The reintroduction of a dung-beetle associated primate increases the recruitment success of large seeds

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Abstract

The biased loss of large and medium frugivores alters seed dispersal and plant regeneration. Species reintroductions have been proposed as a strategy to reverse the consequences of species loss. However, reintroductions effects on ecological processes are seldom accessed, which hinders the comprehension of reintroductions' potential to reestablish functioning ecosystems. In this study, we investigate the effect of howler monkey (*Alouatta guariba*) reintroduction in the plant regeneration of Tijuca National Park (TNP), a highly defaunated Atlantic Forest fragment. Howlers are medium sized folivore-frugivore primates, whose large clumped defecations attract dung beetles. Dung beetles bury seeds present in howlers' feces, a process known as secondary seed dispersal. Thus, we expect that the fate of seeds dispersed by howlers will differ from those dispersed by the other frugivores present in the Park. We followed the fate of seeds between 3 and 14mm in diameter in three steps of the seed dispersal loop, each consisting of a different experiment. First, we estimated secondary seed dispersal and burial depth probabilities according to the frugivores' defecation pattern; then,

predation probability in different burial depths and defecation patterns; and, finally, recruitment probability in different burial depths. Considering the final result of the three experiments, the howlers' reintroduction affected positively the regeneration of large seeds. 3mm seeds did not benefit as much because they weren't frequently predated at shallower depths and couldn't recruit when deeply buried. Seeds larger than 3mm reached more frequently the seedling stage when dispersed by howlers than when dispersed by other animals present in the Park. Thus, howler monkey reintroduction in defaunated areas, consisting mainly of smaller frugivores, whose defecation pattern doesn't attract dung beetles as frequently, improves the regeneration of large seeds. We hope that this study will stimulate the resuming of howler reintroduction in TNP, as well as new howler reintroductions in defaunated areas.

1 Introduction

Human activities have been interfering with biodiversity for over 50.000 years (Koth and Barnosky 2006). Larger vertebrates are particularly affected due to hunting, habitat degradation and fragmentation (Peres 2002, Peres and Palacios 2007, Ripple et al. 2019). This bias is of major concern, since body size partially determines the contribution of species to some ecosystem process, such as seed dispersal (Vidal et al. 2013, Schleuning et al. 2015). Larger frugivores interact with larger seeds and disperse them through longer distances (McConkey and Brockelman 2011, Dehling et al. 2016). Thus, downsized frugivore communities can not fully substitute the roles of extinct large and medium-sized animals. The reduction of seed dispersal affects plant recruitment and population growth (Traveset and Riera 2005), modifying plant community composition (Terborgh et al. 2008).

In the actual biodiversity crisis, conserving what is left is not enough. The reintroduction of extinct species to restore interactions and its associated functions has been proposed to reverse the consequences of species loss (e.g. refaunation (Oliveira-Santos and Fernandez 2010) and trophic rewilding (Svenning et al. 2016)). Just as the extinction of species leads to losing interactions, the reintroduction of species can rewire these interactions and reestablish functions in ecological processes (Genes et al. 2019, Mittelman et al. 2020). Nevertheless, most reintroductions are focused on species conservation and the effect of reintroductions on ecological and ecosystem functions is rarely evaluated (Polak and Saltz 2011).

A possible way to evaluate the effect of reintroductions on ecological processes is to measure the number of restored interactions (Genes et al. 2017). However, two animals can interact with the same seed species but lead to different seed fates (Culot et al. 2017, Lugon et al. 2017). Thus, another way to evaluate the contribution of a reintroduced frugivore is to compare the fate of their seed dispersal with that of present frugivore species. Nevertheless, accompanying the seed dispersal loop is challenging. This challenge can explain the lack of studies that follow many steps of seed fate (Wang and Smith 2002) or that consider a community perspective and include more than one taxon (Andresen et al. 2018).

Primates are some of the last remaining megafauna in the tropics and among the most threatened groups (Estrada et al. 2017). Although primates constitute a large proportion of the frugivore biomass in tropical forests (Eisenberg and Thorington 1973), only recently the number of seed dispersal studies with primates has increased (Andresen et al. 2018). In the Atlantic Forest, primates interact with all seed sizes (Bufalo et al. 2016), have a generally positive effect on seed germination (Fuzessy et al. 2016) and disperse seeds to long distances (Fuzessy et al. 2017). Nevertheless, there are still many gaps in the knowledge

of primates' role on plant regeneration (Chapman and Dunham 2018).

