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Short communication

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Abstract:

Wildlife species population density estimation is important from both ecological and management perspectives. The pellet-group counting method has been used to evaluate density of the white-tailed deer (*Odocoileus virginianus*). This species is an important component of human diet in tropical habitats. The objectives of this study were to: 1) compare density estimates using four methods: counts in circular plots (FSC, Fecal Standing Crop and FAR, Fecal Accumulation Rate), and counts in transects (LT, Line Transect and ST, Strip Transect); 2) simulate the effect of increased sampling effort on density and precision, and; 3) evaluate the effort required to detect changes in population density using LT. From 2006 to 2007, we intensively sampled a 1 km^2 quadrant in a Mexican tropical dry forest. The results indicate that all four methods produce similar mean population density estimates. However, estimates of precision were dependent on sample size which in turn was associated with the particular counting method used. In descending order of estimate precision, the methods ranked as: LT, ST, FSC, and FAR. To detect population changes of < 20%, we suggest the establishment of 5 to 22 transects (LT) of 390 m during the dry season. To reduce bias in density estimation, it is important to obtain defecation and pellet decomposition rates in the study site.

Key words: fecal counts; sampling methods; density; precision; *Odocoileus virginianus.*

Resumen:

Desde una perspectiva ecológica y de manejo es necesario estimar la densidad poblacional de la fauna. El método de conteo de grupos fecales es empleado frecuentemente para estimar la densidad de muchas especies incluido el venado cola blanca (*Odocoileus virginianus*). Este cérvido es importante en la cacería de subsistencia en regiones tropicales. Los objetivos del estudio fueron: 1) comparar las estimaciones de densidad empleando cuatro métodos: conteos en parcelas circulares (FSC, "Fecal Standing Crop" y FAR, "Fecal Accumulation Rate"), y conteos en transectos (LT, "Line Transect" y ST, "Strip Transect"); 2) simular el efecto de aumentar el esfuerzo de muestreo sobre la densidad y su precisión, y 3) evaluar el esfuerzo requerido para detectar cambios en la densidad empleando LT. De 2006 a 2007, monitoreamos intensivamente un cuadrante de 1 km^2 localizado en un bosque tropical seco del centro de México. Encontramos que los cuatro métodos proporcionan estimaciones poblacionales similares. Sin embargo, la precisión de las estimaciones dependió del tamaño de muestra, el cual a su vez fue determinado por el método de conteo utilizado. Así, LT produjo la estimación de densidad más precisa, seguido por ST, FSC y finalmente FAR. Para detectar cambios poblacionales < 20%, sugerimos poner 5-22 transectos (LT) de 390 m de longitud durante la época seca. Para reducir el sesgo de la estimación es importante obtener las tasas de defecación y de descomposición de los excrementos en el área de estudio.

Palabras clave: grupos fecales; métodos de muestreo; densidad; precisión; *Odocoileus virginianus.*

Introduction

Efficient and accurate population density estimates of wildlife species are an important aspect of management planning [1]. In areas where direct observation of animals is difficult, due to either low local abundance of the species, secretive behavior, low visibility, or human disturbance, the use of indirect methods such as the quantification of tracks and feces (pellet groups) can be more appropriate [2,3]. Pellet-group counting methods have been assessed to accurately evaluate the population density of both old and new world ungulates, lagomorphs, and elephants [e.g., 4-9]. In the field, pellet groups have been quantified in sampling units (e.g., circular plots, strip transects, ST) and for estimating decay rate (FSC, Fecal Standing Crop), or in re-sampling the same plots two or more times (FAR, Fecal Accumulation Rate) [10]. However, these methods do not take feces located outside the sampling units into account, and this can reduce the sampling size and affect the accuracy of density estimation, particularly in those sites with low abundance of animals. As an alternative, the line transect (LT) method has been applied to count pellet groups, and is a technique that has been shown to achieve an increase in precision of density estimates [11,12].

