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MAXIMIZING NONHUMAN PRIMATE FECAL SAMPLING IN THE REPUBLIC OF CONGO

Sarah H. Olson,[1] Kenneth Cameron,[2,3] Patricia Reed,[2,3] Alain Ondzie,[3] and Damien Joly[1,4]

[1] Wildlife Conservation Society, 900 Fifth Street, Building 373, Nanaimo, BC V9R 5S5, Canada
[2] Wildlife Conservation Society, Global Health Program, 2300 Southern Boulevard, Bronx, New York 10460, USA
[3] Wildlife Conservation Society, B.P. 14537, Brazzaville, Republic of Congo
[4] Corresponding author (email: djoly@wcs.org)

ABSTRACT: Techniques for detection of pathogens in wildlife feces allow disease surveillance of species that are difficult to locate and capture (e.g., great apes). However, optimal strategies for detection of feces in logistically challenging environments, such as the forests of Central Africa, have not been developed. We modeled fecal gorilla sampling in the Republic of Congo with computer simulations to explore the performance of different fecal sampling designs in large tropical landscapes. We simulated directed reconnaissance walk (recce) and line-transect distance-sampling survey designs and combinations thereof to maximize the number of fecal samples collected, while also estimating relative ape density on a virtual landscape. We analyzed the performance of different sampling designs across different densities and distributions of ape populations, assessing each for accuracy as well as cost and time efficiencies. Past ape density surveys and fecal deposition rates were used to parameterize the simulated fecal sampling designs. Our results showed that a mixed sampling design that combines traditional transect and a directed reconnaissance sampling design maximized the number of fecal samples collected and estimates of species density. Targeted sampling produced strongly biased estimates of population abundance but maximized efficiency. This research will help design the fecal sampling component of a larger study relating great ape density to Ebola fecal antibody prevalence.

Key words: Congo, Ebola, feces, *Gorilla gorilla gorilla*, pathogen, scat, spatial.

INTRODUCTION

Fecal detection methods for low host density and low-visibility systems (e.g., great apes living in tropical forests) have not been developed, even as laboratory diagnostic capacity for pathogen detection from feces has grown. A few examples include a 4-day turnaround time to detect unknown viral pathogens (Nakamura et al., 2009), identification of norovirus antigen by reverse-transcription PCR in a matter of hours with 100% sensitivity and specificity (Schmid et al., 2004), and at the commercial scale, an antigen test for canine parvovirus requiring 10 min at $20 per sample (SNAP® PARVO, IDEXX Laboratories, Inc., Westbrook, Maine, USA). Diagnostics for viral pathogens, antigens, and antibodies are becoming increasingly specific, faster, and cheaper.

Researchers performing large-landscape fecal sampling will need to evaluate the number of fecal samples collected, estimates of species density (to calculate the size of the population), and the number of samples collected per unit time. Fecal detection in low-visibility forest cover is not trivial. Fecal samples degrade quickly in the tropics due to high temperatures, humidity, rainfall, and sunlight (Kuehl et al., 2007; Michalski et al., 2011). In addition to rapid decay rates, researchers are in direct competition with fecal consumers; within 24 hours dung beetles effectively removed 71% of scat from landscapes in Mato Grosso, Brazil (Norris and Michalski, 2010). Thus, achieving a targeted sample size may be challenging under conditions that often arise in regions where emerging infectious disease risk is high (Jones et al., 2008).

Large-landscape fecal surveys are traditionally used to estimate the abundance or density of elusive species. The calculation thereof is based on fecal counts along line transects, as well as average fecal production and decay. The purpose of transects is to estimate species density, not to collect copious amounts of fecal samples. However, an accurate estimate of host abundance

would be a value-added component of fecal detection methods designed to maximize the number of fecal samples collected. Certain sampling strategies may be more effective at fecal collection, density estimation, or both.

