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MEASURING UNDERSTORY LEAF OBSTRUCTION IN ECOLOGICAL STUDIES: A COMPARISON BETWEEN TWO METHODS

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Abstract: The square wooden frame and the digital photography are methods widely used to measure density or foliar obstruction of the understory (FOU) in structurally complex habitats. Here we compared these two methods by measuring FOU in two grids in the Garrafão, an area of Atlantic Forest in the Serra dos Órgãos National Park, state of Rio de Janeiro, Brazil. Measures of FOU obtained were described by calculating the mean, median, and minimum and maximum values. FOU were compared between grids and methods using Mann-Whitney U tests for independent samples. Spearman correlations were used to compare methods. We found that FOU measurements obtained through the two methods are correlated, suggesting that both methods provide a measure that reflects the FOU. The advantages and disadvantages of using each method are discussed.

Keywords: digital photography; foliar obstruction; habitat complexity; microhabitat; square wooden frame.

Tropical forests have a great three-dimensional complexity (*sensu* August 1983), with several plant species and different life forms composing the vertical strata. The vertical strata of forests can vary in density, with the lower stratum (*i.e.*, understory) frequently being denser than the following strata, such as the canopy. The highest density or foliar obstruction of the understory (FOU) can be attributed to species composition, as this stratum is composed by herbaceous and shrub species but also by young individuals of canopy tree species and adult individuals of understory species (Guilherme *et al.* 2004). Additionally, FOU can be affected by canopy structure (Nuñez *et al.* 2021) or by the relief (Silva *et al.* 2007), which can directly regulate incoming solar radiation in this stratum.

In ecological studies, FOU is frequently used to correlate the occurrence of animal species and community parameters (*i.e.*, species richness and abundance) to local habitat characteristics, such as for non-volant small mammals (Aprigliano 2003, Delciellos *et al.* 2016), bats (Marciente *et al.* 2015), birds (Pinto *et al.* 2013), anurans (Scriven *et al.* 2018), and invertebrates (Gries *et al.* 2012). For instance, some Atlantic Forest non-volant small mammal species seems to prefer habitats with less FOU from the ground to *ca.* 1 m height, as found for the Cursor grass mouse *Akodon cursor*, Brazilian common opossum *Didelphis aurita*, Brown four-eyed opossum *Metachirus myosuros*, and Southeastern four-eyed opossum *Philander quica* (Freitas 1998, Aprigliano 2003, Moura *et al.* 2005).

Several methods can be used to measure FOU, providing quantitative or qualitative measurements (Nudds 1977, August 1983, Marsden *et al.* 2002). Among the methods widely used in ecological studies are the square wooden frame (SWF; Freitas *et al.* 2002) and the digital photography (DP; Marsden *et al.* 2002, Zehm *et al.* 2003). Here we compared these two methods in an Atlantic Forest area, evaluating if the measurements of FOU obtained are correlated between them, and analyzing if both provide representative measurements of FOU. Additionally, the advantages and disadvantages of using each method are discussed.

The study was carried out at the locality of Garrafão, in the Serra dos Órgãos National Park, municipality of Guapimirim, state of Rio de Janeiro, Brazil. Vegetation in the locality is dense evergreen forest belonging to the Atlantic Forest biome, but in an old-growth successional stage due to the history of human occupation in the locality (Cronemberg & Castro 2007). The climate is mild humid-mesothermic, with a super-humid season from October to March and a wet season from April to September (Nimer 1989).

FOU was measured in two grids (A – 22°28’12” S; 42°59’50” W and B – 22°28’29” S; 42°59’08” W; *Datum* WGS84) which were established in 1997 for a long-term project of capture-mark-and-recapture of non-volant small mammals (Gentile & Kajin 2015). Each grid had 0.64 ha, in a 5 x 5 design, with stations 20 m apart (Ferreira *et al.* 2016). Grids A and B were located at 748 and 652 m a.s.l., respectively. In each station, the central point was marked with a stake and other four points (North, South, East, and West) were established 3 m apart from the central point with the help of a compass. FOU was measured using the two methods at these four points (North, South, East, and West) in each station, only once during the period between February and April 2017, totaling 50 stations and 200 points measured per method.

