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ORIGINAL ARTICLE

On the way to systematize habituation: a protocol to minimize the effects of observer presence on wild groups of *Leontocebus lagonotus*

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Abstract

Habituation is used in most field research with primates to minimize observer effects on their behavior. Despite its importance, there is little published on the methods used to habituate different taxa of primates or how these methods vary in different habitat types. We assessed changes in behavior and space use of two groups of *Leontocebus lagonotus* in the Ecuadorian Amazon in order to document this process. Although the subjects had not been studied before, visitors and researchers were more frequently in the home range of Group 1 than of Group 2. We followed both groups for 2 months, collecting behavioral data through scan sampling and recording the use of space (ground, understory, subcanopy, and canopy) and the routes along which we followed the groups. We then divided our data into two equivalent stages, randomized the data for each stage and looked for significant differences using Wilcoxon tests. Our results show a significant decrease in submissive behaviors toward the observer for both groups and a significant increase in resting and foraging for Group 1. In addition, Group 2 used the subcanopy significantly less and the understory more during the second stage. The routes the animals used were significantly longer in the second stage for Group 1, but not for Group 2. We conclude that our methodology is adequate to advance in the habituation of *L. lagonotus* in less than 2 months and that a group will habituate more quickly if it has had some previous neutral exposure to humans.

Keywords Habituation · Habituation protocol · Observer effects · Saddleback tamarins · *Leontocebus lagonotus*

Introduction

In the study of animal behavior, habituation is the process by which wild animals accept the presence of human observers as a neutral element in their environment (Tutin and Fernandez 1991). The methodology of habituation proposes exposing the animals to neutral observers (i.e., without impacting their behavior), repeatedly and long enough to observe natural behaviors. In this way, the effects of the observer's presence on the behavior of animals is eliminated or, at least, minimized (Williamson and Feistner 2003). Since Carpenter (1934) put this mechanism into practice for the first time to study wild primates, habituation has become common practice for many studies of wild animals including birds (Ellenberg et al. 2009), carnivores (Hofer and East 2008), and cetaceans (Connor and Smolker 1985).

Currently, most field studies of primates are preceded by a habituation period (Jack et al. 2008; Aguiar and Moro-Rios 2009) to reduce the effects of the observer's presence on the animals and to facilitate data collection and identification of individual study animals (Goldsmith 2005). Despite its widespread use, there is no published systematized method to habituate a group of primates. Researchers seldom describe the habituation process or the results, thereby limiting our knowledge of this mechanism.

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The effectiveness of habituation varies according to different factors, especially the study species, type of habitat, visibility in the habitat, and the animals' previous experiences with humans (Tutin and Fernandez 1991; Williamson and Feistner 2003). Souza-Alves and Ferrari (2010) found that vegetation impedes the habituation of *Callicebus coimbrai* (Kobayashi and Langguth 1999), as the titi monkeys were able to hide in vegetation and avoid contact with the observers. Blom et al. (2004) concluded the opposite in the case of gorillas (*Gorilla gorilla gorilla* Savage, 1847), assuming that abundant vegetation facilitated habituation by making it a less stressful process for the animals, as they could hide from observers even when they were in close proximity. In addition, arboreal and/or gregarious primates are more easily habituated than terrestrial and/or solitary ones (Aguiar and Moro-Rios 2009). Furthermore, other researchers have found that habituation will be more difficult if animals are afraid of humans due to hunting activities, or if humans are considered competitors due to their foraging behavior (Tutin and Fernandez 1991). Therefore, previous negative encounters with humans could be an important obstacle to the habituation of primates. Conversely, neutral encounters (Jack et al. 2008) and positive stimuli, such as provisioning, are usually considered favorable (Goodall 1986; Whittaker and Knight 1998; Bertolani and Boesch 2008). However, provisioning is rejected by most researchers since it can spread diseases (Wrangham 1974) and change the behavior of the animals (Sugiyama 2015).

African apes require the longest habituation periods, sometimes up to 10 years (Doran-Sheehy et al. 2007; Williamson and Feistner 2003). In some cases, such as with western gorillas, even a decade may not be enough to achieve habituation, due to low population density, large home ranges, and poor visibility, which make it difficult for researchers to locate and observe animals (Doran-Sheehy et al. 2007; Tutin and Fernández 1991).