To better understand trade-offs between different fecal sampling designs in a logistically challenging environment we simulated fecal sampling for western lowland gorilla (*Gorilla gorilla gorilla*) feces in the forests of the Republic of Congo (RC) as a case study. Ebola hemorrhagic fever (EHF) is a putative driver of great ape population decline, and has emerged repeatedly in central Africa with high human and ape mortality rates. During one outbreak a well-studied population of gorillas at the Lossi Reserve, RC is reported to have declined by 95% (134/143), whereas another population in Minkébé Forest, Gabon, is believed to have dropped by 99% (Walsh et al., 2003). The apes most threatened by EHF are western lowland gorillas, currently listed as critically endangered by the International Union for Conservation of Nature (IUCN) and predominantly found in Gabon, RC, and Cameroon (IUCN, 2011). Despite the magnitude of these mortality events and the conservation risk, very little is known about the ecology of Ebola virus (EBOV) in great ape populations.

A new, noninvasive fecal assay is in development that may help shed some light on the history of EBOV in gorilla populations. The Wildlife Conservation Society and the National Institute for Allergy and Infectious Diseases Vaccine Research Center are jointly developing a test to detect EBOV antibodies from gorilla feces. With this test the immunological status of populations may be used to establish the geographical distribution of EBOV, identify immunologically naive populations, and determine which populations survived virus exposure. Furthermore, variation in levels of exposure between different populations could then be identified. In anticipation of this new diagnostic tool, the next step to understand the EBOV disease ecology enigma will require the collection of many fecal samples. To our knowledge there has not been a formal assessment of fecal sampling designs to efficiently maximize sample size and estimate the abundance of the host population, nor has there been a sensitivity analysis of different fecal sampling designs applied to different levels of population density and variation in home-range size.

Efficient fecal sampling is increasingly important as fecal diagnostics can provide a wealth of biological host information in addition to pathogen detection. This includes tests for physiologic stress, reproductive productivity, sex, and genetic differentiation of individuals (Wasser et al., 2004; Le Gouar et al., 2009). Herein, our objective was to provide recommendations on choosing a fecal sampling design that maximized the number of fecal samples, the accuracy of population density estimates, and the efficiency of the sampling teams. We applied these criteria and report on spatial simulations to evaluate four different fecal sampling designs: a classic, systematic line-transect distance-sampling survey, two nontraditional reconnaissance (recce)-based survey designs already used for fecal sampling in RC, and a mix of the transect and recce designs. To simplify our analysis we approximated ape abundance using feces detection. Our hypotheses were that traditional line-transect distance sampling would produce the most accurate estimate of ape abundance and targeted recce-based sampling would produce the largest number of fecal samples in a given landscape. We predicted that a combination of both line-transect and recce-based designs would produce quality estimates of ape abundance as well as a large number of fecal samples.

MATERIALS AND METHODS

Fecal sampling designs

Distance-based line-transect surveys are the ecologist's primary tool for estimating species

density, but the design is not well suited to the forest floor conditions of a tropical jungle (Buckland et al., 2001; Thomas et al., 2010). The sampling design requires strict adherence by observers to a linear path between two points, perpendicular measurements from that path to a detected object sighted by an observer, and statistical calculations that account for the higher probability of observing sample objects that are located closer to the path. A gorilla survey team may only travel 1 or 2 km per day, literally cutting their way through swamps and thick vegetation, and traveling so as to not leave a path for poachers to follow (Maisels, pers. comm.).

The directed recce sampling design is a survey suited to the realities of jungle travel. As described by Walsh and White (1999), "in recce sampling, researchers follow a path of least resistance along game trails, and natural features (e.g., watercourses and ridges), cutting only enough vegetation to maintain a general compass bearing…because it does not require hacking a perfectly straight line through thick vegetation and because perpendicular distances to dung piles are not measured, the recce design requires much less effort per unit distance than transect sampling." Although a recce may require less effort per unit distance, "recces do not provide an unbiased sample of the area, and variation in recce encounter rates is very likely to result from a variety of sources and not just variation in density" (Kühl et al., 2008). A mix of recces and line-transect distance sampling may be the solution to produce efficient and accurate density estimates.

Our starting point was over a dozen recce surveys conducted in RC to collect the feces that were originally used to develop the fecal antibody assay. Each survey covered ~400 km^2 and required 1 month in the field. The large spatial scale and short duration of the fecal surveys was intended to reduce the likelihood of sampling individuals more than once. Although most researchers prefer to use nest detection over feces for primate population surveys, we analyzed feces to generalize our results to nonnesting species (Kühl et al., 2008; Todd et al., 2008).