Besides its ecological roles as herbivore, seed disperser, and prey [13], the white-tailed deer (*Odocoileus virginianus*) is an important component of human diet in the tropical habitats of Mexico, Central, and South America [14-16]. Due to the consequent interest in the management of natural populations of this species, accurate and precise estimates of population density are required. The aim of this study was to intensively sample a 1 km^2 area in order to identify the most appropriate pellet-group counting method for white-tailed deer in a tropical dry forest. Specifically, we 1) compared density estimates using four methods: counts in circular plots (FSC and FAR), and counts in transects (LT and ST); 2) simulated the effect of increased sampling effort on precision; and 3) evaluated the effort required to detect changes in population density using the LT method. The parametric value of density was unknown and therefore in this study we did not estimate the bias of each method.

Methods

The study was carried out in central Mexico, in the Mixteca region located in the southern part of Puebla state (18° 12' N and 98° 46' W; Fig. 1). The topography of the region is characterized by numerous hills and narrow valleys of elevations ranging from 600 to 2750 m. The mean annual precipitation and temperature are 817 mm and 21°C, respectively. The climate is warm sub-humid with rains in summer, and the predominant vegetation is seasonal-dry tropical forest. Estimates of white-tailed deer density (1 to 7 ind/km^2) had been previously obtained in several locations in this region [17,18]. According to the findings of these studies, we selected a specific location (Jolalpan, Fig. 1) with the highest deer density in order to intensively sample pellet groups within a 1 km^2 experimental plot. Habitat characteristics within this plot are relatively homogenous: the dominant vegetation is tropical dry forest with a well-developed understory that provides food and shelter to the deer, while the terrain is very irregular with pronounced slopes. As is normally the case throughout this region, the principal human economic activity is livestock production. Conversely, hunting activity is low in this location.
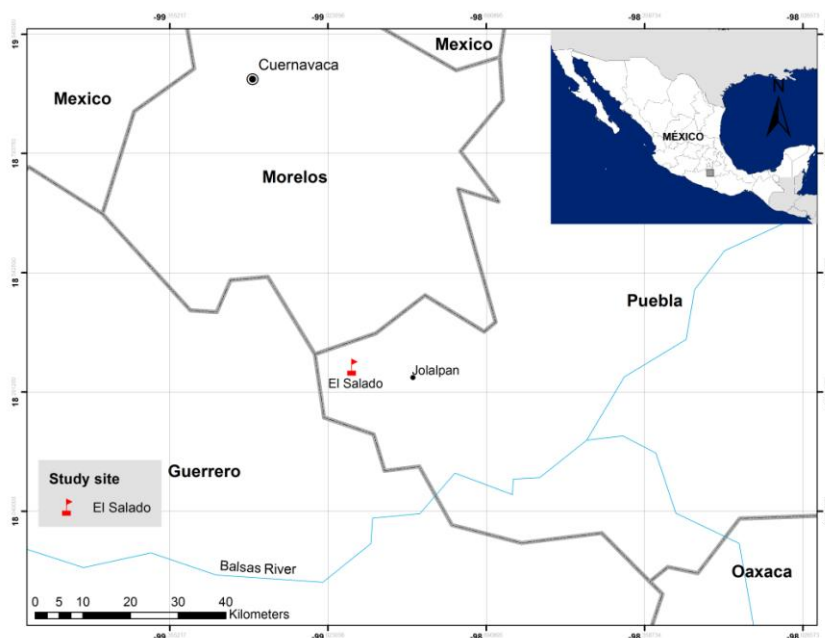
Fig. 1. Geographic location of the study site (El Salado, Jolalpan) in the southern part of Puebla State, Mexico.