In contrast, if subjects are easily located and observed, it may be possible to habituate animals more rapidly: for example, Narat et al (2015) habituated a group of chimpanzees (*Pan paniscus* Schwarz 1929) in a period of 18 months. Nocturnal primates and most diurnal and cathemeral lemurs require the shortest habituation periods, from only a few hours to less than a month (Andrews and Birkinshaw 1998; Williamson and Feistner 2003). Thus, environmental conditions as well as species-specific reactions to observers can impact habituation processes. Williamson and Feistner (2003) suggested that callitrichines require less time to be habituated than most anthropoids, with a period of 2–5 months often being sufficient time for habituation to occur (*Callimico goeldii* Thomas 1904 is an exception since it usually requires 7 months or more to become habituated (Porter, 2001; Porter and Garber, 2010)). This conclusion, however, is based on limited data, as there are few publications concerning the habituation process for this taxonomic group (Rylands 1986; Passamani 1998; Rasmussen 1998).

Rasmussen (1998) describes habituation of Geoffroy's tamarins (*Saguinus geoffroyi* Pucheran 1845) that had been reintroduced on an island in Panama. During this study, researchers reduced the approach distance to the study group from 100 to 30 m in a month and a half. In addition, the tamarins significantly reduced their effort to flee from the observers in this period of time. It is necessary to point out that, according to the authors, the subjects of this study had probably been exposed previously to both human hunters (although it is unknown whether they targeted tamarins) and researchers studying other taxa on the island.

Little is known of *Leontocebus lagonotus* (Jiménez de la Espada 1870), the red-mantled saddleback tamarin, except for its distribution (De la Torre 2017) and its ability to live in disturbed forests (Aquino et al. 2014). In spite of its adaptability, if the level of disturbance is severe, its population density decreases (De la Torre 2017).

Our study aims to establish habituation protocols for this species. For this purpose, we studied the habituation process of two groups of red-mantled saddleback tamarins in the Ecuadorian Amazon. Our specific objectives are (1) to assess changes in the behavior of the study groups throughout the process of habituation, especially in the agonistic behaviors caused by the presence of the observer; and (2) to assess the changes in the groups' use of space, analyzing the use of forest strata and the daily path lengths (calculated from the routes along which the groups were followed for) throughout the process of habituation.

Methods

Study zone

The Jatun Sacha Biological Station (JSBS) is a 2200-ha reserve of tropical rainforest and a biological station located in the Ecuadorian Amazon (01° 4 'S; 77° 36′ W; Napo, Ecuador) (Fig. 1). It has a very humid uniform megathermal climate, with average annual temperature and precipitation of 25 ºC and 4500–5000 mm (Murray 2000; Jatun Sacha Foundation 2018), and its altitude ranges between 400 and 450 masl (Murray 2000). Only 70% of the original primary forest remains. The other 30%, mostly corresponding to the territory borders, is composed of a mosaic of secondary forest, scrubland, plantations, and pastures (Murray 2000).

The reserve harbors an exceptionally diverse variety of flora and fauna (Pearman et al. 1995), particularly herpetofauna (Vigle 2008). Concerning primates, three groups of *Leontocebus lagonotus* have been detected, as well as a single howler monkey (*Alouatta sp.* Lacépède