Simulating gorilla populations on the landscape

We designed a virtual 65-km × 65-km (4,225 km^2) landscape that we populated with different densities and distributions of gorillas. Stokes et al. (2010) reported densities of 0–7 individuals/km^2 for similar-sized landscapes within northeastern RC, so we selected 13 gorilla densities ranging from 0.005 to 7.0 individuals/km^2. Fecal production rate was set at five piles/day (Todd et al., 2008). The fecal assay requires a sample collection within 24 hours of deposition; therefore we simulated 1 day's worth of fecal production. Due to uncertainty in the spatial configuration of daily gorilla activities, and consequent fecal deposition, we then created 16 template landscapes with at least 147,875 fecal piles (equivalent to 7 individuals/km^2) using a 1–40-km range of clustering radii with the Thomas cluster algorithm, a uniform Poisson point process from the spatstat R package, set to five parent points and an expectation of 50,000 child points per parent (Baddeley and Turner, 2011). A random spatial distribution was used to create the 17th landscape template. Gorilla density determined the number of fecal piles sequentially retained from each template landscape (Fig. 1).

Simulating field-sampling designs

We examined four fecal sampling approaches: 1) classic line-transect distance sampling, 2) a recce-based random IronX, 3) a recce-based targeted IronX, and 4) a mix of line-transect distance sampling and recces. To simulate classic line-transect distance sampling, we implemented a standard, gridded line-transect distance-sampling survey similar to those executed by Stokes et al. (2010). An evenly distributed column of 4- × 2-km transects spaced 16.25 km apart was randomly created using the DSpat R package (Johnson et al., 2008). The transect column was then replicated three times, creating a total of 16 transects, with 14 km separating each transect column (Fig. 2A). The total length of distance sampling within a landscape was 32 km.

The recce-based sampling design resembled an iron cross (IronX) with eight 10-km segments radiating from a central point every 45° and four 7.6-km segments connecting alternate ends. We used sine curves to approximate the wandering path of a collector, theoretically following the route of least resistance (Fig. 2B). For each simulation, the field team walked 112.2 km per IronX. The central point of the random IronX was selected from the area 10 km from the edge of the landscape, to avoid sampling outside the landscape (Fig. 2C). The central point of the targeted IronX was randomly selected from all the piles greater than 10 km from the edge of the landscape (Fig. 2B). In actuality surveyors centered the targeted recces on gorilla carcasses. The number of IronXs per landscape was set to one.

The mixed design replicated the distance sampling design (see above) but added 15 recce