Howler-monkeys (*Alouatta* spp.) are folivore-frugivore medium sized primates. They swallow up most of the seeds from the fruits they eat and affect positively seed germination (Arroyo-Rodríguez et al. 2015). Howlers usually defecate simultaneously, which generate large clumped defecations and high seed densities (Julliot 1996). This pattern attracts rodents and other seed predators, but also dung beetles (Scarabaeidae: Scarabaeinae) (Andresen 2002a). Dung beetles feed on feces and bury seeds while building their nests, a process known as secondary seed dispersal (Andresen and Feer 2005). Secondary seed dispersal alters seed survival and recruitment probability (Shepherd and Chapman 1998). While seed burial decreases predation by rodents, it decreases seedling recruitment, often to very low rates when lower than 3cm in depth (Andresen and Feer 2005). Thus, when comparing the role of seed dispersers it is essential to take into account the secondary seed dispersal probability associated with each primary disperser (Culot et al. 2018).

In this study, we investigate the effect of the reintroduction of red howler monkeys (*Alouatta guariba*) on the plant regeneration of a highly defaunated Atlantic Forest fragment. We compare the fate of seeds dispersed by howlers with other frugivores already present in the fragment through three experiments. First, we estimate the probability of secondary seed dispersal according to the frugivore's defecation pattern; then, we estimate the predation probability for different defecation patterns and burial depths; and finally, the recruitment probability in different burial depths. Each step consists of a different experiment. We hypothesize that howlers clumped defecation will have a different effect on plant regeneration than the other animals already present in the fragment.

2 Methods

Study site

This study was carried out from September 2019 to December 2021 at Tijuca National Park (TNP, 3.953 ha). Mean temperature varies between 18 and 26°C, with rainy summers, surpassing 1.200mm yearly (ICMBIO 2008). TNP is situated within the city of Rio de Janeiro and its history is intertwined with the citys. The area that now corresponds to the Park used to be explored for coffee farming and coal production until the middle of the 19th century, when it began to be reforested (Peres 2002). Despite some exotic species introduced during reforestation, the vegetation is mainly composed of native Atlantic Forest species (Freitas et al. 2006). Since the TNP is surrounded by a metropolitan matrix, the missing fauna has been unable to recolonize. Although there are still seed dispersers present (e.g. the lowland paca (*Agouti paca*) and the brazilian squirrel (*Sciurus aestuans*)), the Park lacks medium and large frugivores. Recently, three vertebrate species have been reintroduced to TNP, starting with agoutis (*Dasyprocta leporina*) in 2010 (Cid et al. 2014), followed by howler monkeys (*Alouatta guariba*) in 2015 (Genes et al. 2019) and, more recently, in 2019, yellow-footed tortoises (*Chelonoidis denticulatus*).

The experiment

We compared the seed fate of seeds dispersed by howler monkeys and the mammal and avian species at TNP whose fruit diet most overlapped with that of howlers: capuchin monkey (*Sapajus nigritus*) and rusty-margined guan (*Penelope superciliaris*). Diet overlap was based on data from the Atlantic Frugivory data set (Bello et al. 2017), the most complete set of frugivory interactions from

the Atlantic Forest so far. The selection of seed species was based on the howlers credit of ecological interactions in the TNP (Genes et al. 2017), that is, the list of TNPs plant species with which the howler has the potential to interact. We chose species of plants whose genera (or species) were also part of the capuchin monkey and guan diet. Additionally, we only selected tree species of recalcitrant seeds that were dispersed by zoochory.

Three experiments were set to follow the seed path from seed deposition by the primary disperser (howlers, capuchins or guans) to its recruitment into seedling. The seed fate and predation experiments were both set in TNP, in the same plots, 48hr apart; whereas the seedling recruitment experiment was set indoors. In order to understand if the howlers effect on seed fate and seedling recruitment varies with seed size, all experiments were assembled for seeds with four diameters: 3mm (*Psidium cattleianum*, Myrtaceae), 7mm (*Cordia superba*, Boriginaceae), 10mm (*Nectandra membranacea*, Lauraceae) and 14mm (*Virola bicuhyba*, Myristicaceae). All seeds used in the experiments were donated from partner greenhouses.