In May of 2006, we selected a random point location at which to establish a set of five parallel 1 km long transects, evenly spaced 250 m apart. These were used to apply the four quantification methods: FSC, FAR, ST, and LT. In the case of FSC and FAR, we marked 100 circular plots each of 9.3 m^2 along each transect, spaced every 10 m (center to center) and counted all pellet groups within each plot. For ST, we defined a width of 1 m along the length of transect, considering this width to be adequate to census complete pellet groups in the dense vegetation present. For LT, we measured the perpendicular distance from the transect line to every fecal group observed along the length of transect. Particular attention was paid to meeting the assumptions of this method [19]. In May 2007, we repeated the process in the same transects and plots as recorded the previous year. Following this methodology, we obtained two snapshots for the FSC, ST, and LT

methods. Since the FAR method employs an estimate of pellet group accumulation between two points in time, we identified and marked the pellet groups in the circular plots in January 2007 in order to quantify the new groups found after 60 and 120 days. The surveys were carried out by three to five people; to reduce observer bias, the same observer (ACS) counted the pellet groups for each treatment in each year. During this survey, we recorded the time necessary to count pellet groups in both the circular and transect methods.

To estimate population density (D, individuals km^{-2}), we applied the equation proposed by Eberhardt and Van Etten [20] as: $D = (NP \times D_{pg})/(T \times dR)$, where NP = number of plots (circular or strip transects) per square kilometer, D_{pg} = mean pellet groups; T = either deposition time of fecal groups in the case of FAR, or mean decay time in FSC; and dR = defecation rate. In the case of FSC and FAR, D_{pg} was estimated considering each circular plot as a sampled unit. To estimate D_{pg} with the ST method, we used the equation: $D_{pg} = n/2Lw$, where n = number of fecal groups, w = transect width (1 m) and L = total transect length sampled. In this case, the standard error (Se) was estimated using each individual transect as a replicate. For the LT method, we used the equation: $D_{pg} = nf(0)/2L$, where f(0) = probability density function at 0 m. We used the DISTANCE 5.0 software to estimate D_{pg}, f(0) and Se [21]. We used likelihood ratio tests and Akaike's Information Criterion test as objective and quantitative methods for model selection (Uniform + cos) using non-truncated and truncated (5% and 10%) data.

For each method, we estimated the annual variance of density (D) according to Plumptre [22] as: $Var(D) = [(NP \times D_{pg})/(T \times dR)]^2 \times [(CV(D_{pg}))^2 + (CV(dR))^2 + (CV(T))^2]$. The estimates of $CV(D_{pg})$ for FSC and FAR were calculated following a negative binomial distribution to estimate Se with the equation: $Se = \sqrt{[(x + x^2)/(k/n)]}$, where k = parameter of the binomial negative, and n = number of plots; also $k = D_{pg}^2/(S^2 - D_{pg})$, and S^2 = variance. For the ST method, Var(D) was estimated using each individual transect as a replicate, while we used the DISTANCE 5.0 software [21] for the LT method. Estimation of CV(dR) was carried out according to a previously estimated defecation rate using tame deer close to the same study region, as the mean of 17 ± 4 (Se) fecal groups/individual/day [23]. Finally, CV(T) was first estimated for pellet decomposition rate by marking 50 freshly deposited pellet groups and revisiting these until their total decomposition. A Z-test was used to test whether the two density estimates differ utilizing the same method in different years, using the equation: $Z = (D_2 - D_1)/\sqrt{[Se(D_2)^2 + Se(D_1)^2]}$; while two-way ANOVA was used to test density estimates between methods and years.

To assess the effect of sampling effort (number and length of transects) on density precision, we re-sampled the database obtained in May 2006 with a code written in R [24]. We simulated (N = 100 replicates) sampling from one to 10 transects of 390 m and from one to five transects of 790 m in length. The simulation transects were placed randomly along the 5 km of field sampled transects. To determine the transect length (L) necessary in order to detect changes in population density using the LT method in particular, we followed Buckland et al. [19] as: $L = L_0 \times CV(D)^2 / CV_t(D)^2$, where L_0 and CV(D) are the transect length and coefficient of variation of density of the pilot study, respectively, while $CV_t(D)$ is the selected coefficient of variation of population density (10%, 20%, and 30%). We estimated the required L, considering data from 2006 and 2007 as a pilot study.

