontally in opposite directions. Nine recording devices were placed at heights of ~2 m, but the other three (R6, R9, and R10; Fig. 1) were located in areas with more human activity and, therefore, were placed 5 m above ground to prevent theft. Although Darras et al. (2018) found that height (~ 20-26 m) was a positive predictor for alpha richness in recorder species detection, the minor positioning differences in our study are less than local differences in altitude between sampling sites, and are therefore

unlikely to have an effect on detection distances. Blake (1992) found that tropical birds are most active from a half hour before sunrise until three hours after sunrise, and that species detection does not vary significantly during this period. Therefore, we conducted all data collection between 05:30 and 09:30 to ensure later surveys did not skew detection results, and to sample the dawn chorus characteristic of tropical forests (Berg et al. 2006).

We programmed the autonomous recorders to record continuously from 05:30 to 09:30 each morning over an 11-day survey period from 12 to 23 April 2018. This timing was derived from the results of our pilot study where we programmed recorders to record continuously (24 h/day) and decided that, given the quantity of audio data combined with our desire to compare recorder data directly with point-count data, recording from 05:30 to 09:30 was most appropriate for our study. For point counts, we divided our study site into two areas based on accessibility and visited them alternately. Overall, we conducted three point counts at each of our 16 pointcount locations during the same 11-day period. Our survey team consisted of four observers and one scribe, including an experienced Peruvian ornithologist who specialized in white-sand birds, to ensure maximum and accurate detection of species at each point. When arriving at a point, each observer faced in one of the cardinal directions, and the scribe immediately began recording species seen and/or heard by the four observers. We did this because Ralph et al. (1995) explained that acclimation periods used in other studies under-sampled species richness because the initial human disturbance upon arrival may flush more secretive species. Each point count lasted 6 min because studies have shown that the maximum number of new individuals visiting an area peaks ~ 5 min after beginning a survey, and declines substantially over the 5-10 min period (Lynch 1995). Species observed flying over a point location were not counted because

they were not considered to be using the surrounding habitat (Ralph et al. 1995). Point counts were not conducted during heavy rain (Ralph et al. 1995).

Data analyses. We imported all audio data into Kaleidoscope Pro (v. 4), software that clusters audio files based on similar spectrograms and acoustic patterns. Audio data from each recorder were analyzed separately. We manually identified all vocalizations that were classified as the most central to each cluster as defined by the minimum "Top Distance" statistic generated by Kaleidoscope. Pattern recognition software such as Kaleidoscope produces false positives and negatives, but this was not a concern for our analysis because we identified all species manually and did not use advanced classifiers. After identifying all species present in the first audio file of each cluster, we recorded the total species list for that recorder. This under-samples the available data and additional analysis may yield a larger species list, but, with the time constraints of our rapid inventory, this was not possible.

Due to our low overall survey point sample size, we used a Mann-Whitney U test to compare mean species richness per point between the two sampling methods (point counts and autonomous recorders). Furthermore, we plotted all survey points in ArcGIS (sized based on their total species richness) to visually compare differences in total detected species per point across different patch sizes (Fig. 1).

Given the patchiness and variation in patch sizes of white-sand forests, we investigated the effect of edge habitat on avian species richness. Using ArcGIS, we calculated the distance of each survey point from broadleaf *terra firme* habitat and used a regression analysis in R version 3.4.0 (R Core Team 2017) with (a) total species richness and (b) white-sand specialists per point to test for possible correlations.

RESULTS

Overall, we detected 126 species of birds in white-sand forest patches of RNAM using traditional point-count and bioacoustic survey methods. Of these 126 species, we detected 82 during point-counts and 106 using remote audio recorders. We also detected 21 of the 26 species of white-sand specialists. We detected most specialists with both survey methods, but only detected Gray-legged Tinamous (*Crypturellus duidae*), Black-throated Trogons (*Trogon rufus*), Duida Woodcreepers (*Lepidocolaptes duidae*), and Long-tailed Woodcreepers (*Deconychura longicauda*) using autonomous recorders (Table 1). Conversely, we only detected Short-billed Leaftossers (*Sclerurus rufigularis*) during point counts (Table 1). We recorded two of the three white-sand forest specialists endemic to Peru (Mishana Tyrannulet, *Zimmerius villarejoi*, and Allpahuayo Antbird, *Percnostola arenarum*), from multiple sites in the reserve, but did not detect Iquitos Gnatcatchers (*Polioptila clementsi*), despite their presence near the location of recorder R9 (Fig. 1) in February 2018 (Percy Saboya, pers. oberv.).

