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Release and follow-up of a rehabilitated two-toed sloth (*choloepus hoffmanni*) in a tropical dry forest in Ecuador

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Short title: *Cholloepus hoffmanni* release and follow-up

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Abstract
We present the first record of post-release follow-up and monitoring of a rehabilitated two-toed sloth (*Choloepus hoffmanni*), first record of freezing behavior and first antagonistic interaction of the genus *Choloepus* with an owl (*Pulsatrix perspicillata*). Two-toed sloths are nocturnal and arboreal mammals which survival relies on their capability to remain undetected by predators. Unfortunately, in the Guayas province of Ecuador, they are the among the species most abundantly received in rehabilitation centers. The liberation of animals back to the wilderness is the main goal of rehabilitation, and the follow-up, or, post-release human presence/support of animals, facilitates their reestablishment in their natural habitat. Follow-up, direct observation and Bluetooth-based monitoring of the sloth secured the survival of the two-toed sloth. The range of detectability of the device used indicates its suitability for tracking low-mobility animals. After the first five days, the number of trees used per day increased, and 21 trees within 1152 m^2 were visited. Daylight and movement time range showed correlation towards detectability. The follow-up effort permitted to maintain safe the two-toed sloth during 12 days after release. Due to the difficulty monitoring nocturnal animals, economic constraints in conservation, accessibility, and safety of the animals, biodegradable Bluetooth based backpacks are recommended to ease the location of the animal and support its survival in the wild.

Keywords: Blue-tooth, Choloepus hoffmanni, follow-up, release, rehabilitation, monitoring.

Introduction

Comparative rescue and wild population abundance modeling predict that the activity of rehabilitation and release of rescued animals can have an important supporting influence on declining wildlife populations, especially long-living species (Paterson et al. 2021). Besides the anthropogenic threat level, certain characteristics of the biology of the species, such as the reproduction rate, determine the vulnerability of species towards local and global extinction (Caughley 1994). The combined efforts of veterinarians, rehabilitators and ecologists can build strategic interventions to maintain wild populations

(Paterson et al. 2021) and, therefore, their role in the protection of ecosystem processes (Caughley 1994; Vizcaíno and Bargo 2014; Superina and Loughry 2015).

Sloths are species that rely on their camouflage and slow movements to remain undetected and survive in forests with a high diversity of predators. The two-toed sloth (*Choloepus hoffmanni*) and other sloth species have been shown to survive in secondary forests (Plese et al. 2016). Various felids and other terrestrial predators that are native to Ecuador's coastal region, such as *Puma concolor*, *Leopardus pardalis* and *Eira barbara*, prey on two-toed sloths (Voirin et al., 2009; Pacheco Jaimes et al. 2018). Several camera trap studies have shown relative abundancy of ocelots (*Leopardus pardalis*) in various areas of the Ecuadorian coast (Cervera et al. 2016). On the contrary, the neotropical cougar (*Puma concolor*) has been elusive, even in the protected areas of Guayas province (Cervera et al. 2016; Mendoza et al. 2017; Solórzano et al. 2021).

The follow-up, or, post-release human presence/support of animals, involves a set of actions implemented in situ, ahead of the time of their release, to support their reestablishment in their natural habitat. The first weeks or months after release to the wilderness are a stressful and critical period of time during which animals suffer a high mortality rate due to behavioral deficits, predation, accidents and other reasons (Pottie et al. 2021). Species are considered for release according to their specific health and ethological characteristics (Choperena-Palencia and Mancera-Rodríguez 2018; Pottie et al. 2021). Nevertheless, survival of individuals in the wilderness is not guaranteed. Thus, to increase the likelihood of survival, minimal monitoring of body condition, behavior and important events (mating, death or birth, for example) is implemented to facilitate accident prevention and enable external aid and rescue according to every situation (Bello et al. 2018; Pottie et al. 2021). Logically, post-release follow-up for the animal increases the likelihood of its survival. In this regard, the presence on-site of monitoring teams and follow-up by rehabilitators has been identified as an important factor in the establishment process and survival rate for these animals (Bello et al. 2018; Pottie et al. 2021).