Results

In May 2006, we counted 71, 135, and 326 fecal groups with the FSC, ST, and LT methods, respectively, while in May 2007, 48, 101, and 293 fecal groups were recorded. In May 2007, 21 and 48 fecal groups were quantified with the FAR method at 60 and 120 days of accumulation, respectively. Pellet decomposition rate was estimated as 123 ± 2.4 days.

As expected, the number of pellet-groups counted decay with the increase in perpendicular distance from the center of the transect (Fig. 2). According to LT, detectability probability must to be 1.0 at 0 m, but our observations indicate that complete observation of pellet groups was obtained between 0 to 1.0 m of perpendicular distance.
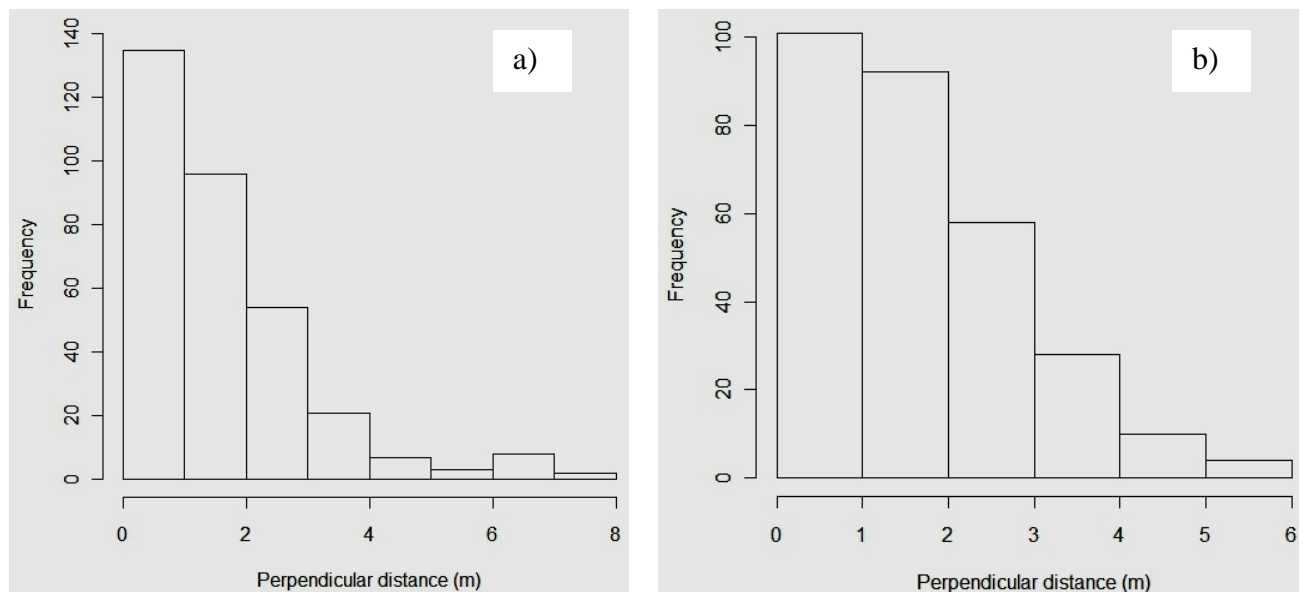
Fig. 2. Histograms of the frequency of counted pellets-groups at different perpendicular distances in the LT method 2006 (a) and 2007 (b). 100% of the pellet-groups between 0 and 1 m were counted in the ST method.

According to data from January 2007, the mean sampling time per 500 m long transect to count pellet-groups in circular plots was 1.48 h (range 1.08 to 2.34 h), while in transects (LT) this was significantly different (Wilcoxon test, W = 48.5, P = 0.03) at 2.14 h (range 0.42 to 3.0 h). Thus, sampling in the transects was 1.2 times more time-consuming than in circular plots; however, the number of pellet groups counted was 6.6 times greater in the transects than in the circular plots (Fig. 3).