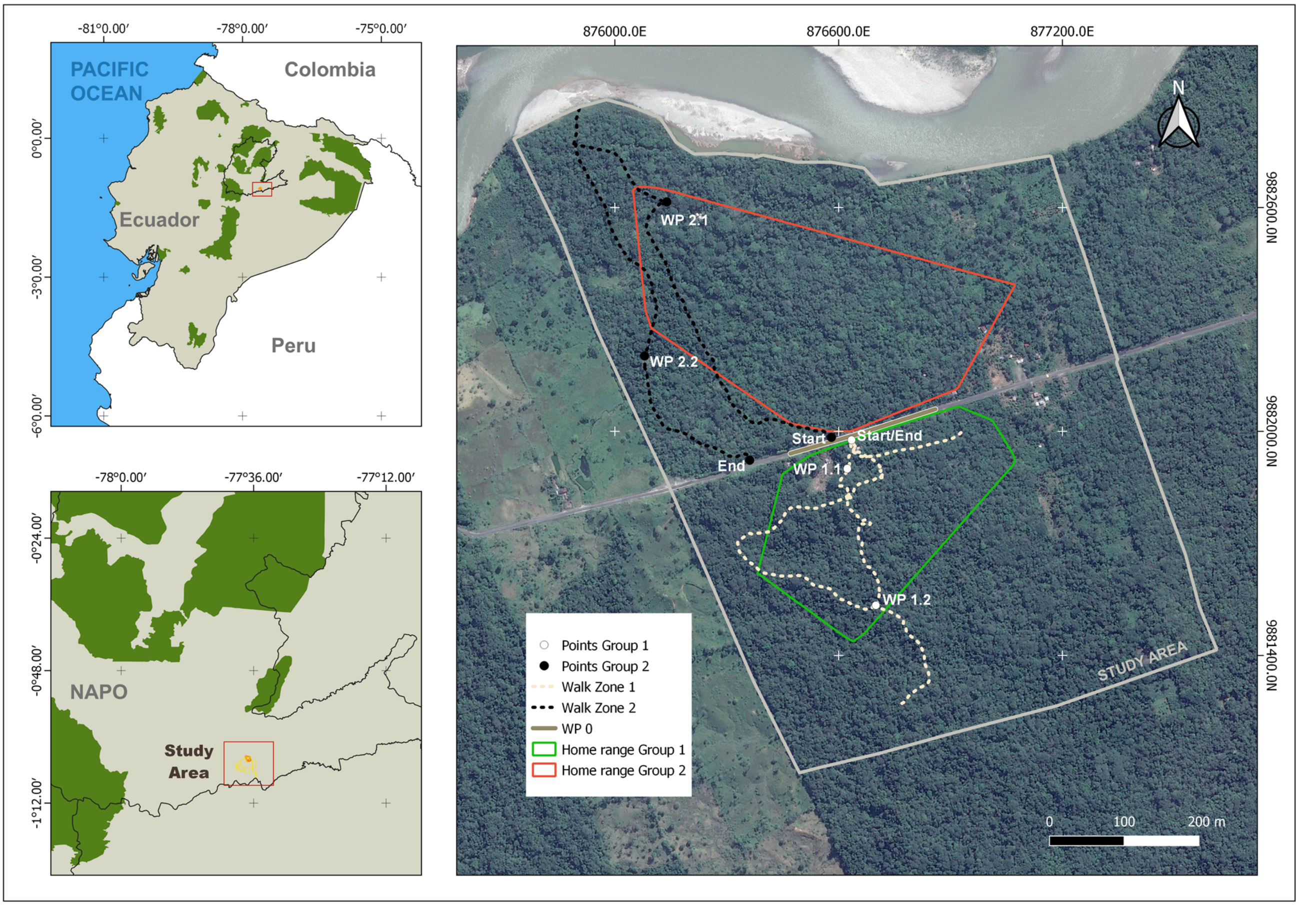

Fig. 1 Location of the study area; waiting points (WPs), start/end points of the walks and trails of the walks for both study groups, and home ranges of the two study groups

1799) and some squirrel monkeys (*Saimiri cassiquiarensis* Lesson, 1840). Mammals and birds in the area are hunted currently (A. Suárez, personal communication) and historically (Devries et al. 1997) by surrounding Kichwa populations.

The JSBS was divided into two fragments by the recent construction of a highway. The first fragment, Zone 1, covers most of the extension of the JSBS and includes almost all the reserve infrastructure (the biological station, the observation tower, and most of the trails, among others). The second fragment, Zone 2, comprises a smaller area and is less frequented than Zone 1 by both visitors and reserve employees. The reserve employees consist of a group of three members of the Kichwa community (sometimes joined by one or two foreign volunteers) whose main duties are the maintenance of the station facilities, the maintenance of the paths running through the reserve, and guiding the occasional visitors. In 2017, the JSBS received a total of 553 visitors (Jatun Sacha Foundation, personal communication).

Study subjects

We chose two *L. lagonotus* groups to begin this habituation project. During the 2-month study period, Group 1 consisted of eight subjects, including adults and subadults. At the end of the study, two offspring were born into the group. This group occupied Zone 1 of the JSBS and was regularly seen by the reserve employees (between 0 and 3 times per day).

Group 2 consisted of nine individuals during the study period. Most were adults, but we presumed that it also included subadults and juveniles (it was difficult to accurately determine the age of all individuals due to their elusive behavior; however, based on very rough estimates of individual body sizes, we assume that most group members were adults). This group occupied Zone 2 of the JSBS, and thus it was seen less frequently by visitors and reserve employees. Like in Group 1, a pair of twins were born into Group 2 at the end of the study period.