Seed burial and predation

We set up 120 stations in TNP, 50m apart. Each station contained a combination of one seed/bead size and one primary disperser treatment. Primary disperser treatments were set according to the disperser feces deposition pattern. Howlers feces were placed in plots of $2m^2$ in 10 piles containing 10gr each. Capuchins feces were set in 100m transects each, with 8gr of feces (Mikich et al. 2015) every 10m. Guans feces were also set in 100m transects, but with 7gr in each pile (Mikich 2002). Stations were replicated 10 times for each seed/bead size and primary disperser combination (but we only replicated the *V. bicuhyba*s predation experiment 5 times due to limitations in seed availability). In total, we used 1.500, 900, 600 and 300 beads of 3, 7, 10 and 14mm of diameter, respec-

tively, 1500 seeds of *P. cattleianum*, *C. superba* and *N. membranace*a, and 450 seeds of*V. bicuhyba*.

Seed burial experiments occurred from September to November 2019. We collected howlers, capuchins and guans feces from populations at Rio de Janeiros Zoo in the mornings. The feces were used on the same day in the experiments at TNP. Since in this first step we only wanted to analyse the probability of secondary seed dispersal and depth, we used plastic beads with the seeds diameters (3, 7, 10 and 14mm). We varied the number of beads in the piles of feces according to their sizes: smaller beads were more abundant than bigger ones. In each pile one bead was tied with a 20cm nylon thread with a colored tape in the tip. We returned to the experiments 48hr after they were set, to check if the beads had been buried or not. When positive, we would also measure the burial depth.

Seed predation experiments were arranged following the data collection from bead burial. Seeds were buried 1, 3, 5 and 10cm deep, 20cm from each other, at the same location where the dispersers feces had been set. We also included one unburied seed to estimate the probability of predation with no secondary seed dispersal. Seeds were cleaned of their pulp and glued (Tekbond 793) to a 20cm nylon thread with a colored tape in the tip, and left to dry for over a week. We checked the experiments every month for 6 months. Seeds were considered as preyed upon if they were not found in a 1m radius, if we found the nylon thread but the seed had been partly or fully consumed, or if they presented holes in their surface.

Seedling recruitment

Seedling recruitment experiments were set indoors to test the effect of burial depth on recruitment probability. Seeds were buried on 1, 3, 5 and 10 cm and unburied, to test the recruitment probability of seeds that did not go through secondary dispersal. Seeds were watered daily and monitored weekly. We con-

sidered recruitment the appearance of the first leaves and ceased monitoring for a given species and depth after two weeks without new recruitments. In total, we used 550 seeds of *P. cattleianum*, 400 of *C. superba* and *N. membranacea* and 180 of *V. bicuhyba*.

2.1 Statistical analysis

We estimated the recruitment probability of a seed with diameter x dispersed by a primary disperser j considering the results from the three experiments. Let $B_{x,j}$ be the burial depth, $S_{x,j}$ the seed survival and $R_{x,j}$ the recruitment. To estimate the final recruitment probability, it is necessary to first determine the burial depth distribution, the seed survival at those different depths and, finally, the recruitment probability of seeds buried at those depths. We set $S_{x,j} = 1$ if the seed survived and $S_{x,j} = 0$ if it was preyed upon. Similarly, $R_{x,j} = 1$ if the seed recruited and $R_{x,j} = 0$ otherwise. By taking the conditional probabilities, we derive our equation for the final recruitment probability, represented by $Y_{i,j}$, as:

$$Y_{i,j} = \sum_{p} \mathrm{P}\big[\, B_{x,j} = p \,\big]\ \mathrm{P}\big[\, S_{x,j} = 1 \,|\, B_{x,j} = p \,\big]\ \mathrm{P}\big[\, R_{x,j} = 1 \,|\, S_{x,j} = 1 \,,\, B_{x,j} = p \,\big] \,, \tag{1}$$

where the sum is carried out over all burial depths p. Recall that in our experiment p takes the values 0 (unburied), 1, 3, 5 and 10cm.

<u>Seed burial</u>

All statistical analysis was done using R 3.6.2 statistical environment (R Development Core Team 2019). To estimate the burial probability we ran a Generalized Linear Model for proportional data (i.e. the proportion of beads that were buried in each station containing 10 piles of feces). For estimating the probability of burial depth, we ran a Cumulative Link Model for ordinal data using the

ordinal package (Christensen 2019). As we wanted to calculate the final probability of seedling recruitment considering the results from all experiments (see equation (1)), we categorized the response variable burial depth into the 5 treatment levels from the predation and recruitment experiments: unburied and 1, 3, 5 and 10cm burial depths. In both analysis we did a χ^2 to test the effects of the explanatory variables in the models. We estimated the treatment combinations marginal means through the emmeans package (Lenth et al. 2020) as a post-hoc test.

