

## Estimating Population Density of Amazonian Titi Monkeys (*Callicebus discolor*) via Playback Point Counts

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### ABSTRACT

We describe a new method for estimating population density of vocally responsive animals, which is based on playback calls coupled with auditory point transect sampling. The method yielded, for red titi monkeys (*Callicebus discolor*), better estimates than traditional line transect surveys. We propose it as an effective alternative for sampling certain cryptic, but vocally responsive animal species.

Abstract in Spanish is available at <http://www.blackwell-synergy.com/loi/btp>.

*Key words:* abundance; census; distance sampling; Ecuador; point transects; primates; sampling method; vocalization.

ACCURATELY ASSESSING POPULATION DENSITY IS IMPORTANT FOR MANY STUDIES IN ANIMAL ECOLOGY AND CONSERVATION. Distance sampling methods (Buckland *et al.* 2001) are widely used for estimating population density of wildlife in tropical environments. In dense forest habitats, however, where visibility is limited, the population density of elusive or cryptic species might be severely underestimated in surveys that rely on the direct vision of the observer. If a species produces vocal cues, observers can estimate distance via aural detection, a common practice among avian surveys (Simons *et al.* 2009). A low encounter rate, however, can render surveys costly or time consuming, in which case playbacks can be used to elicit a vocal response from animals (*e.g.*, Turcotte & Desrochers 2002). In this study, we describe a new method for estimating population density that uses playback calls combined with point transect sampling (Buckland *et al.* 2001), which should be of broad interest and applicability to tropical biologists working with elusive or cryptic, but vocally responsive animal species. While playbacks have often been used to evaluate species presence/absence in an area and relative abundance (particularly for avian taxa), they have seldom been combined with point transect distance sampling for estimating animal population densities.

Titi monkeys (genus *Callicebus*) are fairly inconspicuous animals, apart from their loud early morning territorial duet calls (Robinson 1981). The cryptic behavior of *Callicebus* might lower the probability of detection during traditional line transect surveys,

the most widely used method for estimating primate population densities (Marshall *et al.* 2008, Buckland *et al.* 2010). In fact, previous surveys using line transects have reported suspected underestimates of density (Freese *et al.* 1982, Price *et al.* 2002), and in some localities, the presence of *Callicebus* could only be assessed via their vocalizations (Freese *et al.* 1982, Chiarello 2000, Moura Martins 2005). Given that titi monkeys display territorial vocal behavior, and respond vocally to playbacks performed within their home ranges (Robinson 1981), playbacks can be used to increase encounter rates during population surveys.

We conducted the study at the Tiputini Biodiversity Station (TBS, 0° S 76° W), a 650 ha study site located within a large, contiguous expanse of primary Amazonian rain forest in Ecuador, where a long-term comparative study of the behavioral ecology of socially monogamous primates is being conducted (Carrillo-Bilbao *et al.* 2005, Di Fiore *et al.* 2007, Fernandez-Duque *et al.* 2008). We conducted 48 point transect samples along the existing TBS trail system during 8 d of July 2007 (Fig. 1). Playbacks were conducted between 0600 and 1700 h. The locations of consecutive point transects were spaced 150–300 m apart, to maximize the number of potential titi monkey territories covered. Once at a point transect, the observer (A. Dacier) placed a portable speaker (Anchor-Audio PB-25 MINIVOX) on a tree branch at a height of 1.5–2 m, hid behind suitable vegetation, and started a point playback session. During each session, we played back four 2-min titi duet calls, separated by —2–3 min intervals, at a standardized sound pressure of 100 dB measured at one meter. This protocol had previously been successful in stimulating vocal responses and in locating titi groups at the site (A. Di Fiore & E. Fernandez-Duque, unpubl.