In 2006, the population density estimates were statistically similar between the FSC, ST, and LT methods (Appendix 1, F = 0.146, df = 2, 12, P = 0.87); and also in 2007, when the FAR estimation was included (F = 21.35, df = 4, 20, P = 0.11). Mean density estimates were similar between years in each method (paired Z-test, P < 0.05).

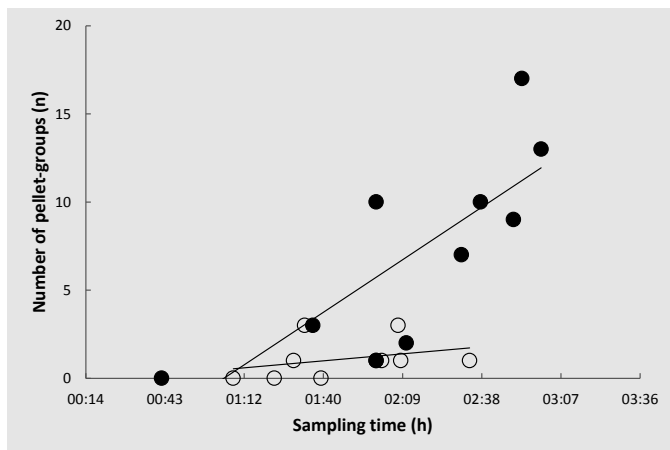**Fig. 3. Relationships between sampling time and number of pellet-groups counted in transects (●) and circular plots (○) during January 2007. Sampling time is given considering a 500 m transect.**
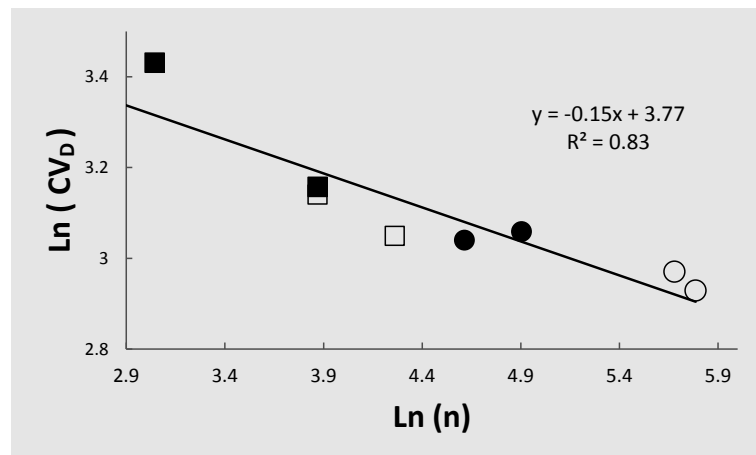