Seed predation

We ran several χ^2 tests to evaluate the effect of the explanatory variables: primary seed disperser, seed size and burial depth. For estimating the probability of predation we ran a Generalized Linear Model for proportional data (i.e. the proportion of seeds that were predated in each station containing 10 piles of feces). The differences among treatments were calculated by estimating their marginal means through the emmeans package (Lenth et al. 2020).

Seedling recruitment

Finally, to estimate the probability of seed recruitment considering burial depth, we first did a χ^2 test to select the explanatory variables and then ran a Generalized Linear Model for binomial distribution. A post-hoc marginal means test was done to calculate the difference between treatments through the emmeans package (Lenth et al. 2020).

3 Results

The primary disperser and bead size both affected bead burial probability (primary disperser: $\chi^2 = 317.27$, $df = 2$, $p < 0.001$, bead size: $\chi^2 = 31.14$, $df = 3$, $p < 0.001$) and burial depth (primary disperser: $\chi^2 = 56.51$, $df = 2$, $p <$

0.001, bead size: $\chi^2 = 42.56$, $df = 3$, $p < 0.001$). Burial probability was higher for dispersal by howlers in all bead sizes and decreased with increasing bead size (Figure 1). In howler treatments bead burial probability varied between 77 $\pm$ 6.8%, for 3mm beads, and 54 $\pm$ 10.0%, for 14mm beads. For capuchins the probability varied from 32 $\pm$ 8.2% to 14 $\pm$ 5.4% and for guans from 22 $\pm$ 6.9% to 9 $\pm$ 3.9%, for 3 and 14mm beads, respectively.