Monitoring animal survival after release is essential for recording whether the rehabilitation process has been accomplished, but it is rarely done in practice, given the amount of funds required (Choperena-Palencia and Mancera-Rodríguez 2018). Conservation projects often suffer from a lack of resources for implementing their objectives (Campos-Silva et al. 2018; Choperena-Palencia and Mancera-Rodríguez 2018). To monitor *Choloepus hoffmanni*, telemetry (based on satellite and radio signaling) and direct observation are commonly used methodologies (Choperena-Palencia and Mancera-Rodríguez 2018). Today, new tracking technologies that can be helpful for wildlife conservation are appearing on the market. Bluetooth tracker devices are cheap and are extensively used to locate missing objects at distance of up to 120 m, through Bluetooth technology, in which the location of the tracked object is sent to a smartphone. Use of this technology has already been proposed for monitoring patients with dementia (Shu and Woo 2021).

In this study, we report on an experience of following up and monitoring the release of a rehabilitated two-toed sloth (*Choloepus hoffmanni*) in the Prosperina protected forest (Bosque Protector Prosperina, BPP), Guayaquil, Ecuador. Additionally, we share our experience of in-situ use of a Tile-brand Bluetooth-based tracker device, attached to a handmade biodegradable backpack, in the reestablishment process for this two-toed sloth.

Methods

The two-toed sloth that was to be released arrived at the Mansion Mascota veterinary clinic (which is equipped for wildlife treatment and rehabilitation) and was intensively monitored, supported individually by a caregiver, and was treated by veterinarians from the institution. “Bravo”, a male two-toed sloth, was received weighing 750 g as an infant at 1-2 months of age. After 11 months, Bravo weighed 3.8 kg and showed strength, competence in movement on trees, active seeking of food (native leaves used in its diet) and aggressivity towards humans in general, but significant tolerance towards the caregiver. Bravo was also transported for further evaluations by an external veterinarian upon request from the Guayas province unit of the Ecuadorian Ministry of the Environment, Water and Ecological Transition (MAATE - acronym in Spanish).

The BPP is a 323-ha protected dry tropical forest inside the precincts of the ESPOL Polytechnic University in Guayaquil, which has been under restoration since 1999. It is connected to the 6078-ha Cerro Blanco protected forest (Bosque Protector Cerro Blanco, BPCB) reserve. The release point inside the BPP was selected for its connectivity and tree species. Various itineraries within the area of the BPP were analyzed through walks covering 11 km, and five different release points were considered. *Choloepus hoffmanni* is present in the BPP, which is favorable for this species to take up suitable habitats, but specific sloth observation points were avoided to prevent territorial conflicts (Pottie et al., 2021). The area selected for release (see map 1), contained abundant bototillo (*Cochlospermum vitifolium*), ceibo (*Ceiba trichistandra*), Saman (*Samanea saman*) and jocote (*Spondias purpurea*), which are important species in the diet of two-toed sloths. Bravo was allowed to get to know these species during the rehabilitation process (Bello et al. 2018; Choperena-Palencia and Mancera-Rodríguez 2018). In addition, the structure of the habitat was considered: this site presented different habitat levels, at heights ranging from 6 m to 22 m, with connectivity to surrounding patches of taller trees. To transport this two-toed sloth, we used a kennel transportation box with a fixed stick positioned transversally, which the individual could then grapple to, inside the cage, thus minimizing the stress of movement.

To ensure that the rehabilitated two-toed sloth became established in its habitat, follow-up was performed. The activity of two-toed sloths is predominantly nocturnal. They are awake from 9 p.m. to 4 a.m., with activity peaks between 9-10 p.m. and 2-3 a.m. (Sunquist and Montgomery 1973; Mosquera et al., 2019; Martínez et al. 2020). Thus, we conducted follow-up every day after release, from 5:30-6:00 p.m. to 4-6 a.m. Through continuous direct observation and annotations every 30 min, the sloth’s movements, interactions and vocalizations were recorded (Martínez et al. 2020).