We did not follow the third group of *L. lagonotus* because its home range included private land and marshy areas which were difficult to access. None of these three groups had been studied or habituated before.

Data collection

We collected data from October 12 to December 16, 2017. To avoid the bias that the increase in the experience of the observer could cause, as well as the possibility that the subjects become habituated to a specific person, we replaced the first observer with a second person (who had the same level of previous experience) halfway through the study. Observers worked from dawn (5:45–6:00 am) until sunset (around 6:00 pm) or until the arrival, if observed, of the followed group to the sleeping tree. Each day the observer completed a fixed trail that was designed to systematize and facilitate locating the study groups. This trail included waiting points (WP; specific locations where the observer remained stationary for 20 min) and walks made at a speed of approximately 1.25 km/h (Peres 1999), all of which were established based on the information the reserve employees provided as to where they had seen the tamarins. Five WP were set: two in Zone 1 (WP 1.1 and WP 1.2), two in Zone 2 (WP 2.1 and WP 2.2), and one on the road that separates the two zones (WP0) (Fig. 1). WP0 differed from the rest of the points in two aspects: (1) it consisted of a small section (405 m) that the observer walked over and over again during the waiting time (instead of a point where the observer remained); and (2) the wait lasted 1 h 45 min (instead of 20 min, since, according to the employees, this was a location that both groups visited very frequently).

Specifically, our daily schedule was as follows: 5:45–8:00 am, waiting and walking along WP 0; 8:00–12:00 am, walking along Zone 1 (2385 m; it includes waits at WP 1.1 and WP 1.2); and 13:30–18:00 pm, walking along Zone 2 (2495 m; it includes waits at WP 2.1 and WP 2.2). We alternated the schedules so that if the walk along Zone 1 was completed in the morning, the next day it would be in the afternoon and vice versa. WP0 remained fixed every day at 5:45–8:00 am because, according to the reserve employees, this was the zone of maximum sighting of both groups at this time.

When a group was located, the observer would halt the search procedure to follow the group. The observer would follow the group close enough to be able to observe the behaviors of the subjects and at the same time be well visible to them. This distance ranged from 5–15 m depending on the vegetation cover of each area, since this affected the visibility of both the observer and the subjects. When the group was lost, the observer would resume the search protocol at the stage where they had left off. The observers wore clothes in tones in accordance with the surroundings (brown and dark green), just as the reserve employees and other researchers usually did. However, these clothes were not intended to camouflage the observers in the environment, but to standardize the appearance of all researchers, thus guaranteeing that the habituation results are homogeneous and independent of who is observing. These measures were intended to prevent the subjects from generating specific behavioral responses to specific people. The observers never tried to hide from the subjects by concealing in the vegetation or walking stealthily. On the contrary, they took care to remain clearly visible, although maintaining a calm attitude and avoiding causing disturbances such as loud noises (slight noises occurred when walking and dictating the observed behaviors to a recorder) or sudden movements. They also avoided interacting with the subjects in any way.

We collected behavioral data through scan sampling (Altmann 1974), recording the behavior of all visible individuals every minute. An ethogram developed specifically for this study was used (Appendix 1). We also recorded other remarkable information ad libitum (Altmann 1974) to ensure that if any event that could affect the habituation process took place (e.g., hunters, movement into a neighboring group's home range), it would be noted. Subjects were not identified at the individual level. Individuals carrying offspring were counted as a single individual in the scans.

Additionally, we collected information with respect to the group's use of space, both vertically and horizontally. Regarding the vertical use of space, or forest strata, we considered four categories: (1) ground; (2) understory (from ground level to the lower half of the arboreal canopy, approx. 15 m high); (3) subcanopy (the immediately higher stratum, partially exposed to direct sunlight, approx. 15–25 m high); and (4) canopy (the highest stratum of the arboreal canopy, exposed to direct sunlight, approx. 25–27 m high). The observers were previously trained to estimate measures with the same criteria.

Regarding the horizontal use of space, we used a GPS device (Garmin 64s) to record the routes along which a group was followed for from the moment it was located until it was lost (tracks). On occasions when it was not possible to follow the group, we recorded the group's location where it was observed (sighting point).