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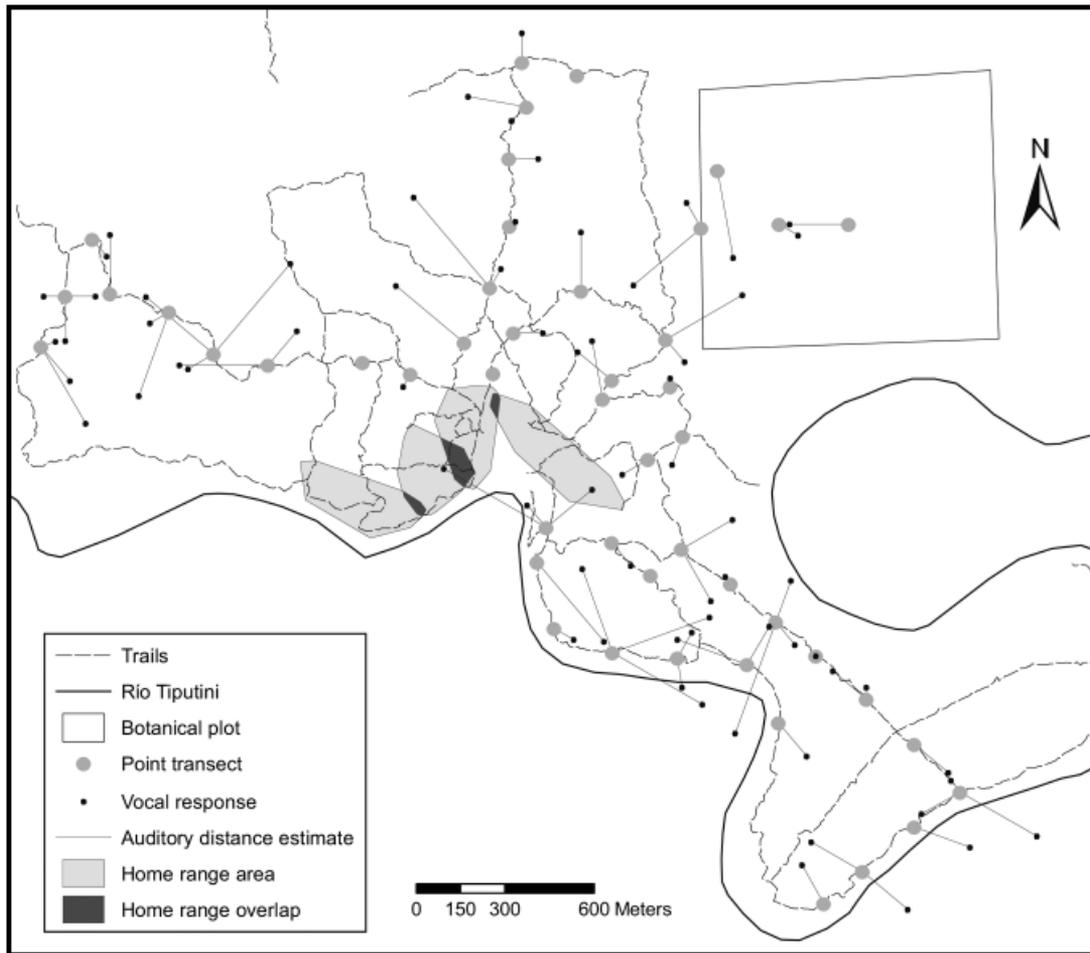


FIGURE 1. Map of the Tiputini Biodiversity Station study site showing the locations of point transects at which vocalizations of *Callicebus discolor* were played back and the locations of responding groups, based on auditory estimation of distance. The map also shows the home ranges of four radio-collared *Callicebus* groups, which were used to derive an independent estimate of titi population density.

data). As soon as a group responded, the group's location was estimated based on aural detection, with distances being rounded to the nearest 10 m within the first 50 m radius, the nearest 25 m for distances between 50 and 100 m, and the nearest 50 m for distances  $> 100$  m. The observer had practiced estimating distances at the site during a 1-mo pilot playback experiment, in which the estimated distance was confirmed by subsequently locating the calling group. The duet call used in playbacks had been recorded from a single group within the same *Callicebus discolor* population, but all point transect playback sites were located outside of the home range of this group (Fig. 1). The program Distance v. 5.0 (Thomas *et al.* 2010) was used to estimate population density via statistical modeling of the generalized 'detection function'  $g(r)$ , which estimates the probability of an object being detected as a function of its distance  $r$  from the sample point. Response distances were grouped in the analyses to account for rounding of the data. The most appropriate model for the detection function was selected based on Akaike's Information Criterion (AIC).