Additionally, in order to track Bravo, a handmade biodegradable backpack with Bluetooth signal transmission capacity was fitted to his body (fig. 1C). The 34-g backpack was manually designed, cut in flexible fake leather and zipped with Velcro to facilitate release. The central area contained a cardboard slide with four Velcro junctions positioned to seal the backpack, so it was biodegradable and decayed without human manipulation. The device used was a Tile-brand tracker and its position was correlated with the location of the tree with the highest intensity of signal. To locate the animal more precisely, a Garmin GPS (+/- 3 m) was used. The Rcommander software was used to analyze the data (Fox, 2005).

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Results

The follow-up lasted for 13 days, over which the animal's wellbeing was confirmed on 11 days. The monitoring was concluded due to a nearby vocal threat from a felid. Subsequently, on days 11, 12 and 13 three daytime searches were performed with just one detection of the sloth. New interactions and behaviors were recorded during the monitoring, such as antagonism with owls (*Pulsatrix perspicillata*) (fig. 1C), a consequent fall from a tree (Table 1), and previous motionless defensive behavior (fig. 1D). The combination of detections and movement observation was highest at 7 p.m. (fig. 1A). Total detection and detection of the animal relative to the monitoring effort showed similar results; with two peaks that concurred with the observed movement of the animal (fig. 1B).