Data analysis

We divided the collected data into two equivalent stages of 25 days each. In this way, data were classified according to whether they belonged to the first or second habituation stage, a methodology used previously in habituation projects (Jack et al. 2008). With these data we elaborated two datasets, one for each study group, composed of the following variables: "Fleeing from Observer", "Aggressive toward Observer", "Type of Aggressive toward Observer" (display, alarm call or display with alarm call), "Traveling", "Foraging", "Resting", "Other Behaviors", "Out of Sight", and “Forest Strata” (see Appendix 1 for definitions of the behaviors mentioned). We calculated percentage frequency distributions of all the behaviors, the type of aggressive behavior toward the observer and the use of the forest strata.

In order to evaluate whether behaviors changed throughout the habituation process, we used Wilcoxon tests (since data were not normally distributed) to assess the differences in the variables between the first and second stages. To carry out these tests, data were paired at the scan level: for each scan of the first stage, we noted the number of subjects that were performing each one of the specific behavioral variables mentioned in the previous paragraph (each variable in a separate column), and we did the same for each scan of the second stage. Then we paired the column of scans of the first stage with those of the second stage for each of the behavioral variables, and we performed a Wilcoxon test per variable.

In the case of “Forest Strata”, a Wilcoxon test was carried out for each stratum. We noted separately the number of individuals that were occupying a specific stratum during each scan and paired these data between the first and second stages. Finally, differences in daily path lengths between the two stages were also evaluated with a Wilcoxon test, pairing the lengths of the tracks made in the first stage with those of the second.

The effects of the independent variables were considered significant when $p \leq 0.05$. Since the second stage of habituation contained a greater amount of data than the first stage, we randomly selected the exact number of cases among the data for the second stage to make it equivalent to the first one for all analyses. In addition, a randomization process was also carried out with the data for the first stage of habituation for all analysis.

All statistical analyses were carried out using IBM® SPSS® Statistics Version 21.0 (Armonk, NY, USA) for Macintosh. For some of the descriptive analyses, Microsoft® Excel Version 15.37 for Macintosh was also used. Spatial data were introduced in QGIS 2.18.13-Las Palmas © 2002–2016 for Macintosh. Home range was estimated from the tracks and throughout sighting points using QGIS “Minimum Bounding Geometry” complement.

Results

We collected data on 50 days (25 for each stage) for a total of 500 hours of fieldwork and 947 scans. Table 1 summarizes the number of days each group was seen in each stage of habituation as well as the number of scans performed per group and stage.

Progression in behavior and reactions to the observer

Group 1

We registered 11 of the 18 behaviors of the ethogram, with “Traveling” and “Fleeing from Observer” being the most frequent (37.8% and 20.6% of the total cases, respectively). The remainder were “Resting” (12.9%), “Out of Sight” (12.2%), “Aggressive toward Observer” (8.9%), “Foraging” (3.4%), and “Other Behaviors” (4.2%). We did not observe the other behavioral categories in the ethogram (Appendix 1).

Comparing the two stages, there were significant differences in the proportion of “Fleeing from Observer” ($z = -2.451$, $p = 0.014$), which decreased from the first stage of habituation (36.4% of total records) to the second (18.2%). Significant differences were also found for “Resting” ($z = -2.100$, $p = 0.036$), with a lower frequency in the first stage (11.3%) than in the second (19.3%). Finally, “Foraging” showed a significative increasing trend ($z = -2.120$, $p = 0.034$) as well, being less frequent in the first stage (2.5%) than in the second (7.0%). No significant differences were found for “Aggressive toward Observer”, “Out of Sight”, “Traveling”, or “Other Behaviors”.

The predominant type of aggressive behavior toward the observer was an alarm call in both the first and second stages (67.9% and 77.5% of the total cases, respectively). Alarm calls with display were the second most frequent type for both stages (21.4% and 17.5%, respectively), while displays without vocalization were the least recorded type (10.7% and 3.75%, respectively).

Group 2

We registered 10 of the 18 behaviors of the ethogram, with “Traveling” and “Fleeing from Observer” being the most frequent (29.4% and 23.5% of the total cases, respectively),

Table 1 Number of days in which the subjects were seen and number of scans performed for each group and habituation stage

	Days observed			Number of scans		
	First stage	Second stage	*Total*	First stage	Second stage	*Total*
Group 1	9	15	24	122	429	551
Group 2	8	18	26	115	281	396

as for Group 1. The remainder were "Out of Sight" (14.7%), "Aggressive toward Observer" (13.3%), "Resting" (9.5%), "Foraging" (6.0%), and "Other Behaviors" (4.2%). We did not observe the other behavioral categories in the ethogram (Appendix 1).