We recorded a total of 71 vocal responses (mean  $\pm$  SD per point transect:  $1.5 \pm 0.4$ , range: 0–3; Fig. 1), which were emitted

approximately in equal frequencies throughout the survey hours ( $\chi^2 = 2.218$ ,  $df = 9$ ,  $P = 0.99$ ). All vocal responses were given by at least a pair of calling individuals, except on one occasion in which the response was given by a solitary animal. Given the distribution of point transects, it is possible that a single group was recorded twice from two adjacent points, but never from the same point. Although recording the same group from two different points is a violation of statistical independence, it usually results in small bias effects and has been empirically demonstrated to be of 'little practical consequence' (Buckland *et al.* 2001, p. 176). The model chosen according to AIC was based on a hazard-rate key function (AIC = 324.67). Models based on a half-normal key with cosine and a uniform key with cosine adjustments had only slightly higher AICs (325.19 and 325.55, respectively), and all three models gave similar estimates of population density relative to the 95% CI. The hazard-rate function generates a broad shoulder that in this case extends to approximately 50 m (Fig. 2), which accurately reflects a high probability that a group will respond to a playback if it is done close to a group's current location (*i.e.*, within 40 m; Robinson 1981). Based on the derived detection function, we estimated the

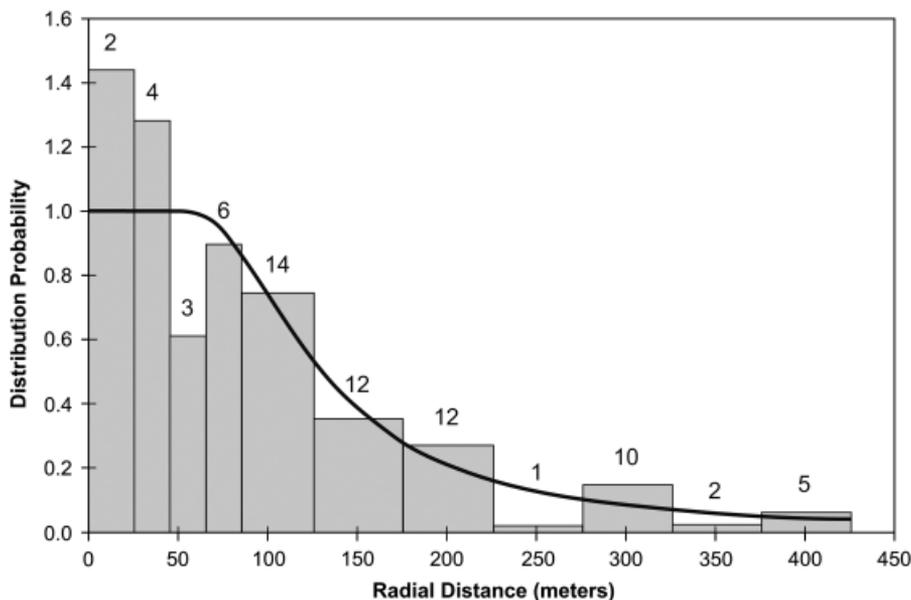


FIGURE 2. Detection distances for *Callicebus* groups based on vocal response to playbacks and the fitted model for the detection function  $g(r)$  using a hazard-rate key function, as selected by minimum Akaike's Information Criterion. Histogram frequencies are scaled by dividing the number of detections in each class interval (noted above the bars) by the midpoint of the corresponding distance interval (see Buckland *et al.* 2001). Data were grouped so as to account for rounding in auditory detection.

population density of *C. discolor* as  $13.6 \pm 3.7$  groups/km<sup>2</sup>, with a 95% CI of 8–23 groups/km<sup>2</sup>, and a coefficient of variation of 27 percent. Based on our long-term, simultaneous study of four radio-collared titi groups at the study site, the average number of individuals per group is  $3.5 \pm 0.6$ , yielding a density estimate of  $47.6 \pm 15.3$  individuals/km<sup>2</sup>.

In order to evaluate the accuracy of the playback point count method, we also estimated density based on home range size, which is generally considered the most accurate method for estimating wild primate population densities (Whitesides *et al.* 1988, Brugiere & Fleury 2000, Fashing & Cords 2000, Hassel-Finnegan *et al.* 2008). Home ranges (Fig. 1) and range size estimates were derived using the minimum convex polygon method implemented in ArcGIS 9.2, based on 100 mo of ranging data pertaining to four different radio-collared groups of *C. discolor* at the site. Ranging location records were collected at 20-min intervals during group follows on multiple days each month, and each group contributed at least 20 consecutive months of locations to the home range size data set. A total of 4755 group location records were collected, with group K being followed for 275 d, resulting in 3467 data points, group L for 80 d yielding 671 data points, group H for 74 d yielding 289 data points, and group B for 70 d yielding 328 data points. To account for home range overlap between groups, we divided the area of overlap by the number of groups sharing that area, and then added the results to the nonoverlap home range area of individual groups. We estimated density by dividing the average of the readjusted home range size of the four groups by the average number of individuals within the four groups ( $3.5 \pm 0.6$ ). The average home range size of *C. discolor* at TBS was estimated as  $6.1 \pm 0.8$  ha, yielding

a population density estimate of  $16.4 \pm 2.2$  groups/km<sup>2</sup> and  $57.4 \pm 12.4$  ind/km<sup>2</sup>.