Fig. 4. Relationship between simple size (number of pellet groups counted, n) and coefficient of variation (%) of the population density (CV_D). Sampling pellet groups in FAR (■), FSC (□), ST (●) and LT (○) methods. The line represents the best-fitted linear model (coefficient of determination, R^2).
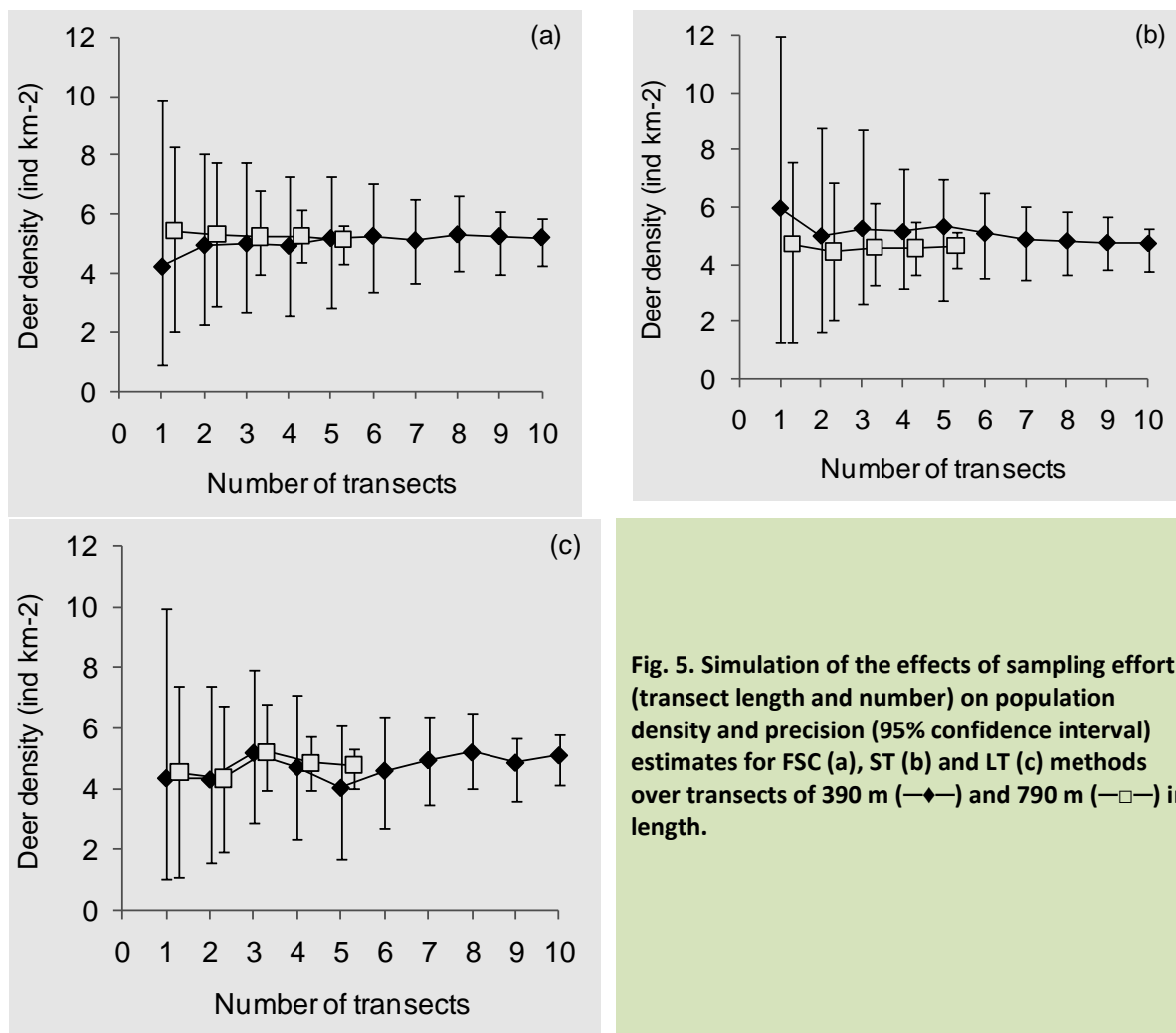
The precision of the density estimates was dependent of the number of pellet groups counted (Fig. 4; $r^2 = 0.83$; F = 28. 4; df = 6; P = 0.002). The methods were ranked in descending order of estimate precision as: LT, ST, FSC, and FAR. .