Autonomous recorders detected significantly more species per survey point than traditional point counts ($U_{26} = 0.5$, P = 0.0001; Figs. 1 and 2). Surveys (both point counts and autonomous recorders) conducted closer to the edges of patches of white-sand forest did not detect more species ($F_{26} = 1.3$, $R^2 = 0.048$, P = 0.26; Fig. 3). Conversely, surveys conducted farther from the edges of white-sand forest patches recorded significantly more white-sand specialists ($F_{26} = 8.5$, $R^2 = 0.24$, P < 0.01; Fig. 3). We also detected Barred Tinamous (*Crypturellus casiquiare*) multiple times (three individual recordings between 02:30 and 04:30) in recorder audio data from our pilot study (placed in IIAP during mid-February 2018) (Audio S1).

DISCUSSION

Autonomous recorders detected significantly more species of birds per survey point than traditional point counts. However, identifying many of the small passerines in mixed-species flocks in audio data was nearly impossible, mainly because the only vocalizations recorded of birds in these flocks were indistinguishable call notes. We know from incidental observations and point-count data that these flocks include canopy-dwelling nectarivores, frugivores, and insectivores such as Purple Honeycreepers (*Cyanerpes caeruleus*), Blue Dacnisses (*Dacnis cayana*), and Paradise Tanagers (*Tangara chilensis*). Thus, some species are easier to identify visually. Possible ways to remedy this limitation would be to place autonomous recorders in the canopy to improve recording quality that might allow easier identification of call notes, and to combine recordings and visual observations using a mixed-methods survey technique.

Our results suggest that autonomous recorders placed in forests with low-lying or undeveloped canopies (like *chamizal*) sample all species recorded during point-counts and, therefore, may stand alone for surveys conducted in these areas. In our study, recorders placed in *chamizal* in IIAP detected all the species recorded during nearby point counts plus additional species like Mishana Tyrannulets. This highlights an advantage of autonomous recorders over point-counts for avian monitoring (especially in certain forest types), and we therefore recommend that, in future studies of tropical avian assemblages, investigators use autonomous recorders as their primary survey method. Relying on recorders not only leads to detection of more species, but also eliminates the unnecessary components of traditional surveys (i.e., walking between survey points), thereby optimizing survey time and effort. Haselmayer and Quinn (2000) also suggested that, in most circumstances, autonomous recorders should be used to monitor tropical birds rather than point counts. We failed to detect rare Iquitos Gnatcatchers in areas of RNAM where they have been recorded historically. This underscores the time required to accurately monitor rare species, and suggests that the short duration of our study likely resulted in incomplete presence/absence data. Alternatively, we may have detected Iquitos Gnatcatchers, but were unable to identify them in Kaleidoscope. Like tanagers, Iquitos Gnatcatchers frequently forage with canopy-dwelling mixed flocks and do not routinely sing, but utter call notes that can be confused with those of many other small passerines and, therefore, can be difficult to identify from audio data (Percy Saboya, pers. observ.). Given their preference for edge habitat in *varillales* (Percy Saboya, pers. observ.), Iquitos Gnatcatchers may be easier to detect using a combination of autonomous recorders placed higher in trees and visual observations.

Although autonomous recorders failed to detect Iquitos Gnatcatchers, they did detect Barred Tinamous (*Crypturellus casiquiare*), a species undetected in RNAM prior to our study (Audio S1). This, combined with the number of unsuccessful point-count surveys conducted in this area to detect this species (Percy Saboya, pers. observ.) highlights the efficacy of autonomous recorders at detecting cryptic species compared to traditional point-count methods. The three separate detections of Barred Tinamous occurred over a short time period during the evening (between 02:30 and 04:30), highlighting both the potential and utility of setting remote audio recorders on a 24-h schedule. Powering autonomous recorders externally with a car battery allows recording of audio data for 24 h/day for up to 2 or 3 months, serving as an efficient alternative for accurately surveying avian assemblages rather than relying on human surveyors.

Edge habitat. Bird assemblages along the edge of white-sand forest are likely influenced by the surrounding broadleaf *terra firme* habitat, and white-sand forests are less diverse than broadleaf *terra firme* forests (Alonso et al. 2013). Our test of the effect of edge proximity on

species diversity yielded a general, negative relationship between distance from patch edge and total species count, suggesting that as patch size decreases, avian diversity increases. Surveys conducted in small patches are likely to detect some species from the broadleaf *terra firme* as well as white-sand specialists, effectively leading to an increased sampled diversity. Although greater diversity is generally considered a positive result, increased species diversity in this case may not reflect ecosystem health. Some bird species like facultative specialists may benefit from smaller patches because they may use both white-sand forest and broadleaf *terra firme*. Conversely, strict specialists will likely suffer population declines in smaller patches, given their specific requirement for white-sand habitat and increased competition with broadleaf terra firme species that infiltrate edge habitat. This is further emphasized by our regression analysis including only white-sand specialists where we found a significant increase in the total number of white-sand specialists as the distance from patch edge increased. Therefore, conserving large, continuous patches may increase the likelihood of survival of white-sand specialists. As whitesand habitats suffer further degradation, understanding the bird assemblages in small white-sand patches is essential. We hope that the significant, positive relationship between white-sand specialists and distance from edge serves as a baseline for future studies investigating habitat use by white-sand specialists.