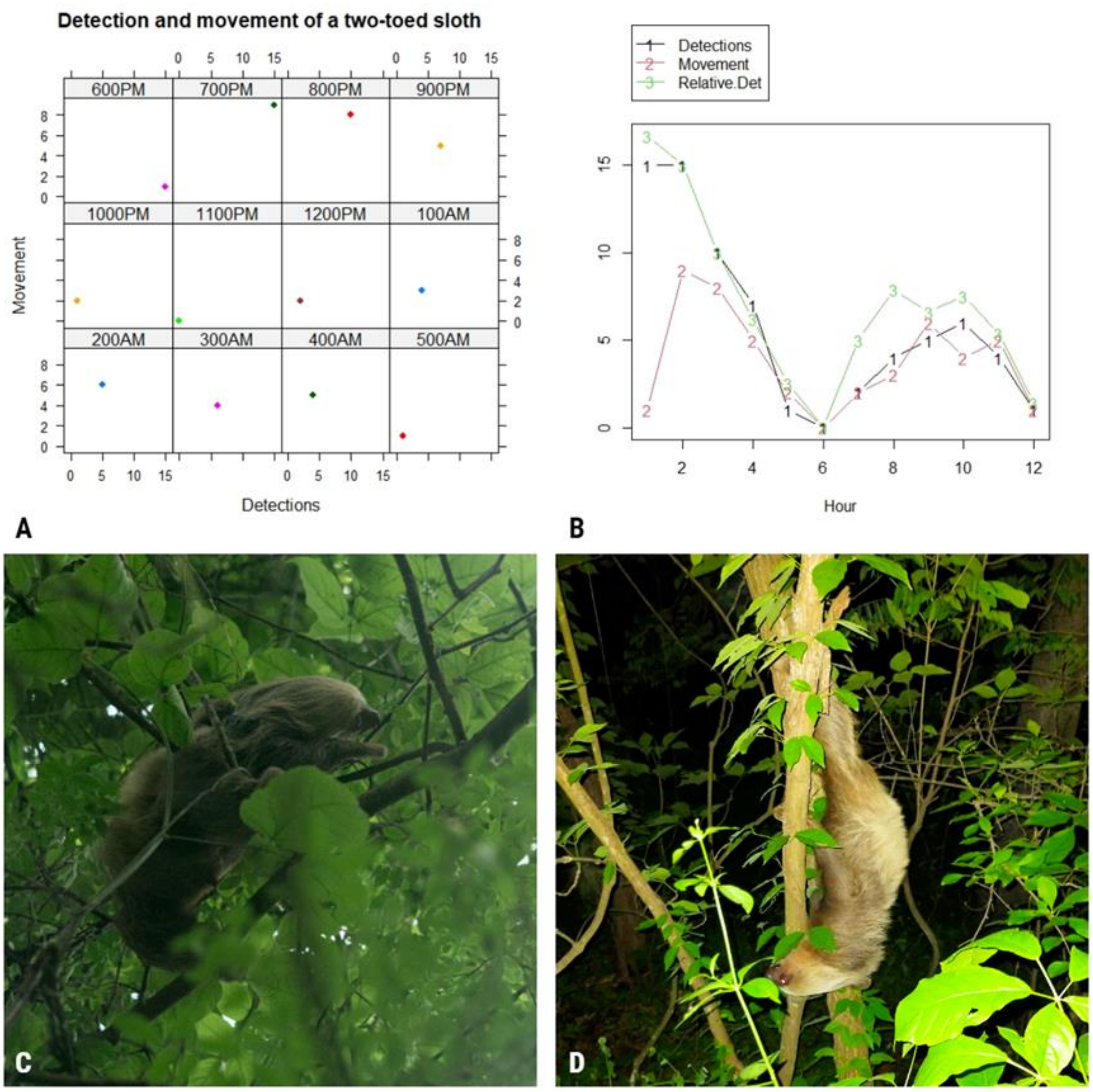

Figure 1. Follow-up and monitoring of the rehabilitated two-toed sloth (Choloepus hoffmanni). A) XY graph showing evaluation of the combination of detections and movement. B) Graph showing the movement observations, total detections and the relative detections during night monitoring (10 days). C) Bravo, the two-toed sloth moving through the trees on the day of release with a biodegradable backpack that

supported the Bluetooth detection device (Day 1). D) Motionless defensive behavior of Bravo, the two-toed sloth, under threat from an owl (Day 6). Source: R. Villalba-Briones.

The Shapiro test revealed that the detection and movement data were normally distributed (with p values of 0.08 and 0.58 respectfully). The Pearson test showed a mild correlation between the two parameters ($p < 0.1$; $p = 0.06$).

All the monitoring starting at 6 p.m. revealed that the two-toed sloth was sleeping. On the first two days, the sloth’s first movement along the tree was recorded at 8 and 9 p.m., but on the rest of the days its movements started at 7-7:30 p.m. (75% of the records).

Table 1. Record of observations during follow-up of the released two-toed sloth (*Choloepus hoffmanni*).

Days after release	Time (24-h clock)	Observation
Day 1	18:17	Release on the tree T1
	20:18	Efficient use of Tile device registered
Day 2	21:03	Movement along T1 registered
	3:09	Elusive behavior
	1:25	Urine odor detection (one site)
Day 3	18:00	Same initial site on T1
	21:30	Electric storm and abandonment of the follow-up
Day 4	22:43	First possible tree change detected on the Tile device
Day 5	20:45	First confirmed tree change visually detected
	0:08	Throughout the day, owl calls were recorded
	2:36	Urine odor detection (two sites), 20 m south of T1, uphill
	3:07	1.5 m height descent by the sloth, staring at the caretaker
Day 6	18:00	Tile backpack found degraded
	19:00	Light use increased for monitoring
	19:17	Descent for pooping.
	19:25-20:00	0-1 m height above ground, feeding (fig. 1C)
	21:36-4:36	Approximately five owl call events; far away and very close
	21:38-0:41	2 events of motionless sloth (fig. 1D)
	1:56	Fall from 6 m height from T6 subsequent to very close owl call
	2:03-2:17	Fast movement, going out of sight
Day 7	16:00-00:04	Active search until the sloth was found on T15
	0:48- 3:32	Faraway owl calls
Day 8	19:21	Very faraway owl call
	2:21	Very faraway owl call
	3:32	Medium-distance owl call and increased movement by sloth
Day 9	17:50	Urine markings detected (3)

	21:38	Medium-distance felid call recorded
	21:39	End of monitoring
Day 10	11:00	Search started
	11:28	Sloth detected sleeping in T19
Day 11	14:00-16:04	Search without detection
Day 12	12:36-13:31	Search without detection
Day 15	14:34-17:07	Search without detection
	16:35	Big mammal resting place found
	17:07	Roar at 5 m distance, at 2 m from the exit of the forest, and end of the follow-up.

