Staining Method for Assessing the Ecological Function of Excrement Removal by Dung Beetles (Coleoptera: Scarabaeidae: Scarabaeinae)

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Dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) perform several ecological functions that can be interpreted as environmental services, since they provide direct benefits to humans (Nichols et al. 2008). These benefits are associated with excrement and carcass removal, since these items are their primary food source, and this behavior has positive implications for matter decomposition and nutrient cycles (Yamada et al. 2007; Hanafi 2012), secondary seed dispersal (Vulinec 2002; Andresen 2003; Slade et al. 2007), soil aeration, and biological control of flies (Braga et al. 2012, 2013). Since these functions are related to dung beetle feeding habits, quantifying excrement removal has been a common approach in studies of ecological functions (e.g., Klein 1989; Horgan 2001; Yamada et al. 2007; Amézquita and Favila 2010) provides greater contrast between soil and excrement; however, this resource is recommended for pasture environments, since dung beetles prefer omnivorous animal excrement in forest areas (Filgueiras et al. 2009, Bogoni and Hernández 2014). In the Atlantic Forest fragments of southern Brazil, the crab-eater fox, *Cerdocyon thous* (Linnaeus, 1766), is a common native omnivore (Graipel et al. 2001; Cherem et al. 2004) and tolerant of disturbed habitats (Dotta and Verdade, 2011). The excrement from *C. thous* is a highly attractive resource for dung beetles in the Atlantic Forest (Bogoni and Hernández 2014), but acquiring this material for experimental reasons to evaluate removal is not very feasible. A viable alternative is to use excrement from domestic dogs (*Canis lupus familiaris*) raised in animal facilities (bioterium) (Batilani-Filho et al. 2014). This type of resource has the advantage of being safe in regards to the spread of pathogens in the natural environment. To increase the contrast of omnivore excrement with the soil to allow faster quantification of the resource buried by the paracoprids separate from that used by the telecoprids, we present here a removal evaluation technique that involves prior staining of these excrements. For this technique, we used domestic dog excrement collected at the Central Animal Laboratory of the Federal University of Santa Catarina.

The staining technique consisted of mixing edible, odorless liquid blue stain to domestic dog excrement (40 mL/kg). This stain is commonly used in cakes and has a neutral ethyl alcohol base mixed with organic colorants. The color blue was chosen instead of purple, yellow, or red because it provides a greater contrast with the ground after mixing, displaying a green color. We tested the effect of the stain on excrement attractiveness and manipulation by dung beetles in September 2013, in an area of Atlantic Forest in southern Brazil.
(Environmental Conservation Unit Desterro, Florianópolis, Santa Catarina). We used ten pairs of collection and assessment arenas, which were installed and exposed for 48 hours (Fig. 1). Each arena consisted of a buried 1.5-L plastic container filled with sieved soil (maintaining a 5 cm height on the margins so that the relocated material and telecoprids remained in the arenas) and protected from rain with a suspended plastic cover. A spacing of 5 m was maintained between pairs of arenas and 30 m between the ten pairs. In each arena pair, one arena was baited with 50 g of unstained excrement and the other with 50 g of blue-stained excrement. For each treatment, four control arenas were installed, of which the value of excrement dissection was calculated, and subsequently this value was subtracted from the excrement removal data. The control arena differed from others in having its opening sealed with voile fabric, preventing the entry of insects.

Upon 48 hours in the field, arenas were inspected, and the insects that remained active on the surface of the area around the arenas were noted as showing various behaviors, such as feeding, rolling food balls, underground gallery construction, and fighting. The beetles on the ground were collected, and

![Fig. 1.](image-url)
the ground surface material was separated into non-removed resource (that stayed within the arena) and resource allocated by telecoprids, which were the rolled food balls made by this guild, but were not buried. After this procedure, the arenas were capped and taken to the laboratory for screening and measurements, and differentiation of removed excrement was conducted. During this process, a brush and a spoon were used to carefully remove 1 cm of soil at a time in order to find the material buried by paracoprids and possible balls buried by telecoprids, as well as beetles that were buried. For arenas with unstained excrement, the soil was removed with a spoon when it presented a pasty consistency; during the screening process, this was observed under a magnifying glass due to the low contrast with the soil, in order to isolate excrement using small brushes and forceps. Triage in arenas with stained excrement followed the same protocol. In both cases, when the material appeared to form a spherical shape, the allocation was attributed to telecoprids, and material with an amorphic shape was attributed to paracoprids (Fig. 1).