Occupancy by white-sand specialists and conservation implications. Habitat use for many of the described white-sand species remains largely unknown, and autonomous recording could be useful for acquiring this information. Interestingly, one recorder placed in *chamizal/irapayal* white-sand forest in our study recorded most of the detections of two Peruvian white-sand forest endemics, Allpahuayo Antbirds (*Percnostola arenarum*) and Mishana Tyrannulets (*Zimmerius villarejoi*). These localized detections reaffirm the associations described by Alonso (2002) for Allpahuayo Antbirds, but not for Mishana Tyrannulets that tend to occupy *varillales secos*. Both species could potentially use *chamizal/irapayal* habitat for foraging, especially given its isolation from the surrounding *terra firme* broadleaf forest. *Chamizal* is often embedded within the core of larger white-sand forest habitats (Percy Saboya, pers. observ.), effectively acting as isolated, undisturbed habitat for white-sand specialists. Furthermore, *chamizal* habitat is characterized by short, small trees that could increase detections because birds will be more likely to be near a recorder. However, Allpahuayo Antbirds and Mishana Tyrannulets were also detected by recorders outside of *chamizal* areas, further indicating the need for long-term studies in this habitat.

Although RNAM has protected status in Peru, mining for sand continues. The low occurrence of species endemic to Peruvian white-sand forests in our study highlights the importance of additional research to determine the presence or absence of these species in various patch sizes. The number of functional white-sand forest patches and their connectivity will likely be reduced by continuing anthropogenic pressures (Borges et al. 2016). Because they host a unique assemblage of birds not found elsewhere in Peru, protecting these white-sand forests is crucial. Longer-term use of autonomous recording devices would likely provide the presence/absence information across a gradient of patch-sizes critical to conserving these specialist species.

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Common name	Scientific name	Pointcount	Auton- omous recorder	Specialist Type
Fuscous Flycatcher	Cnemotriccus fuscatus duidae			Strict
Brown-banded Puffbird	Notharchus ordii	x	x	Strict
Gray-legged Tinamou*	Crypturellus duidae		x	Strict
Iquitos Gnatcatcher***	Polioptila clementsi			Strict
Mishana Tyrannulet**	Zimmerius villarejoi	x	x	Strict
Pompadour Cotinga	Xipholena punicea	x	x	Strict
Saffron-crested Tyrant- Manakin	Neopelma chrysocephalum	x	x	Strict
Zimmer's Tody-Tyrant	Hemitriccus minimus	x	x	Strict
Allpahuayo Antbird**	Percnostola arenarum	x	x	Local
Cinnamon Tyrant	Neopipo cinnamomea	x	x	Local
Cinnamon-crested Spadebill	Platyrinchus saturatus			Local
Citron-bellied Attila	Attila citriniventris	x	x	Local
Duida Woodcreeper	Lepidocolaptes duidae		x	Local
Orange-crowned Manakin	Heterocercus aurantiivertex			Local
White-masked Antbird	Pithys castaneus			Local
Yellow-throated Flycatcher	Conopias parvus	x	x	Local
Zimmer's Antbird	Myrmeciza castanea	x	x	Local
Ancient Antwren*	Herpsilochmus gentryi	x	x	Facultative
Black-throated Trogon	Trogon rufus		x	Facultative
Long-tailed Woodcreeper	Deconychura longicauda		x	Facultative
Paradise Jacamar	Galbula dea	x	x	Facultative
Pearly Antshrike	Megastictus margaritatus	x	x	Facultative
Rufous-tailed Flatbill	Ramphotrigon ruficauda	x	x	Facultative
Short-billed Leaftosser	Sclerurus rufigularis	x		Facultative
White-crowned Manakin	Pipra pipra	x	x	Facultative
Yellow-browed Antbird	Hypocnemis hypoxantha	x	x	Facultative

Table 1. Detection of white-sand forest specialists known to inhabit RNAM by point counts and autonomous recorders, and their degree of specialization (adapted from Alonso et al. 2013).

*** = Critically Endangered

** = Vulnerable

* = Near Threatened

Fig. 1. Locations of points surveyed using point counts (circles) and autonomous recorders (squares) from 12 to 23 April 2018 in five patches of white-sand forest in RNAM (-3.95219, -73.43210). Larger symbols indicate more species detected. All white-sand forests are shown in beige and were classified by IIAP prior to our study (IIAP, unpubl. data).

Fig. 2. The number of species detected per survey point by point counts and autonomous recorders. Bold central lines show the median values of each dataset, while boxes show the interquartile range. Dashed lines extending from the boxes correspond with highest and lowest values of each dataset.

Fig. 3. The relationship between (a) the total number of bird species per survey point and the distance from each point to the edge of a patch of white-sand forest, and (b) the total number of white-sand specialists per point using the same edge distances. Distances from survey points to patch edge measured in ArcGIS in meters.





Fig. 2