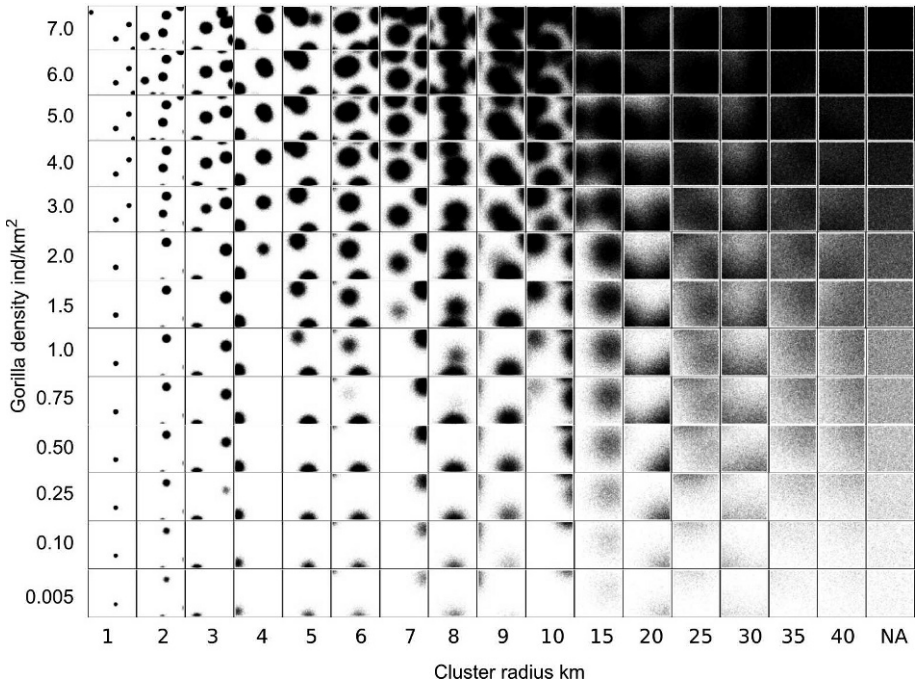

FIGURE 1. Images of each simulated landscape, where each microdot represents one fecal sample. Gorilla populations were clustered at 17 radii ranging from 1 to infinity (NA) and distributed at 13 different densities between 0.005 and 7.0 individuals/km^2.

segments that linked individual line transects with sine curves (Fig. 2D). This design made use of the time the field team spent as they traveled from the end of one line transect to the beginning of the next. The field team traveled 251 km to complete a mixed survey in each landscape.

Full-field fecal sampling simulations

The four fecal survey designs were simulated 15 times on each landscape. The model assumed the collection team detected all fecal samples 1.0 m to the right and left of the sampling path or transect (Kühl et al., 2008). Hence, the total area directly surveyed for the distance, IronX, and mixed designs was 6.4×10^{-2} km^2, 2.2×10^{-1} km^2, and 5.0×10^{-1} km^2, respectively. The rate of field team travel was set to 0.75 km/day for line transects and 4.0 km/day for recces (Maisels and Cameron, pers. comm.). One day of recce sampling was set to 6.5 person-days and 1 day of transect or mixed sampling was set to 8 person-days (Ondzie, pers. comm.). Gorilla density was estimated as the number of fecal samples detected, adjusted for the rate of fecal production and divided by the spatial area surveyed. Mean bias was defined as the mean difference between the known gorilla density and the estimated gorilla density of 15 simulations. Encounter rate was calculated as the number of detections per kilometer, and efficiency was calculated as the number of detections per person-days.

To evaluate the accuracy of estimating density from the IronX at a local scale, we calculated the density of fecal samples within a 10-km radius of its center point (314 km^2). Local bias was determined as the mean difference between the observed and local density. All simulations and analyses were performed using the R package version 2.13.1 (R Development Core Team, 2011).

RESULTS

Number of fecal samples

Clustering of gorilla groups with radii less than 10 km sharply increased the number of samples detected by targeted IronX, but did not greatly affect the number of samples collected by alternative designs. The targeted IronX detected the most samples, followed by the mixed, random IronX, and classic distance designs (Fig. 3A). At the highest expected density