Comparing the two stages, there were significant differences in the proportion of "Fleeing from Observer" ($z = -4.423$, $p < 0.001$), which decreased from the first stage of habituation (40.0% of total records) to the second (14.2%). Also, "Other Behaviors" (which, in the case of Group 2, included "Self-Directed", "Grooming", "Playing", and "Scent Marking") were found to be significantly different ($z = -2.795$, $p = 0.005$), with a lower frequency in the first stage (0.7%) than in the second (10.7%). No significant differences were found for "Aggressive toward Observer", "Resting", "Traveling", "Foraging", or "Out of Sight".

The predominant type of aggressive behavior toward the observer was alarm call in both the first and second stages (67.7% and 65.6%, respectively). Alarm calls with display were the second most frequent type in both stages (27.1% and 32.8%), and displays without vocalization were the least frequent (7.3% and 3.1%).

Progression in tracks and use of space

Group 1

Wilcoxon test results showed significant differences between daily path lengths during the first stage as compared to the second stage ($z = -2.511$, $p = 0.012$). The average length of the tracks in the first stage was 234.0 m (standard deviation = 151.5 m) and in the second, 490.0 m (standard deviation = 408.9 m). Tracks, sighting points, and an estimate of the home range are represented in Fig. 1.

With respect to the use of forest strata, no significant differences were found between stages 1 and 2. In a descriptive manner, Group 1 used the understory slightly more in the second stage (48.7% vs. 51.9%), while the use of the subcanopy was similar between the two stages (48.7% vs. 48.0%). The use of the canopy decreased in the second stage (2.6% vs. 0.5%). The group was never observed on the ground.

Group 2

Wilcoxon test results did not show any significant differences between daily path lengths during the first stage as compared to the second stage. Tracks, sighting points, and an estimate of the home range are represented in Fig. 1.

With respect to the use of forest strata, there were significant differences in the use of the subcanopy ($z = -5.144$, $p < 0.001$), which was lower in the second stage than in the first (58.2% vs. 36.1%). Group 2 frequented the understory more in the second stage than in the first (39.0% vs. 62.0%), while the use of the canopy was similar between stages (2.9% vs. 2.0%). The group was never observed on the ground.

Discussion

We found it possible to make advances in the degree of habituation of *L. lagonotus* in less than 2 months. This was evidenced by the significant differences in the behavior of the groups, the daily distances we were able to follow the groups and the groups' use of forest strata.

We saw both groups more frequently in the second half of the habituation process than in the first. Although we performed a similar number of scans on both groups in the first stage, we collected more records of Group 1 than of Group 2 in the second stage, indicating that Group 1 was more habituated.

We recorded the same behaviors for both groups except for "Fighting", which was only observed in Group 1. However, a comparison of stage 1 to stage 2 demonstrated that the behavior of Group 1 changed more than Group 2. Group 1 fled the observers less, and rested and foraged significantly more in stage 2. Group 2 also showed significantly less fleeing in the second half of the habituation, together with an increase in "Other Behaviors", but neither resting nor foraging changed significantly. These results indicate that there was an improvement in the habituation of both groups, but particularly for Group 1 (Fig. 2).

In the process of habituation of another tamarin species (*Saguinus geoffroyi*), Rasmussen (1998) also found a reduction in the flight responses as the main result after a month and a half of work. In the same way, studies with

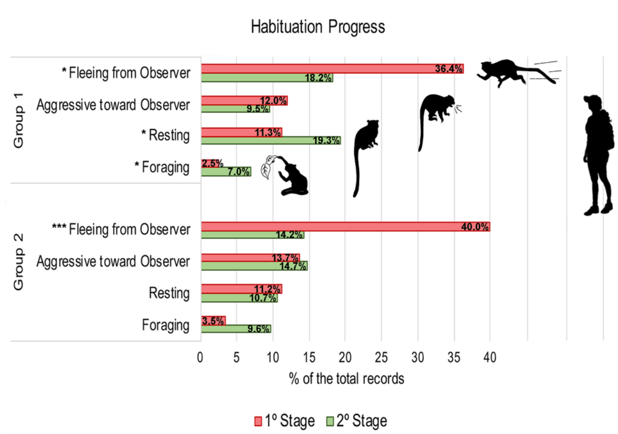

Fig. 2 Comparison between the percentages of several key behaviors to measure habituation that Group 1 and Group 2 showed during the first and second stages of the habituation process. Behaviors with significant changes between the two stages are marked with asterisks: *$p < 0.05$ and ***$p < 0.001$