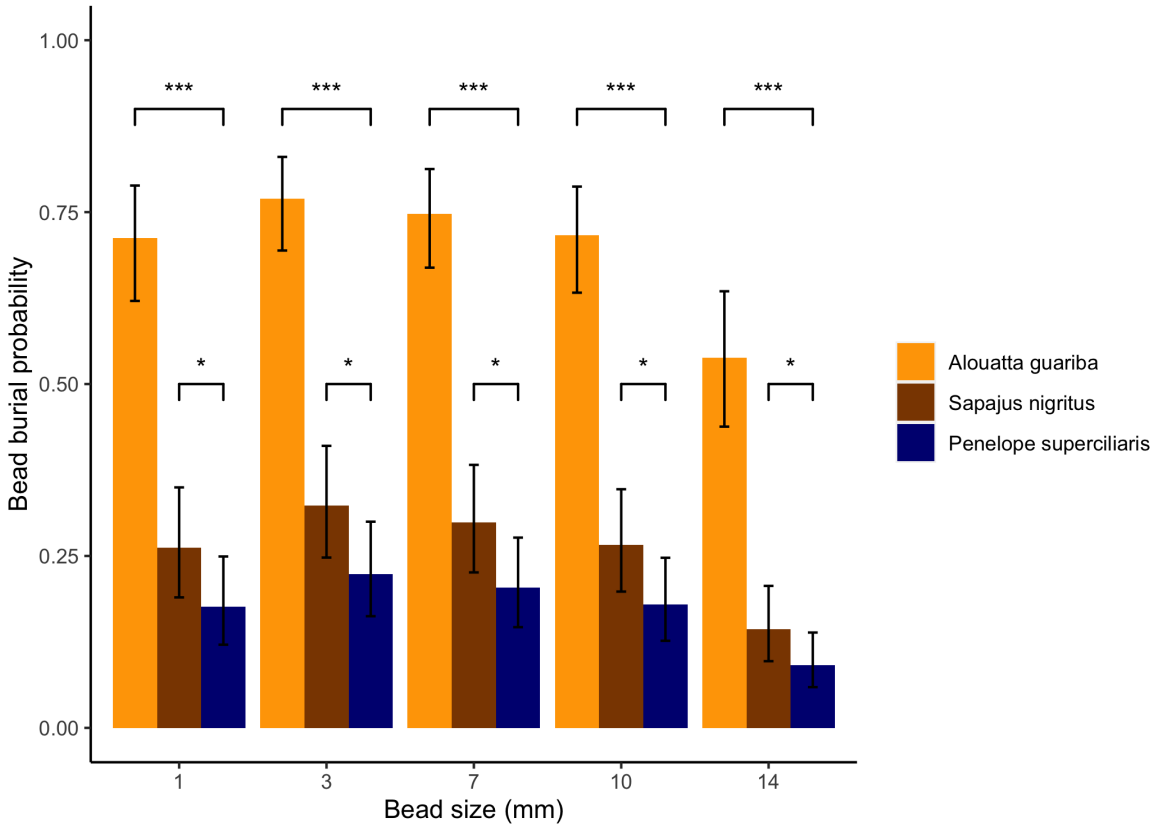

Figure 1: *bead burial probability. Percentage of beads buried in howler monkeys, capuchin monkeys and guans feces for different bead sizes in orange, brown and blue, respectively. *** corresponds to* $p < 0.001$ *and * to* $p < 0.05$.

Bead burial depth pattern varied according to the primary disperser (Figure 2). In the howlers treatments, burial probability increased with depth for 3 (from 16 $\pm$ 5.2% to 35 $\pm$ 8.5%) and 7mm beads (from 21 $\pm$ 6.6% to 27 $\pm$ 7.9%), but decreased for 10 (from 34 $\pm$ 8,6% to 16 $\pm$ 5.6%) and 14mm beads (52 $\pm$ 11,9% to 8 $\pm$ 4.1%). In contrast, in the capuchins and guans treatments, burial probability

decreased with depth for all bead sizes. When beads were buried in guans experiments, it was mostly 1cm deep (from 54.7 $\pm$ 5.2%, for 3mm beads, to 88 $\pm$ 7.4%, for 14mm beads). In capuchins treatments, the difference between probabilities was higher for 3 (from 38 $\pm$ 1.0% to 14 $\pm$ 5.6%) and 7mm (from 46 $\pm$ 12% to 10 $\pm$ 4.7%) beads, than for 10 (from 63 $\pm$ 11,4% to 5 $\pm$ 5.1%) and 14mm beads (from 78 $\pm$ 1.0 to 3 $\pm$ 1.7%).