Our playback point counts yielded density estimates similar to those obtained using the home range size method (Table 1). On the other hand, line transect surveys previously conducted at the same site (Derby 2008) estimated the density of titi monkeys at only 4.6 groups/km<sup>2</sup>, which is more than three times lower than our home range size method estimate (Table 1). We believe that our estimates based on home range size should be fairly accurate, assuming that the pattern of space occupancy by our radio-collared study groups is representative of the population in the study area at large. If so, playback point counts yielded a demonstrably better estimate of true population density than traditional line transect surveys, while also being considerably less time consuming than either the home range or traditional line transect survey methods. Our density estimates are also much higher than estimates from other *Callicebus* studies that used line transect methods (Table 1). Although this result may be reflecting true differences among localities, we think it is more likely an artifact of the line transect method combined with the cryptic behavior of titi monkeys.

Although the method we describe here is based on distance sampling theory, some of the assumptions of distance sampling were violated during this survey (Buckland *et al.* 2001). For example, although point transect samples covered the area thoroughly, they were done along existing trails at the site rather than distributed systematically. Thus, if animals differentially avoided or were drawn to trails, it could result in downward or upward density estimates, respectively. We found no association, however, between the locations of contact with titi monkey groups and distance from

Table 1. Population density estimates of *Callicebus* based on various methods.

Species <sup>a</sup>	Method	Groups/km <sup>2</sup>	Ind/km <sup>2</sup>	Study area <sup>b</sup>	Reference
Amazonian species					
<i>Callicebus discolor</i>	Playback point transect	13.6 ± 3.7	47.6 ± 15.3	TBS, Ecuador	This study
	Home range	16.4 ± 2.2	57.4 ± 12.4		This study
	Line transect	4.6	10.8		Derby (2008)
	Line transect	2.5	7.0	Alto Itaya River, Peru	Aquino <i>et al.</i> (2009)
<i>Callicebus cupreus</i>	Line transect	3.9	14.6	Trapiche River, Peru	Bennet <i>et al.</i> (2001)
	Line transect	—	1.1–30.1	Jurua River, Brazil ( <i>N</i> = 14)	Peres (1997)
<i>Callicebus brunneus</i>	Home range	~40	—	Los Amigos, Peru ( <i>N</i> = 5 groups)	Lawrence (2007)
	Line transect	0.6–5.4	1.8–16.2	Various localities, Bolivia and Peru ( <i>N</i> = 7)	Freese <i>et al.</i> (1982) <sup>c</sup>
<i>Callicebus oenanthe</i>	Auditory triangulation method	—	113	Tarangue, Peru	Aldrich <i>et al.</i> (2008)
<i>Callicebus torquatus</i>	Line transect	0.8–1.2	2.5–2.8	Alto Itaya and Algodon Rivers, Peru ( <i>N</i> = 9)	Aquino <i>et al.</i> (2008, 2009)
	Line transect	0.5–3.8	2.0–15.2	Various localities, Peru ( <i>N</i> = 2)	Freese <i>et al.</i> (1982)
<i>Callicebus purinus</i>	Line transect	—	5.8–10.2	Jurua River, Brazil ( <i>N</i> = 2)	Peres (1997)
<i>Callicebus regulus</i>	Line transect	—	2.8–7.2	Jurua River, Brazil ( <i>N</i> = 4)	Peres (1997)
<i>Callicebus lugens</i>	Line transect	3.5 (min)	-	El Tuparro, Colombia	Defler and Pintor (1985) <sup>e</sup>
		7.1 (max) <sup>d</sup>			
Atlantic Forest species					
<i>Callicebus personatus</i>	Home range	10	34.4	Espirito Santo, Brazil	Price <i>et al.</i> (2002)
<i>Callicebus personatus</i>	Line transect	3.7	12.6	Espirito Santo, Brazil	Price <i>et al.</i> (2002)
	Line transect	—	1.3–10.2	Espirito Santo, Brazil ( <i>N</i> = 4)	Chiarello (2000)
<i>Callicebus nigrifrons</i>	Line transect	—	12.2	Sao Paulo, Brazil	Trevelin <i>et al.</i> (2007)
	Line transect	—	3.5	Sao Paulo, Brazil	Bernardo and Galetti (2004)
	Line transect	—	14.9	Minas Gerais, Brazil	Oliveira <i>et al.</i> (2003)
<i>Callicebus melanochir</i>	Line transect	—	17.0	Bahia, Brazil	Müller (1995)