other primate species also point out that the first obvious change in primate habituation is related to a reduction in flight responses [*Papio cynocephalus* (Linnaeus 1766) (Rasmussen 1979); *Chlorocebus pygerythrus* (Linnaeus 1758) (Mikula et al. 2018)]. Additionally, Williamson and Feistner (2003) indicated that unhabituated primates may alter their activity patterns during the habituation process, for example, by interrupting foraging and resting to flee from observers. Therefore, the increase in both resting and foraging that we observed for Group 1 in the second stage can be considered indicative of its habituation progress.

With respect to the use of space, daily path lengths were significantly higher in the second stage than in the first for Group 1. In the case of Group 2, we observed the same pattern of increase, but it was not significant. In both cases, the factor responsible for this increase could have been the reduction in the flight of the subjects and/or the reduction in the attempts of the group to lose the observer (Marsh 1981; Williamson and Feistner 2003).

In addition, the changes in use of the different forest strata also suggest that there was an advance in habituation. In this case, it was Group 2 which showed the largest change, using the subcanopy significantly less in the second stage and the understory more. Group 1 showed no significant changes in this regard. It is important to note that in the first stage of habituation, Group 1 used the understory and subcanopy with similar frequency, whereas Group 2 used the subcanopy more.

Considering that other studies have shown the saddleback tamarin to use the understory more than other strata (Aquino and Encarnación 1994), the high use of the subcanopy can be understood as an attempt to move away from the observer as if it were a terrestrial predator (Bianchi and Mendes 2007). Therefore, the increased use of the understory in the second stage may indicate that the subjects perceived the observer to be a neutral individual instead of a threat. The use of the canopy was similar between the two stages and the ground was not occupied at any time, which is expected given the presence of terrestrial predators (de Oliveira et al. 2003).

The fact that Group 1 seems to have habituated its behavior more than Group 2 might be due to differences in the initial state of habituation of both groups. Jack et al. (2008) suggest that neutral exposure to humans can increase the speed of habituation. Given that Group 1 inhabits the area with the highest degree of neutral human presence (researchers, volunteers, visitors, and reserve employees), this facilitation would likely have been more important for it than for Group 2.

In addition, it is possible that Group 2 members have undergone more negative previous experiences with humans, since they are located close to a Kichwa community and they were seen several times in their crops. These included *Mauritia flexuosa* Lf, *Bactris gasipaes* Kunth, *Oenocarpus bataua* Mart., and *Theobroma cacao* L. Van der Hoek et al. (2019) found that tamarins feed on insects associated with *M. flexuosa* fruits or stems and that the palm is also commonly used as nest site by primates, among other mammals and birds. If Group 2 fed on these crops, animal-human conflicts could occur, perhaps leading to a slowdown in the habituation of this group (Bertolani and Boesch 2008). However, regarding the use of forest strata, the habituation progress was notable for Group 2. This, together with the fact that Group 1 already used the understory widely in the first stage of habitation, may indicate that normalization in the use of forest strata occurs before normalization in other behaviors during the process of habituation of *L. lagonotus*.

Conclusions

The habituation methodology developed in this study has proven to be effective in reducing observer effects on wild groups of red-mantled saddleback tamarins (*Leontocebus lagonotus*). Given that tamarin species of the genus *Leontocebus* show many ecological and behavioral similarities (Rylands et al. 2016), the methodology described here could be equally useful to habituate all species of this genus and other callitrichines. Additionally, it could provide other studies of wild primates with guidelines from which to elaborate a habituation strategy.

More documentation in the field of habituation could enable researchers to solve problems like estimating the duration of the habituation period and to develop a more efficient design of the habituation process for each case. We hope that our study will encourage other researchers to share their experiences and knowledge in this matter in order to elaborate specific manuals and protocols to carry out the habituation of each species in an appropriate and systematic way.

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Author contributions All authors contributed significantly to the study. The design of the work was carried out by Sara ÁS and SV-A. Data collection was performed by SV-A and LS-S. Data analysis and interpretation was performed by SV-A and Sara ÁS. The first draft was written by SV-A and both Sara ÁS and LS-S contributed to its improvement with comments and work in the elaboration of the figures. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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