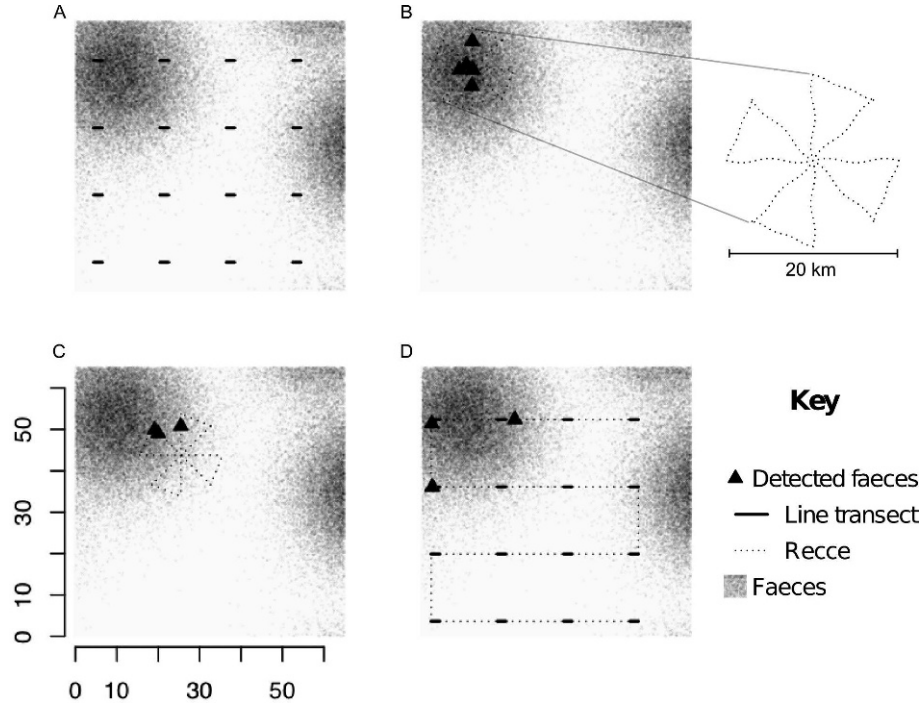

FIGURE 2. Diagrams of the (A) distance line transect, (B) targeted IronX with inset of recce paths, (C) random IronX, and (D) mixed recce and distance line-transect sampling methods. The landscapes are 65 km × 65 km. Dark lines indicate a path that was sampled in a straight line, and dotted lines indicate a path that was sampled along a recce. Feces that were detected during a simulation are indicated with a triangle.

(7.0 individuals/km^2) of a spatially random gorilla population, the mixed strategy detected the largest amount (Table 1). The most samples that classic distance sampling ever detected was 26.

Population density estimates

On average, the line-transect and random IronX designs were the most accurate, or least biased, for estimates of density at different levels of gorilla density and clustering size (Fig. 4). The bias of the mixed design did not vary with density, but tight clustering increased the mean bias. The targeted IronX design, which was centered on a randomly selected fecal pile, always identified at least one sample and overestimated gorilla abundance, especially at high gorilla density and tight clustering. The targeted IronX had the most bias of all designs, both at the landscape (4,225 km^2) and local scale (314 km^2). The performance of the random IronX was similar at the landscape and local scale. With the exception of the mixed design, the density bias of a sampling design increased with increased density and tighter clustering.

Efficiency of sampling teams

Encounter rates (samples per kilometer walked) and sampling efficiency (samples per person-day) were greatest for the targeted IronX and least for the mixed design (Fig. 5). For all sampling designs the encounter rate was directly associated with density and inversely associated with the clustering of gorilla populations. Sampling efficiency trends were similar to encounter rate trends. The encounter rate for distance and the random IronX designs were roughly matched, but the random IronX had a slightly higher sampling efficiency.