The sloth's movement was recorded through an area of approximately 0.12 ha during the first 11 days of monitoring using a total of 19 trees, and feeding was registered in bototillo, saman and lianas.

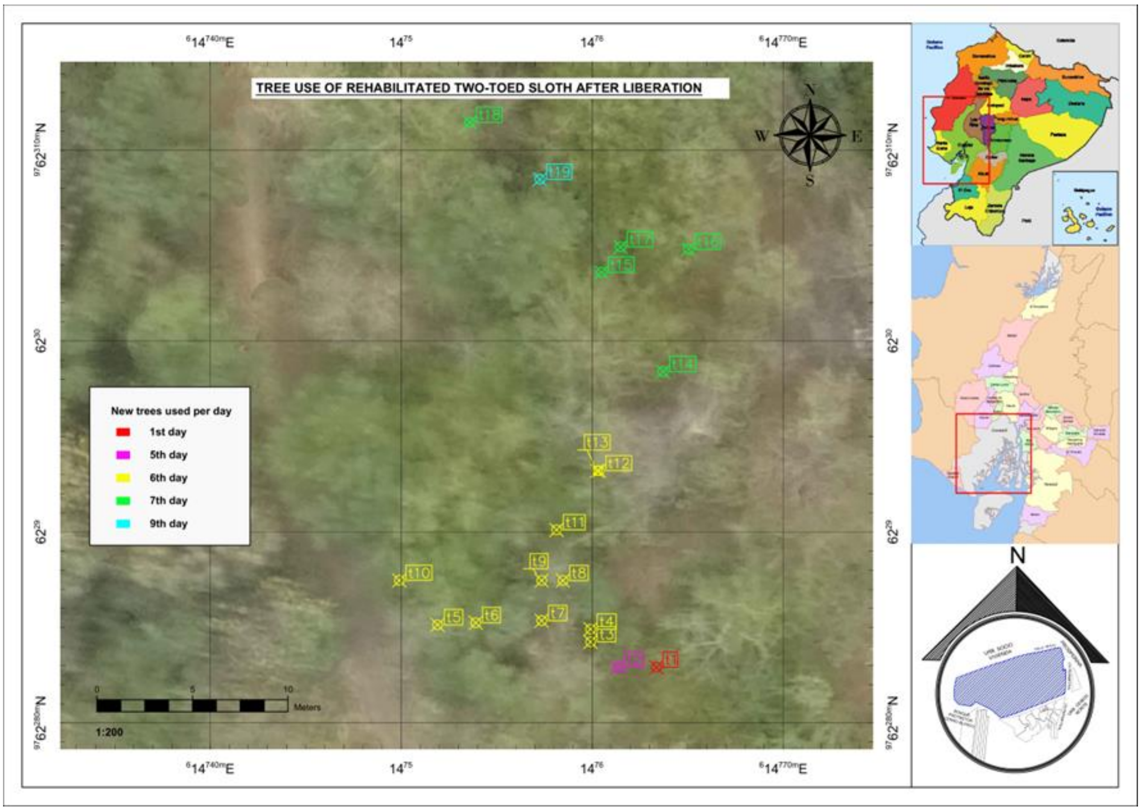

Figure 2.: Map showing the movements and tree use of the rehabilitated two-toed sloth (Choloepus hoffmanni) during monitoring in a dry tropical forest in the coastal region of Ecuador.