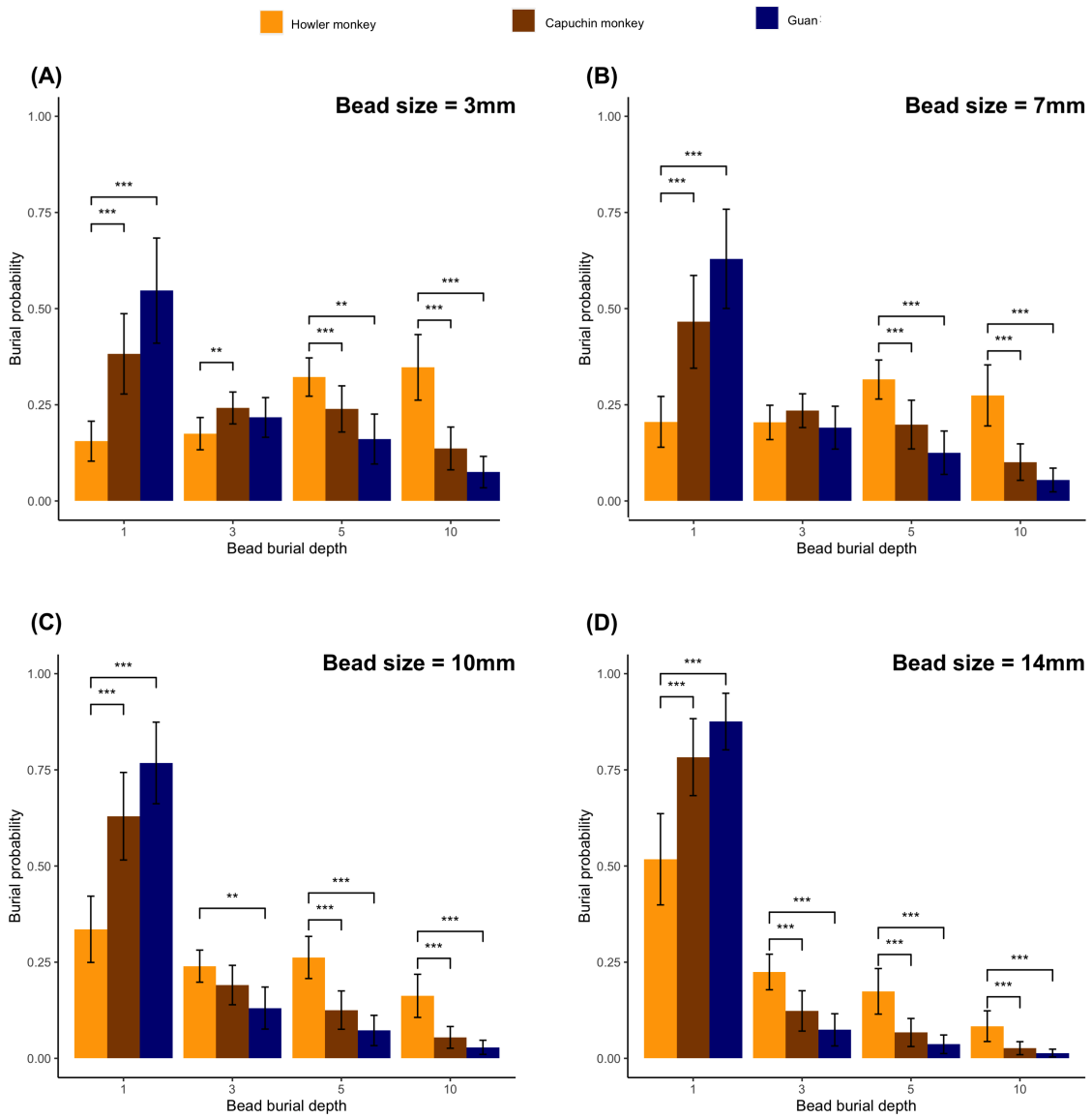

Figure 2: *burial depth probability. Percentage of beads buried at 1, 3, 5 and 10cm for different bead sizes. Results for experiments with howler monkeys, capuchin monkeys and guans treatments are in orange, brown and blue, respectively. Each bead size is represented in a different plot: (A) 3mm, (B) 7mm, (C) 10mm and (D) 14mm. *** corresponds to* $p < 0.001$, *** to* $p < 0.005$ *and * to* $p < 0.05$.

Primary disperser species did not affect predation probability. The model that best predicted predation probability included burial depth ($\chi^2 = 1519.19$, $df = 4$, $p < 0.001$), seed size ($\chi^2 = 211.26$, $df = 3$, $p < 0.001$) and the interaction between these variables ($\chi^2 = 45.01$, $df = 10$, $p < 0.001$). Predation rates decreased with

increasing burial depth (Figure 3). However, unburied seeds were not significantly different from seeds buried 1cm deep ($p > 0.05$) in all seed sizes. Predation probability of unburied and 1cm burial varied between 44 $\pm$ 6.6% and 58 $\pm$ 7.7%, for 3mm seeds, and between 86 $\pm$ 4.5% and 81 $\pm$ 5.5%, for 7, 10 and 14mm seeds. Almost no predation event occurred for 3mm seeds in depths greater than 1cm. Predation rates did not differ significantly between 7 and 10mm seeds in all burial treatments and it was very rare in seeds buried 5cm and 10 cm deep (lower than 22%).