<sup>a</sup>Taxonomic assignment following van Roosmalen *et al.* (2002). When subspecies was not provided in the original study, we have converted taxonomic synonyms based on the study locality and the current species distribution as described by van Roosmalen *et al.* (2002) and the IUCN red list web site: <http://www.iucnredlist.org>

<sup>b</sup>Where density ranges are shown, *N* indicates the number of localities sampled; otherwise, *N* = 1.

<sup>c</sup>Species referred in the study as *Callicebus moloch*.

<sup>d</sup>Authors used three different methods to estimate density from distance data.

<sup>e</sup>Species referred in the study as *Callicebus torquatus*.

the nearest trail (data not shown), suggesting that titi ranging behavior is unlikely to be affected by the position of trails. Furthermore, observer–animal distances were rounded during surveys due to the intrinsic uncertainties associated with estimating distances based on auditory detection (Alldredge *et al.* 2007, Simons *et al.* 2009). Ideally, distances should be exact, without measurement errors or rounding in the data. Still, the effects of rounding can be mitigated by proper treatment of the data during analyses, making reliable estimates of density possible (Buckland *et al.* 2001). In our analysis, data were grouped to accommodate for rounding, such that bin intervals would encompass the error around distance estimates.

Finally, a more serious violation may have been the detection of an animal after its possible movement in response to the playback. In distance sampling, animals should be recorded at their ini-

tial locations, before any movement in response to the observer (Buckland *et al.* 2001). If animals consistently move away from or approach the point transect before detection, this could result in a downward or upward bias in estimates, respectively. Titi monkeys can move in response to playbacks, sometimes approaching the speaker before they respond vocally, which could result in an upward bias in the density estimate. During pilot playback experiments at the site (A. Di Fiore & E. Fernandez-Duque, unpubl. data), the distance traveled by the group in response to the playback was never large when the group was initially far from the source of playback (*e.g.*, > 75 m), although the groups tended to move more when they were closer to the playback source. Thus, it is possible that animal movement resulted in a downshift of the original location of groups, in particular for closer detection distances for which smaller bin intervals were chosen (*i.e.*, detections within 85 m of the

point transect; Fig. 2). The larger bin intervals chosen for detection distances farther from the point (85 m onwards; Fig. 2) should still accommodate the groups' original location, even if they moved slightly in response to the playback before being detected. Furthermore, it is possible that midrange values for each bin, which are more likely to represent the original location of groups, had a higher influence in determining the shape of the detection function (*i.e.*, the function is 'locked' by midrange values, L. Thomas, pers. comm.). Because of these reasons, we suggest that animal movement postplayback and before detection could only have had a small effect on our density estimate, although we cannot quantify the magnitude of such an effect precisely.

Although our results seem promising regarding the advantages of playback point counts for particular taxonomic groups, several factors should be considered before choosing this method for surveying cryptic species. First, whenever possible, a systematic layout of the point transects is preferable to following pre-existing trails. Second, the method will produce better results if animals do not move significantly before emitting vocalizations in response to the playback. Finally, issues related to estimating distance based on auditory cues should also be taken into consideration. One way the method could be improved considerably is by conducting a parallel study that independently estimates the probability of vocal responses to playbacks and thus the effective transect radius. Buckland *et al.* (2006) describe a method that uses field experiments alongside point transects to estimate a transect's effective radius, which is the distance  $\rho$  from the point for which as many objects are detected beyond  $\rho$  as are missed within it. Because the effective radius is estimated independently, one simply needs to record the number of responses that are given at each point transect in order to estimate density, avoiding the potential problems of animal movement and errors associated with auditory detection.

Developing consistent and reliable methods for estimating population density is pivotal for many studies in animal ecology, as well as for efficient population assessment and allocation of conservation efforts. The density of elusive or cryptic species that live in dense forest habitats may be severely underestimated in traditional distance sampling based on line transects. Complementary approaches that use playback calls can be valuable tools for sampling animal populations across different taxa.

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