The SWF method was developed by Freitas *et al.* (2002: See this reference for schematic drawings on the use of this method) and consists of a 0.50 x 0.50 m square wooden structure with a wire mesh divided in 100 units. In this method, measurements of FOU are obtained by counting the number of obstructed units, which are those visually with 50% or more of their area filled (Freitas *et al.* 2002). Measurements of FOU were taken at three different heights in each point (FOU1 = 1.50 m; FOU2 = 1.00 m; FOU3 = 0.50 m) as described in Freitas *et al.* (2002), originating 12 FOU measurements per station. Then, these 12 FOU measurements were summed and the average FOU for each station was calculated.

The DP method used was adapted from that described by Marsden *et al.* (2002: See this reference for schematic drawings on the use of this method) and Zehm *et al.* (2003), aiming to deal with the characteristics of the Atlantic Forest vegetation and terrain, specifications of the camera used and to obtain measurements comparable with those obtained by the SWF method. In this method, a photo from the vegetation is taken in the field and later it is analyzed in the laboratory using specific software. In the field, a Power Shot G11 model camera with 30.5 mm lenses was positioned 0.70 m height with a tripod at the central point of each station, and a white canvas (2 m length x 1.65 m height) positioned 3 m apart. FOU between the camera and the canvas was photographed at the four points. In the laboratory, photos were edited using Adobe Photoshop v. 2017.0.1 (Adobe Systems Inc. 1990-2016) to perform three procedures: (1) the canvas area was selected in the photo and cropped; (2) the photo was leveled in relation to the terrain using a rotation tool because of the terrain slope of the study area; and (3) the “selection” and “brush” tools were used to repair areas of shadow or light that could be clearly mistaken for obstruction or empty space (Suganuma *et al.* 2008). Later, an area of 1.90 m x 1.40 m positioned at the bottom margin and in the center of the photo was selected and cropped for standardization. After this procedure, each image was converted to a binary reading (*i.e.*, black and white), with the percentage of area in black color being the FOU and the area in white color being the empty area, using the software Side Look 1.1 (Nobis 2005). The four FOU measurements obtained in each station were summed and the average FOU for each station was calculated.

For each method, analyzes were performed separately for grids A and B and for the pooled data (grids A+B). The normality of the data was tested using the Shapiro-Wilk test ($p \leq 0.05$). Data was normally distributed only for the FOU measurements obtained with the method of DP for Grid B ($W = 0.963$, $p = 0.479$) and the pooled data ($W = 0.956$, $p = 0.0580$). The mean (± standard deviation), median, and maximum and minimum values were used to describe FOU measurements obtained for grids and methods. Mann-Whitney U tests for independent samples ($p \leq 0.05$) were used to compare: (1) average FOU measurements per station between grids separately by method; (2) FOU1, FOU2 and FOU3 obtained using the SWF method separately between grids; and (3) average FOU measurements between methods separately by grid and for the pooled data. Spearman correlations ($p \leq 0.05$) were used to verify if average FOU measurements are correlated between methods. Statistical analyzes were performed using the software Systat 11.0 (Systat Software, Inc. 2004).

In general, the mean, median and minimum values of FOU measurements for each grid and for the pooled data obtained with the DP were higher than those obtained with the SWF method (Table 1). The exception was the minimum value of FOU for grid B obtained with the DP method, which was lower than that obtained with the SWF (Table 1).

FOU did not differ between grids A and B for both methods (Mann-Whitney U test: SWF = $U_{0.05; 25; 25} = 272.5$, $p = 0.438$; DP = $U_{0.05; 25; 25} = 281.0$, $p = 0.541$). FOU also did not differ between grids A and B when considering measurements taken at the three different heights using the SWF method (Mann-Whitney U Test: FOU1: $U_{0.05; 25; 25} = 262.5$, $p = 0.332$; FOU2: $U_{0.05; 25; 25} = 277.5$, $p = 0.477$; FOU3: $U_{0.05; 25; 25} = 283.0$, $p = 0.567$). FOU measurements differed between methods in grid B (Mann-Whitney U test: $U_{0.05; 25; 25} = 447.0$, $p = 0.009$) and for the pooled data ($U_{0.05; 50; 50} = 1683.5$, $p = 0.003$), but did not differ for grid A ($U_{0.05; 25; 25} = 397.0$, $p = 0.101$). FOU measurements using the two methods were positively correlated for grid A ($r = 0.527$; $p = 0.007$) and for the pooled data ($r = 0.300$; $p = 0.034$), but were not correlated when considering only grid B ($r = 0.076$; $p = 0.719$) (Figure 1).