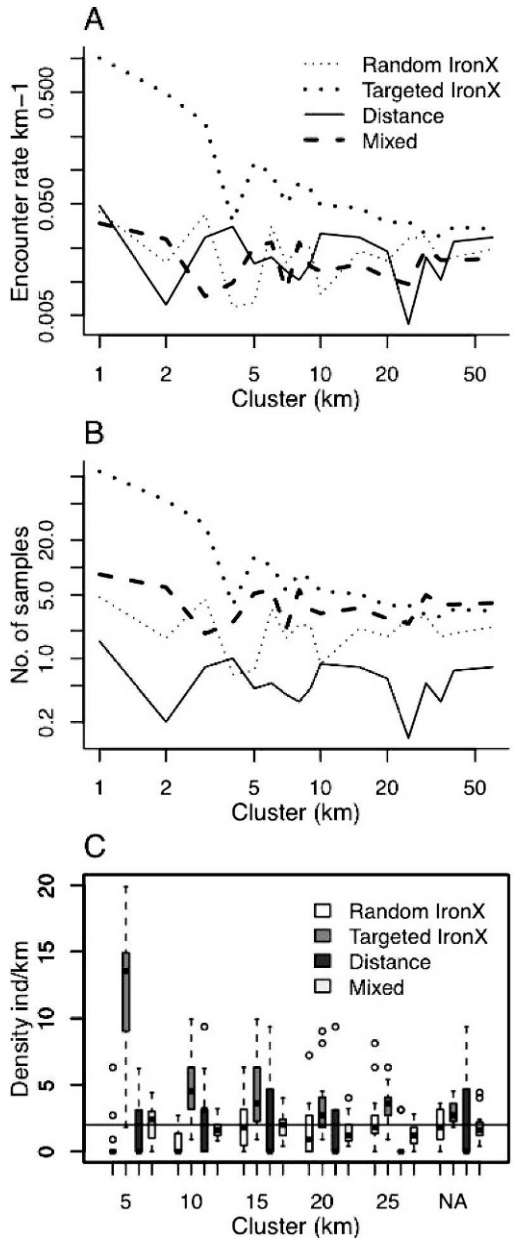

FIGURE 3. Summary of the simulation of four sampling approaches at different levels of clustering within the gorilla population when density is 2.0 individuals/km^2. (A) Encounter rates, or the number of samples detected each kilometer, for each level of clustering and sampling design are plotted. (B) Mean number of samples collected by each sampling approach are plotted at different levels of clustering. (C) A box plot of estimated density is shown with error bars indicating the interquartile range and a thick horizontal bar indicating the median. The horizontal line indicates the true value.

DISCUSSION

We examined the performance of fecal sampling designs for large tropical landscapes using a simple modeling structure and computer simulations. Notably, our study results have yet to be validated on the ground, and therefore the following generalizations should be used with caution. We found that the targeted IronX design detected the greatest number of samples in clustered populations, but it produced the least accurate estimates of gorilla density. Alternatively, we found that distance line-transect estimates of gorilla density were least biased, but detected the fewest number of samples. Finally, a mix design of both recces and distance line-transect sampling held the most promise of optimizing both the number of detections and estimates of population density, but was the least efficient of the four sampling designs as measured by encounter rate or number of samples detected per person-day. These findings suggest that there are no one-size-fits-all solutions for researchers planning fecal sampling at this landscape scale. No single sampling design performs well across all criteria because trade-offs exist between desired sample size, accuracy of the density estimate, and efficiency.

In light of these trade-offs, what approaches maximized fecal sample size and may be useful to improve detection of low-prevalence pathogens? Here we show that a sampling design with recces that link individual line transects may improve sample size and minimize the logistical hassle of transporting teams. For some situations, such as gorilla research, a census approach that intensely samples a specific zone using human trackers and genetic testing may be appropriate (Guschanski et al., 2009; Arandjelovic et al., 2010). Scat detection dogs are another option. Their use is not yet widespread in the tropics but they have been successfully used in temperate regions to collect copious quantities of

TABLE 1. For each sampling design we report the mean number of samples detected, the estimated density, and the encounter rate. A range and standard deviation are also provided for each statistic. This set of simulations was based on a landscape of randomly distributed gorillas set to the highest expected density, 7.0 individuals/km^2.

Design	Mean detected samples (SD)	Range	Estimated density, individuals/km^2 (SD)	Range, individuals/km^2	Encounter rate, km^{-2} (SD)	Range, km^{-2}
Random IronX	7.8 (3.4)	4–15	7.1 (3.1)	3.6–14	0.070 (0.031)	0.036–0.13
Targeted IronX	8.4 (2.3)	5–14	7.6 (2.1)	4.5–13	0.075 (0.021)	0.045–0.12
Line transect	2.4 (1.6)	0–5	7.5 (5.0)	0.0–16	0.075 (0.050)	0.000–0.16
Mixed	15 (5.8)	6–30	7.5 (2.5)	2.4–13	0.062 (0.023)	0.024–0.12

carnivore scat (Smith et al., 2003; Wasser et al., 2004; Michalski et al., 2011). Long et al. (2007) concluded that human searchers found less than one-tenth of the black bear, fisher, and bobcat scat located by detection dogs. Scat detection dogs may translate well into tropical settings, as dogs and handlers typically search transect lengths, specific zones, or grid cells, but important considerations include the cost of training the handler and dogs, boarding and transporting dogs, maintaining the health of the dogs, and ensuring the safety of wildlife (Adams et al., 2003; Smith et al., 2003; Wasser et al., 2004; Michalski et al., 2011).