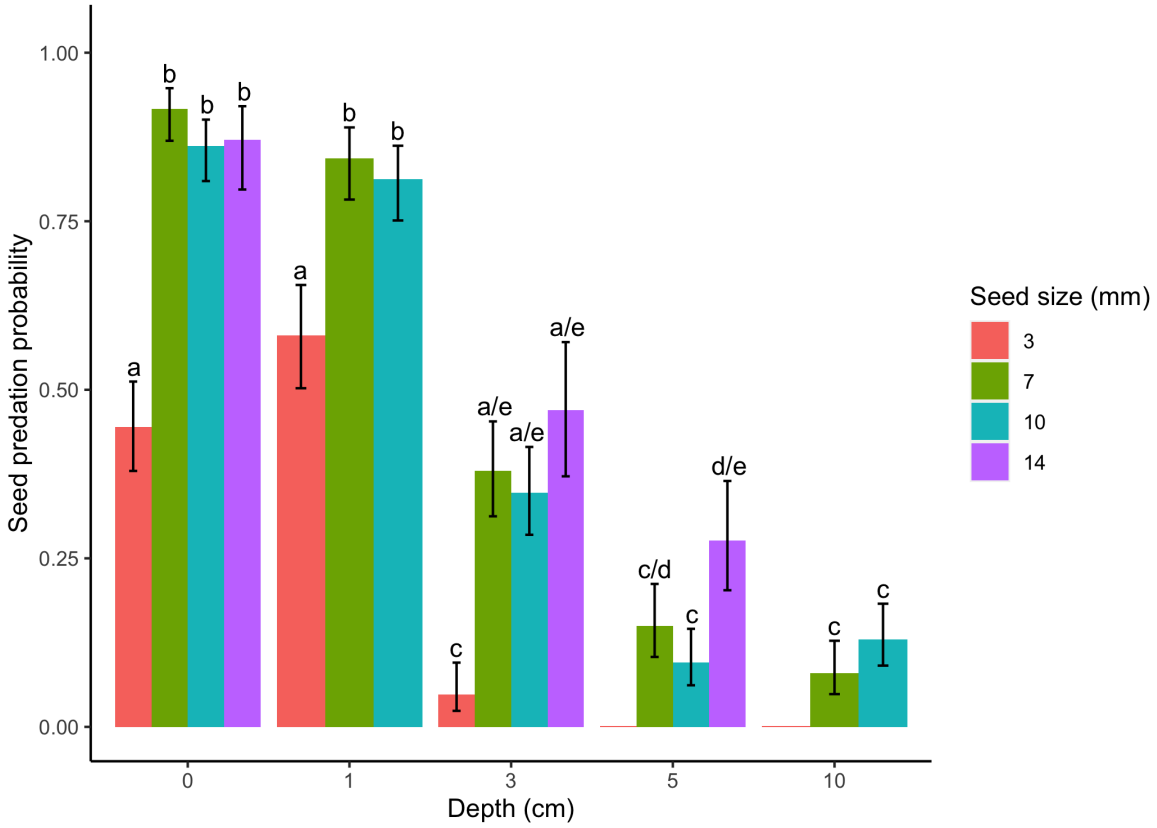

Figure 3: *seed predation probability. Predation probability of 3, 7, 10 and 14mm seeds in red, green, blue and purple, respectively. Due to lack of seed quantity, 14mm seeds were not tested for 1 and 10cm depths. 3mm seeds predation was tested for 5 and 10cm depths, but no predation event occurred during the period of the experiment.*

Escrever resultados de seedling recruitment

4 Discussion

Howler monkeys' seed dispersal in Tijuca National Park improved the recruitment success of large seeds. Seeds dispersed by howler monkeys were buried more often and in greater depths, which increased their survival. However, burial depth decreased the chances of seedling recruitment for all seed sizes. In consequence, the effect of howlers' seed dispersal only differed from capuchins' and guans' for seeds larger than 7mm. Seeds with 3mm had lower predation rates and low recruitment when buried too deep and thus did not benefit from howlers' dispersal. Seeds larger than 7mm, nevertheless, benefited, as their predation rate was greater in shallower depths and recruitment rate didn't decrease as much when deeply buried.

Dung beetles are assumed to be generalists because they depend on ephemeral and patchily distributed resources. However, recent studies suggest that dung beetles are more attracted to omnivorous mammals' feces (Whipple and Hoback 2012, Bogoni and Hernandéz 2014, Wurmitzer et al. 2017). In this study, although capuchins are omnivores and howlers are herbivores, burial probability was significantly higher in howlers' feces (*circa* 40% higher in all bead sizes). This is probably due to dung beetles being more attracted to large piles of moist dung, similar to the howlers' defecation pattern, than pelleted depositions, such as the capuchins' and guans' (Nichols et al. 2009, Santos-Heredia et al. 2010, Lugon et al. 2017, Raine et al. 2020). Indeed, burial probability in capuchins' and guans' feces was more similar (burial probability in capuchins feces was *circa* 10% higher than in guans feces for all bead sizes). Thus, our results indicate that the amount of dung is a better predictor of attractiveness than dung type, as burial probability was higher in howlers' feces than capuchins'. Similar results in tropical forests support this idea (Andresen 1999, Culot et al. 2009, 2018).

In accordance with other studies (Andresen 2001, 2002a,b, Santos-Heredia et al. 2010), burial depth was greater in howlers treatments, which contained larger piles of dung. Large dung beetles usually are more attracted to larger dung piles due to their active foraging (Peck and Howden 1984). They are more efficient at dung and seed removal (Slade et al. 2007) and bury seeds deeper (Feer 1999, Andresen and Levey 2004). Therefore, one can infer that howler monkey defecations, that are larger and clumped, attract large beetles, which in turn bury seeds deeper.

Burial depth also depended on seed size: smaller seeds were buried deeper in all treatments, as larger seeds are more easily detected and removed by dung beetles. This same result was seen in other studies (Andresen 1999, Feer 1999, Andresen and Levey 2004, Lugon et al. 2017). It should be noted, however, that howlers clumped defecation did not attract more seed predators. On the other hand, seed size, burial depth and the interaction between these factors affected predation probability. That is, larger seeds were preyed upon more often and their probability of predation decreased less with increasing depth than for smaller seeds.