Discussion

This is the first record of follow-up of a rehabilitated *Choloepus hoffmanni* and the detectability analysis offers valuable information for future release and follow-up of individuals belonging to the genus *Choloepus*. The movement observations on this two-toed sloth showed that the highest peak was between 7 and 8 pm (8 observations), with a less defined peak between 2 and 4 a.m. (6, 4 and 5 observations) (fig. 1B), which concurs with previous studies on the activity pattern ranges for this and other species of the genus *Choloepus*. (Sunquist and Montgomery 1973; Mosquera et al. 2019). Through implementing direct observation methodology, the first peak of movement was confirmed by Martinez et al. (2020). Subsequent camera trap studies on *Choloepus didactylus* in humid rain forest ecosystem showed higher ground activity (salt licking) with a peak of seven observations at 7- 8 p.m., and less pronounced peaks at 10 p.m. and 3:30 a.m. In our study, detectability and movement correlations showed higher values at 7 p.m. and 3 a.m. (fig. 1A), which were significantly related, thus indicating that the sloth's movements favored the probability of finding sloths and that these were the proper times for searching for two-toed sloths. The discrepancy between detectability and movement over the first hour, at 6 p.m., was due to the higher detectability of sloths under daylight conditions (fig. 1B). In addition, the sloth was always found sleeping at 6 p.m., so it was easier to spot due to the previous night's monitoring observations and the daylight condition. Relative analysis between number of monitoring days and visibility showed consistency with the raw data (fig. 1D).

In this study, it is assumed that a masked owl (*Pulsatrix perspicillata*) showed antagonistic behavior towards *Choloepus hoffmanni*, given its continuous vocalizations coming closer to the sloth and the sloth's subsequent fall. Additionally, previous nearby vocalizations by a masked owl provoked motionless behavior by the sloth (fig. 1D), which is a common strategy among prey animals (Stevens and Ruxton 2019) but had not previously been reported in relation to *Choloepus hoffmanni*. Masked owl predation of sloths has previously been recorded in Panama. In that case, a *Bradypus* sloth (1.25 kg) was found partially eaten and presenting the marks of a masked owl, after a dramatic fall from a tree (Voirin et al. 2009). *Bradypus* individuals can weigh 3.5-4.5 kg. Similarly, our two-toed sloth weighed 3.8 kg. However, in our case, when Bravo fell from the tree, he was quickly found by the caregiver. After resting for a minute, he showed hyperactivity, moved fast and was lost in the direction of the initial owl calls. After this incident, it seems that the masked owls moved to another area. We can suspect that the sloth showed aggressive displays towards the masked owl in its nest or resting area, given that after this event, no more nearby calls were registered (Table 2).

Placing collars and backpacks on animals can be stressful for them, especially when they are still growing. This may therefore be detrimental to their survival in the wild. To avoid unnecessary prolongation of presence of the tracking device on the animal, satellite-controlled gadgets for automatic release are usually added, which significantly increases the total price of the product. Thus, it is important to mention that to avoid invasive extraction of monitoring devices, use of biodegradable material is a useful strategy. The lightweight Tile Bluetooth device did not pose any harm to the sloth, and heavy rains degraded the cardboard attachment, thus releasing the device. While functioning, it helped to locate the two-toed sloth even when the animal was out of sight. Therefore, this device shows potential for use on low-mobility species in future monitoring projects, especially in rehabilitation evaluation and post-release follow-up.

Conclusion

Follow-up is a helpful activity for increasing the survival of released animals. Successful rehabilitation is demonstrated through their establishment in the wild, which can only be verified through monitoring. The relationship between movement patterns and detectability is an important result to consider in the case of two-toed sloths, regarding monitoring through direct observation and follow-up activities. Daylight and knowledge of movement time ranges facilitate detection of two-toed sloths; thus, the best time for detection was found to be 7 pm. Species interactions are part of the process of how biological communities function, as shown in the antagonism between *Pulsatrix perspicillata* and *Choloepus hoffmanni*. Because of the dangers involved in constant exploration of wild forests, especially at night, and because of the influence of human presence on wildlife, we suggest using monitoring devices to locate released animals during the follow-up. For this, a biodegradable backpack with Bluetooth technology is a valuable option to consider, give its accessibility and effectiveness for locating subjects with low mobility. Further evaluation of Bluetooth-based monitoring is needed in order to measure its capabilities. Successful release of animals offers a second chance for these animals and can help in sustaining their populations and ecosystem. Therefore, we recommend that investment in post-release follow-up should be promoted among conservation agents.

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