The results of the simulations indicate that mean density did not vary as the number and length of transects increased in the FSC, ST, and LT methods (Fig. 5). In contrast, the precision of the estimates was found to improve with increased sampling effort. In the case of the LT method, estimates of transect length necessary to achieve 10%, 20%, and 30% of precision (CV%) were: 8.5, 2.1, and 0.9 km respectively, based on the data from 2006, while for 2007 these were 33.8, 8.5, and 3.8 km.

Discussion

Although fecal pellet counts have been widely used to index changes in deer abundance in forests, few studies have modeled the relationship between such indices and deer density [25]. For example, Forsyth *et al*. [26] examined the relationships between 3 fecal pellet indices (total pellets, pellet groups, and pellet frequency) and the density of red deer (*Cervus elaphus scoticus*), and other species, using 4 models (1 linear and 3 nonlinear) to describe the relationships between the indices and deer density. They found that the 4 models explained the relationships between the 3 indices and deer density similarly well, and the slopes of the linear relationships were positive. Therefore, they concluded that fecal pellet counts may be useful as indices of deer abundance. However, two crucial factors are required to convert these indices of deer abundance into population density: pellet decomposition rate and daily defecation rate. Using the FAR method, the deposition time of pellet groups is known; while in FSC, an estimate of the decomposition rate is required. Thus, a recommendation is to calculate this rate at each location where specific climatic conditions, principally precipitation, vary [27]. In general, fecal groups must

be quantified during the dry season when the decay rate of feces is lower in comparison to that under wet season conditions.

Fig. 5. Simulation of the effects of sampling effort (transect length and number) on population density and precision (95% confidence interval) estimates for FSC (a), ST (b) and LT (c) methods over transects of 390 m (—♦—) and 790 m (—□—) in length.

To convert counts of pellet groups to population density, a defecation rate of 12.7 was initially used [20]. However, some other estimates have been: 13.7 [28], 19.6 (CV = 11.7) [29], 26.9 [30], and 34.0 [31] in the United States; 15.2 [32], 17.0 (CV = 9.6) [23], and 20.9 [33] in Mexico; and 14.0 in Venezuela [34]. The defecation rate varied depending on factors such as age, sex, and food quality. In particular, some data suggest that defecation rates are higher in wild deer in comparison with domesticated individuals [29,31]. In consequence, the defecation rate is a crucial parameter in the conversion of pellet groups to population density. In our study, we used the observed defecation rate in tame deer of the same subspecies close to the studied region; but, bias in population density exists in our estimates. We tried to reduce this bias by incorporating the variation of defecation rate. However, it is necessary to estimate the actual defecation rate in the studied site, which is data not easily obtained in wild animals.

Our results indicate that the four methods give similar mean population density estimates. However, in terms of precision, the results suggest that the LT is the most suitable method for fecal group quantification in terms of obtaining more precise density estimations of white-tailed

deer in this tropical habitat. Marques *et al.* [10] suggested that, in sites of low population abundance where a high number of plots would be required to obtain estimates with appropriate levels of precision, it is probable that the LT method would provide a better cost-benefit relationship. Studies that compare the LT with other methods have reached similar conclusions [e.g.*,* 35,36]. The LT method offers more advantages than ST, FAR, and FSC (Appendix 2). Violation of assumptions is a critical factor for selecting a particular field procedure [37]. Methods that used a fixed surface (circular plots and strip transects) seem to be less appropriate in tropical dense forests. As plot size increases there is greater possibility of violating the crucial assumption: all animals or signs, such as feces or tracks, within the sampling areas must be counted (detectability must be equal to 1). Use of small plots seems to reduce this effect, but the low sample size obtained reduces the precision of the density estimation.

In contrast, the robustness of LT assumptions provides a better method for counting pellet groups in this type of habitat. However, very long transect lengths are necessary in order to reach a very precise density estimate (CV < 10%), a factor which could consume more sampling time in comparison to the ST and FSC methods. Although transect length is largely dependent on topographic, vegetation cover, and other conditions, Marques *et al*. [10] suggest that a high number of shorter lines are preferable to a smaller number of longer lines, since the former gives a better measure of spatial variability. To detect population changes of < 20%, we suggest placing from 5-22 transects of 390 m, depending on the size of the study area. An important consideration in the selection of a specific method is the investment of time in sampling. This is particularly important for wildlife management where constrictions exist in terms of time and finance. In our study, counting pellet groups in circular and LT in one transect of 500 m produced sampling times of 1.48 h and 2.14 h, respectively. However, in irregular terrain with severe slopes and dense cover, such as are found in many tropical dry forests, movement between transects was time-consuming and resulted in increased sampling costs. Sampling in line or strip transects increased the number of pellet-groups recorded. As expected, our results suggest that precision improves with an increase in sampling effort, and this concurs with the findings of previous studies [e.g., 10,35].