Although burial depth decreases seed predation, it does not have a positive relation to seedling recruitment. In this and other studies (reviewed by Andresen and Feer 2005) , seedling recruitment had a tendency to decrease with burial depth. However, recruitment probability also depended on seed size: larger seeds recruited more frequently at greater depths. The final outcome was that the proportion of 3mm seeds that recruited did not vary with primary disperser; but, for 7, 10 and 14mm seeds, dispersal by howlers resulted in a higher proportion of recruitment success. Additionally, for all seed sizes, the proportion of seeds that survived but did not recruit was larger when dispersed by howlers. Thus, howlers contribute not only for the recruitment of large seeds but also for the

seed bank composition in TNP.

Ideally, the recruitment experiment should have accounted for primary dispersers. That is, seeds should have been fed to primary dispersers before the experiment, so we could test for the effect of gut passage. We did not include this procedure as we did not have enough seeds. Nonetheless, while howlers have a positive effect on seed germination (Arroyo-Rodríguez et al. 2015), only 34% of seeds remain viable after ingestion by capuchins (Mikich et al. 2015). Thus, the exclusion of gut passage treatment has probably underestimated the positive effect of the reintroduction of howlers in the TNP's seed dispersal.

The biased extinction of large mammals (Dirzo et al. 2014) and birds (Newbold et al. 2013) is a long-time concern. Larger animals have a disproportionate contribution to seed dispersal (Donoso et al. 2017). For instance, smaller animals that remain in defaunated forests are not able to disperse large seeds. Additionally, as seen in this study, the higher probability of secondary seed dispersal in the piles of dung produced by larger animals can increase the recruitment probability of large seeds. Consequently, the densities of saplings of large-seeded plants are severely reduced in defaunated forests in comparison to small seeded ones (Terborgh et al. 2008). A decreased density of large-seeded plants can have cascading effects on the provisioning of ecosystem services due to the positive relation between carbon storage capacity and seed diameter in tropical trees (Bello et al. 2015).

The loss of large animals also affects dung beetle communities (Bogoni et al. 2019). This trend has been observed specifically for howler monkeys presence and abundance (Andresen 2003). Additionally, there is an apparent direction on the reduction of large beetles abundances (Culot et al. 2013). Large beetles have a disproportionate contribution to dung beetle functional activities and a decrease in their abundances can lead to reductions in secondary seed dispersal,

bioturbation and nutrient cycling in defaunated areas (Nichols et al. 2009).

Reintroductions can be applied to reverse defaunation scenarios (Oliveira-Santos and Fernandez 2010, Seddon et al. 2014). Yet, most reintroduction efforts are directed towards the conservation of endangered species, and not the restoration of ecological functions and processes (Polak and Saltz 2011). Furthermore, there is a strong bias towards vertebrates reintroductions (Seddon et al. 2005). Reintroductions projects should have a broader view on ecological processes, including all steps and not just the initial interactions. We propose that dung beetles should be reintroduced after the reintroduction of large animals with which they interact. This management action could improve seed dispersal and regeneration of large seeded plants.

Howler monkeys are good candidates for reintroduction projects to restore seed dispersal and plant regeneration. First, they are resilient and able to inhabit and connect small forest patches (Arroyo-Rodríguez and Dias 2010). Secondly, they swallow seeds up to 4.6cm (Arroyo-Rodríguez et al. 2015) in length and although their clumped defecation results in high seed densities, secondary seed dispersal by dung beetles increases large seed survival and recruitment, as discussed above. In fact, seed dispersal by howlers can partially compensate for the absence of larger seed dispersers (Culot et al. 2017).

The yellow fever outbreak in 2016-2017 (Moreira-Soto et al. 2018) has caused the reduction and extinction of many populations of howlers. The species *Alouatta guariba*, for example, used to be considered of no concern to IUCN and is now vulnerable (Jerusalinsky et al. 2020). Despite howlers' positive attributes, their reintroduction in TNP was the first with the goal to restore ecological processes (Andresen et al. 2018). Howlers' reintroduction in TNP was also affected by the yellow fever outbreak: it was interrupted and only 5 individuals are currently in the area. We hope that our results will encourage the resuming of howlers

reintroduction in TNP as well as new reintroductions in different areas.

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