The main objective is maximizing sample acquisition, but researchers collecting samples for pathogen detection may also want to opportunistically estimate host density. Distance line-transect sampling is the gold-standard sampling design to

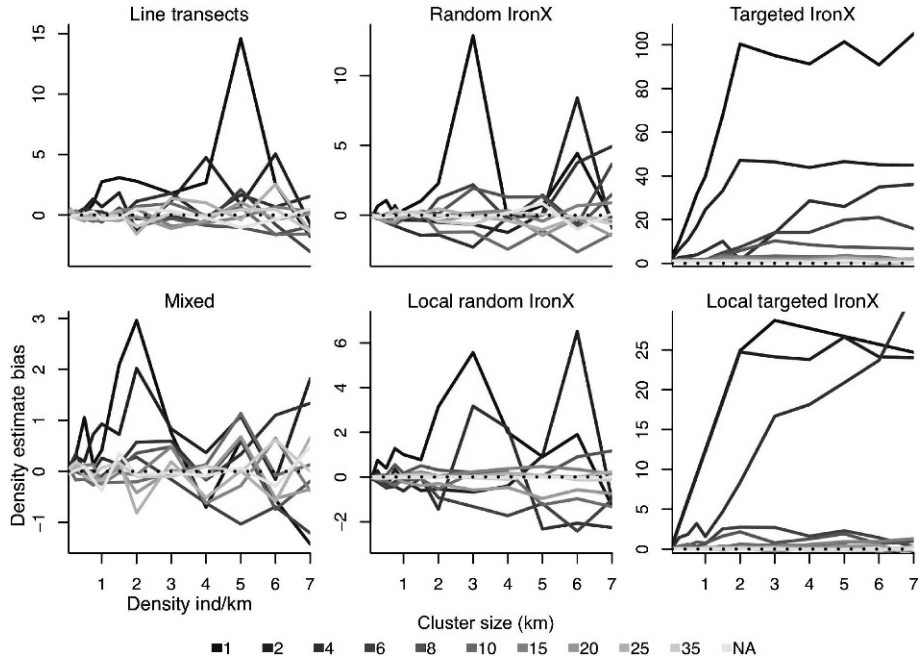

FIGURE 4. Mean density estimate bias for each sampling design at all levels of gorilla density and 11 levels of spatial clustering. The darkest line indicates the accuracy of the sampling method at 1 km of clustering and the lightest line indicates the accuracy of the sampling method with no clustering. The dotted line indicates no bias and as distance from the line increases, the density estimate becomes more biased.

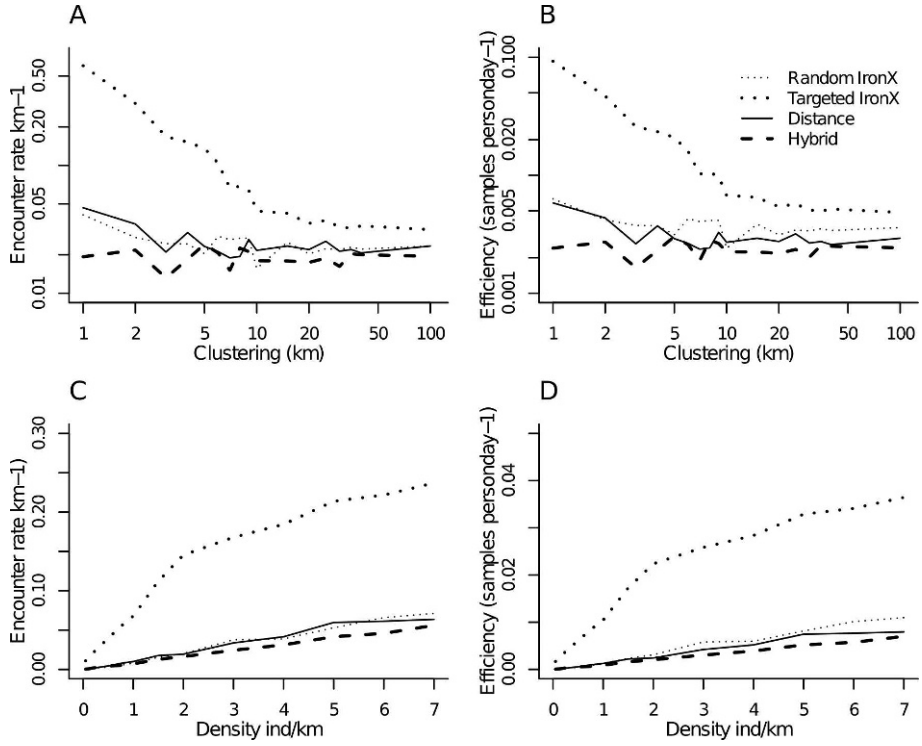

FIGURE 5. Encounter rate (samples/km) and efficiency (samples/person-day) of each sampling design by clustering and density of gorilla populations. The panel shows the relationship of each sampling method with clustering and encounter rate (A), clustering and efficiency (B), density and encounter rate (C), and density and efficiency. A legend is provided in panel B.