The highest FOU values found for the DP method compared to the SWF method probably are related to the fact that the software Side Look 1.1 converts any obstruction within the photo area into a black pixel (Zehm *et al.* 2003). Nevertheless, FOU measurements obtained using the SWF and DP methods were generally correlated, suggesting that both methods provide useful measurements that reflect foliar obstruction in the understory.

The absence of correlation in FOU measurements between methods for grid B may suggest that only for grid B methods are reflecting different responses. Factors that may be causing this absence of correlation when grid B is analyzed alone were not evaluated in the present study. For instance, this result could be related to differences in structural heterogeneity between the grids, such as luminosity and terrain inclination (Aprigliano 2003). According to Aprigliano (2003), grid A has a more stable microclimate and lower terrain slopes than grid B, indicating that grid B would be structurally more complex than grid A. These factors could be affecting some steps of photo analysis and, consequently, measurements obtained with the DP method. However, these relationships remain to be evaluated in future studies.

For the SWF method, measurements taken at the three different heights did not differ between grids A and B. However, a previous study that evaluated spatial heterogeneity between the grids located in the Garrafão found that grids A and B differ regarding FOU2 (Aprigliano 2003). The author

Table 1. Mean (± standard deviation), median, and maximum and minimum values of understory foliar obstruction (%) for grids A and B and for the pooled data (Grids A+B) obtained through the square wooden frame (SWF) and digital photography (DP) methods, in the locality of Garrafão, Serra dos Órgãos National Park, state of Rio de Janeiro, Brazil.

Method	Grid	Mean ± SD	Median	Maximum value	Minimum value
SWF	A	27.95 ± 14.69	21.17	64.25	7.42
	B	24.06 ± 9.66	22.67	48.92	11.58
	Pooled data	26.00 ± 12.46	21.21	64.25	7.42
DP	A	31.95 ± 9.87	32.98	63.47	9.63
	B	30.39 ± 8.90	31.36	46.85	11.31
	Pooled data	31.17 ± 9.33	31.85	63.47	9.63