Species richness, beetle abundance, and amount of excrement removed (g) were analyzed with t-tests to compare treatment means. Community structure similarity was assessed using a Bray-Curtis distance in an ANOSIM similarity analysis (Clarke 1993; Warton et al. 2012).

The arenas with unstained excrement captured 100 dung beetles representing ten species, and the arenas with stained excrement captured 142 individuals of nine species. No differences in species richness \( t = 1.6; p = 0.5 \), abundance \( t = 0.9; p = 0.3 \), or community structure attracted by the different treatments \( \text{ANOSIM } R = 0.05; p = 0.7 \) were detected. The mean removal of 33.1 g of unstained excrement did not differ from the mean removal of 33.3 g of stained excrement \( t = 0.05; p = 0.9 \) (Fig. 2). The use of the stain had no effect on the removal of material depending on the functional guild. On average, 21.7 g of unstained excrement were buried by the paracoprids, which did not differ significantly from the average of 15.1 g of removed stained excrement \( t = 1.08; p = 0.3 \) (Fig. 2). The telecoprids relocated an average of 6.8 g of unstained excrement, which also did not differ from an average of 12.4 g of relocated stained excrement \( t = 1.72; p = 0.1 \) (Fig. 2).

Our results contribute to dung removal studies by demonstrating that using stains facilitates visualization of removed and accelerates relocated excrement quantification. Both the triage and identification of stained excrement allocation was faster and more effective than unstained excrement, where the use of brushes, magnifying glasses, and forceps were necessary to separate the excrement from the soil. Moreover, when the buried material was found, it was easier to isolate stained excrement from the soil to measure the excrement’s true weight due to its elevated contrast, therefore, the proportion of stain used (40 mL/kg) was considered effective.

![Fig. 2. Mean (± SD) amount of excrement removed and amount relocated between treatments according to two functional guilds. The same letters indicate no significant differences \( p > 0.05 \) between treatments within a variable. UE = unstained excrement; SE = stained excrement.](image)
In using this technique, it is possible to differentiate and quantify the removed resource by functional guild, whether it is panacoprid or telecoprid. This approach is conducted without having to manipulate the density or restrict organism access to exclusively assess its participation, as was done in similar studies (Horgan 2001; Yamada et al. 2007; Slade et al. 2007; Dangles et al. 2012). This is a more realistic approach, since paracoprid and telecoprid species compete for resources at the same time. Another advantage of using this staining technique is that it is possible to calculate the resource quantity that dung beetles are actually using, since the difference between what is removed by the community and the sum of the allocation refers to the resource quantity that was immediately consumed or fractioned by beetles.

In using this methodology, it is possible to simultaneously acquire data for community and ecological function analysis during the same period and in the same arena. In regards to the type of resource used, due to the high attractiveness of omnivore excrement, the use of domestic dog excrement in removal studies in forest areas is a good alternative, since there is no difference in removal when compared to native omnivore excrement (Batilani-Filho et al. 2014). Additionally, this resource is easy to acquire, and since they are from a laboratory animal facility, their use is safer in regards to the presence of pathogens, allowing handling and staining.

Lastly, excrement staining, with quantitative detailing by functional guild, may be used in studies that investigate other ecological functions associated with removal, such as secondary seed dispersal (Vulinec 2002; Andresen 2001; Slade et al., 2007), soil aeration, and biological control of flies (Braga 2001; Andresen 2001; Slade et al., 2007; Dangles et al. 2012). This is a more realistic approach, since paracoprid and telecoprid species compete for resources at the same time. Another advantage of using this staining technique is that it is possible to calculate the resource quantity that dung beetles are actually using, since the difference between what is removed by the community and the sum of the allocation refers to the resource quantity that was immediately consumed or fractioned by beetles.

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References Cited


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