estimate species density on large landscapes (Buckland et al., 2001, 2010; Stokes et al., 2010). Except at extreme levels of clustering, line transects performed well when it came to estimating density even though some simulations of gorillas at maximum abundance did not detect a single fecal sample (Kühl et al., 2008; this study); the most number of detections at peak abundance was 26. Notably, Buckland et al. (2001) state that 60–80 detections are necessary to parameterize the modeling component of distance line-transect sampling, suggesting that the number of fecal detections along 32 km of transects is insufficient for traditional line-transect distance-sampling analysis. Increasing the number of transects will increase the number of samples at the expense of cost and time, and elevate the risk of duplicate sampling. Thus, acquiring reasonable density estimates from fecal samples alone is likely wishful thinking.

Our results suggest that utilizing the transect sections to estimate density from fecal counts and the recce sections to maximize sample detection could result in a win-win scenario for pathogen detection. Ideally a team would perform best-practice distance surveillance, including nests and other signs, to acquire a density estimate from design-based inference and use the recce sections to harvest fecal samples (Kühl et al., 2008). The recce sections are not compatible with design-based inference, but calibrating the recces against nearby distance transects with model-based inference may help detect changes in density better than distance transects alone (Kühl et al., 2008; Walsh and White, 1999). In terms of selecting an appropriate sampling scale, as expected, density bias of

IronXs decreased when population was estimated at more local scales.

We can make a few observations on efficiency, but our measures did not encompass numerous field-mission cost considerations. Sample size and encounter rate were roughly correlated with sampling effort, an inverse measure of efficiency, in terms of person-days across all designs. We found, not surprisingly, that acquiring more samples required more sampling effort, which equates to higher costs. Beyond person-days, an ideal measure of sampling effort would incorporate anything altering the overall rate of sample detection independent of population abundance, for example, the logistics of moving between sampling locations, the skill level of the observers, and the duration of the sampling period. Even though the mixed design in our simulations performed poorly on efficiency as measured by samples detected per person-day, the connectivity of the design could offer logistical benefits to field teams that we were unable to quantify. Thus, our simulations offer crude guidelines for evaluating the efficiency of different fecal sampling designs. The comprehensive efficiency of any design should be carefully reviewed on a case-by-case basis.

Fecal sampling and our modeling approach have some additional limitations. Primarily our findings are not based on empirical data nor are they externally validated on the ground. We have only simulated how gorillas are distributed on the landscape and different sampling methods, on the basis of a mix of scientific observations and assumptions. Secondarily, the "known" scientific observations are rife with variability. Dung pile production rate has strong associations with rainfall and feeding behavior and striking differences between age classes, varying from 2–11 piles a day (Todd et al., 2008). Once deposited, climate, environment, and fecal consumers affect sample longevity in the field. All together this variability makes it difficult to compare surveys conducted at different times of the year or between areas with different food sources (Kuehl et al., 2007). Further, only relative comparisons can be made between: 1) our modeling method, which used feces detection, and the density estimates based on nest detection, and 2) our simulation of distance sampling and true distance sampling, which would incorporate sampling probabilities. Finally, the role of spatial design was isolated by assuming perfect detection of piles within 1.0 m of sampling paths and no detection of piles beyond 1.0 m. Our models may not be valid if distance-sampling probability curves of recces and line transects do not align well.

In conclusion, we have assessed the performance of various sampling designs in fecal collection and population density estimation across a range of gorilla densities and spatial distributions. IronXs were considerably better at estimating local density than estimating the density of larger spatial scales, whereas mixed approaches produced accurate estimates of density as well as maximized the number of detections. Line-transect distance sampling detected such a low number of samples that classic distance modeling was untenable. The design of future fecal sampling in low-visibility systems should be tailored to the characteristics of each target population.

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Disclaimer

The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government. The authors declare no competing interests.

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