Implications for conservation

The white-tailed deer is not considered an endangered species by the IUCN [38]; however, local extinctions as a result of over-hunting and habitat loss are common in many sites in the Neotropics [39]. Of the 38 recognized subspecies, at least 20 have a Neotropical distribution [39], but few (<6) have been studied [15,40], and population density data is not available for many sites [16]. Therefore, suggestions on methods and field procedures to estimate population densities of the white-tailed deer are a crucial aspect for the conservation of this species. This is particularly urgent in sites where subsistence, commercial, and game hunting of this deer are common. For example, in Mexico the white-tailed deer is one of the most important hunted species in Management Units for the Conservation of Wildlife (in Spanish: "Unidades de Manejo para la Conservación de la Vida Silvestre" or UMA). Important concerns and limitations of this model have been discussed [41], but one common aspect emphasized by these studies is the need for accurate density estimations. Another example, also in Mexico, is that even Protected Natural Areas are important conservation assets for this species and provide an opportunity to study deer in less perturbed sites [42], although few are of sufficient area to support a hypothetical minimum viable population [43]. In this case, accurate population density estimates are required for management purposes. A similar situation occurs in other Neotropical regions such as Costa Rica, Colombia, and Venezuela [16].

Fig. 6. Two important variables to convert an index of abundance into population density are the rate of defecation and the rate of decomposition of fecal groups. An example of old (a) and fresh (b) fecal groups of white-tailed deer in captivity (c, d).

We strongly suggest replicating our study in other tropical areas in order to test these sampling methods. An important requirement is the comparison of these methods under different levels of white-tailed deer abundance. For example, when we revised methods of density estimation, quantification of fecal groups in circular plots was used successfully in tropical dry forests with higher population densities of > 11 white-tailed deer km^{-2} [37]; while strip transects were used in sites with medium densities of 6-9 individuals km^{-2} [44]. López-Tellez *et al*. [17] estimated an imprecise density of < 3 ± 3 individuals km^{-2} using counts in circular plots. Considering that fecal count is actually a common method of density estimation of ungulates in tropical wet forests [e.g., 45-47], and that white-tailed deer density seems to be very low (< 1 deer km^{-2}) in this habitat [14,48,49], we suggest that in tropical forest with medium-high abundance, the standard method of counts following ST or FSC would be appropriate; while in forests with low abundance, the LT method is more suitable (Fig. 6). We suggest estimating density precision incorporating the variation of three parameters: numbers of pellet groups per sampling unit, daily defecation rate, and fecal decomposition rate, according to Plumtre [22].

Acknowledgments

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Appendix 1. Fecal group number (n), mean density (D, ind km^{-2}), and coefficient of variation (CV) obtained by the Fecal Standing Crop (FSC), Fecal Accumulation Rate (FAR), Strip Transect (ST), and Line Transect (LT) methods, over two years in the study site.

Method	2006				2007			
	n	D	range	CV	n	D	range	CV
FSC	71	7.3	5.9 – 9.6	21.1	48	6.1	4.9 – 7.9	23.1
FAR (60 d)	-	-	-	-	21	4.3	3.5 – 5.6	30.9
FAR (120 d)	-	-	-	-	48	4.8	3.9 – 6.3	23.5
ST	135	6.5	5.2 – 8.4	21.3	101	4.8	3.9 – 6.3	20.9
LT	326	7.3	5.9 – 9.5	18.7	293	5.4	4.4 – 7.0	19.5

Appendix 2. Advantages (+) and disadvantages (-) of pellet-group counting methods evaluated in this study. Abbreviation: Fecal Accumulation Rate (FAR), Fecal Standing Crop (FSC), Strip Transect (ST) and Line Transect (LT).

	Circular plots		Transects	
Criterions	**FAR**	**FSC**	**ST**	**LT**
Procedures assumptions	-	-	-	+
Required time to sample in field	+	+	+	-
"Doubtful" pellet-groups registration	+	-	-	+
Sample size	-	-	-	+
Accurate and precision of estimation	-	-	-	+
Statistical power	-	-	-	+