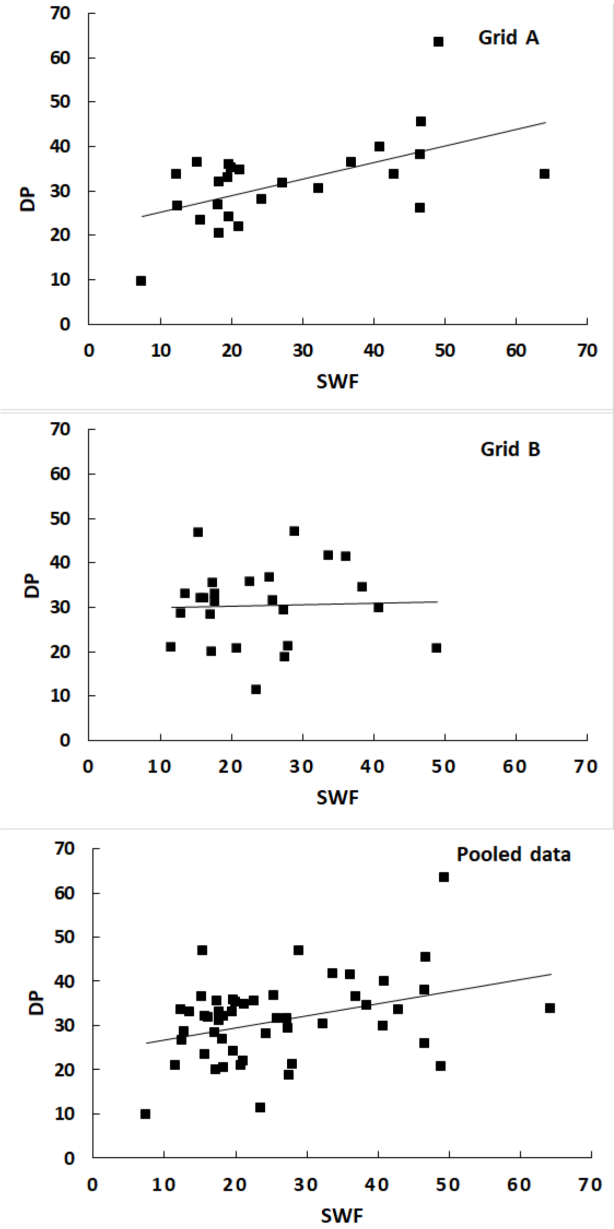

Figure 1. Spearman correlation for foliar obstruction of the understory (%) obtained through the square wooden frame (SWF) and digital photography (DP) methods for grid A (r = 0.527; p = 0.007), grid B (r = 0.076; p = 0.719) and the pooled data (Grids A+B; r = 0.300; p = 0.034), in the locality of Garrafão, Serra dos Órgãos National Park, state of Rio de Janeiro, Brazil.

found that the understory was more developed in grid A, which was attributed to a more open canopy in this grid, having a higher luminosity (Aprigliano 2003). Since those observations were made almost 20 years ago, it is reasonable to assume that the structure of the habitat has changed in the grids.

As both methods provide measurements that reflect FOU, the researcher may choose the most viable method for his research. The SWF method has the advantage of the practicality and agility in data collection, because it was designed to be replicable and to require less effort per man or field hours in data collection (Freitas *et al.* 2002). Also, the SWF method apparently allows a broader view of the understory by the researcher, as there is no limitation imposed by the canvas used in the DP method. Additionally, it seems to be less invasive or destructive, as it is not necessary to place the canvas behind the vegetation as in the DP method. These questions remain to be evaluated in future studies.

On the other hand, the SWF method has as disadvantage the fact that the decision of whether or not there is an obstruction is made by the observer, since the measurement consists of counting the obstructed squares considering a square as obstructed if more than 50% of its area is visually filled (Freitas *et al.* 2002). Another disadvantage, perceived by the authors in the field, but not tested, is the fact that the SWF method does not to allow obtain all the information of the vertical obstruction, such as thinner branches that do not fill more than 50% of the square and that, therefore, are disregarded. This fact may explain, for example, the lower values of FOU found using SWF than DP.

The disadvantages of the DP method, in turn, are related to the fact that it can cause some disturbance in the vegetation for canvas placement and, consequently, some sample bias if the measurements have to be replicated in other campaigns or climatic seasons. It is a method relatively quick and easy to apply, except in conditions of strong wind or moist vegetation (Zehm *et al.* 2003). In the present study, it was not possible to use this method on foggy or rainy days, because the interior of the forest becomes very dark to take photographs, and the rain can damage the photographic equipment. Additionally, Warmink (2007) suggests that the DP method has a bias in describing FOU measurements because of the occlusion effect, *i.e.*, the overlap of individuals on the photos, excluding other individuals that should be measured within the area of interest. Still, some photos may contain shadows depending on the position of the sun, which can be mistaken for foliar obstruction (Warmink 2007). On the other hand, reflections of sunlight on trees and branches, in turn, can be mistaken as an unobstructed area

(Zehm *et al.* 2003). These two factors can lead to an overestimation or underestimation of the FOU measurements, respectively.

Both methods provided representative measurements of foliar obstruction in the understory. Thus, the choice of which method to use should be based on factors such as the time available for data collection during the study (*e.g.*, the SWF method requires fewer hours of fieldwork), available equipment and financial resources (*e.g.*, the DP method demands more hours of field work, photographic equipment and software) and climatic and terrain conditions where the study will be carried out (*e.g.*, areas with high levels of precipitation may prevent the use of photographic equipment and a very rough relief the placement of the canvas for the use of the DP method), as discussed above.

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