Primate Conservation 2022 (36): 23-35

Golden-headed Lion Tamarins, *Leontopithecus chrysomelas* (Kühl, 1820): 27 Years of Experience in Methods for Their Capture and the Collection of Biological Material

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Abstract: The capture of free-living animals allows for the collection of important information that includes group composition, reproductive and health status, and biometric data. It also allows for the collection of biological material, individual marking, and the placement of radio-telemetry equipment to facilitate monitoring and subsequent ecological studies. Human-induced stress in free-living animals can arise due to their fight or flight reaction during capture, handling, confinement, or transportation. It is imperative that a detailed plan be established prior to any capture to ensure the safety of both the animal and the team. In this study, we report on 25 years of experience in capturing, collecting biological material, and releasing free-living golden-headed lion tamarins (*Leontopithecus chrysomelas*) in field projects in southern Bahia, Brazil. In this report, we describe the main activities we carry out in preparation for capture, handling, release and post-capture monitoring of these primates. We include detailed information about the bait used in trapping, chemical immobilization, and the collection of biological material, biometrics, tattooing, and radio-telemetry collar placement. Our hope is that this manual will help guide researchers working in the field with small Neotropical primates.
Key words: Neotropical primates, immobilization, trapping, Callitrichidae, protocol

Resumo: A captura de animais silvestres permite coletar dados importantes sobre a composição de grupos, estado reprodutivo, dados biométricos e estado de saúde de indivíduos. Além disso, permite a coleta de materiais biológicos, marcação individual e colocação de equipamentos de radiotelemetria para facilitar monitoramento e estudos ecológicos posterior a soltura. O planejamento detalhado de uma captura é fundamental para garantir tanto a segurança dos animais como a segurança da equipe. Este trabalho tem como objetivo relatar a experiência de métodos de captura, coleta de material biológico e soltura de grupos de mico-leão-da-cara-dourada em vida livre que vem sendo empregado em projetos de campo há mais de duas décadas anos no sul da Bahia, Brasil. Este relato aponta as principais atividades que devem ser realizadas antes da captura (planejamento), durante e após a captura (soltura) destes primatas. Estão incluídas ainda informações detalhadas sobre montagem de girais para captura de animais através de armadilhas, contenção química e monitoramento anestésico, coleta de materiais biológicos, biométricos, marcação de indivíduos e colocação de radio colar. Espera-se que este manual possa orientar outros projetos que trabalham com primatas neotropicais.
Palavras-chave: Primatas neotropicais, contenção química, armadilhas, Callitrichidae, protocolo

Introduction

In situ wildlife studies require a variety of field methods, oftentimes including the capture of individuals for handling (Brazil. MS, SVS, DVDT 2014). Obtaining biometric data and biological samples, for example, can require physical and/or chemical restraint of the animals (Brazil, MMA, ICMBio 2012). The publication of protocols, manuals and/or reports of capture experiences, such as handling methods for the collection of biological material, tends to reduce errors (Watsa *et al.* 2015) and unintended injury to, or even the death of the animal (Fedigan 2010). Unfortunately, most of the capture protocols for small Neotropical primates are either unpublished or published with insufficient detail (Dietz *et al.* 1996, 1997; Franklin *et al.* 2007; Monteiro *et al.* 2007), although there are exceptions, such as those of Savage *et al.* (1993) for cotton-top tamarins in Colombia and Watsa *et al.* (2015) for *Saguinus*, and Stone *et al.* (2015) for *Saimiri* in the Amazon region.

The lion tamarins, *Leontopithecus* Lesson, 1840, are endemic to the Atlantic Forest. There are four species, *Leontopithecus caissara*, *L. rosalia*, *L. chrysomelas*, and *L. chrysopygus*. *Leontopithecus caissara* is Critically Endangered and the other three are Endangered (EN) (IUCN, 2020; Brazil, MMA (2014b). Golden-head lion tamarins, *L. chrysomelas*, have a restricted range of *c.* 19,462 km² in the south of the state of Bahia, Brazil (Pinto *et al.* 1997). They occur in three protected areas: the Una Biological Reserve, the Una Wildlife Refuge and the Serra das Lontras State Park, with the remainder of their range composed of forest patches on private land.

The first field study of this species was in 1980, at the Lemos Maia Ecological Station in the municipality of Una (Rylands 1982, 1989). Studies on the ecology. demography, and health of *L. chrysomelas* in different habitats have continued to the present day (Dietz *et al.* 1996; Raboy and Dietz 2004; Raboy *et al.* 2004; Guidorizzi 2008; Oliveira *et al.* 2011; Tisovec *et al*. 2014; Catenacci *et al.* 2016; De Vleeschouwer and Oliveira 2017). Largely based on these studies, it is currently known that golden-headed lion tamarin groups consist of 3–15 individuals occupying home ranges of 22 to 197 ha, which they defend aggressively (Oliveira *et al*. 2011). The daily distance traveled by the groups ranges from 1,342 to 2,175 m and travelling accounts for about one-third of the time spent in their daily activities (Raboy and Dietz 2004). Individuals feed on fruits, seeds, gums, insects and small vertebrates, and they are important seed dispersers in the Atlantic Forest in southern Bahia (Rylands 1989; Catenacci *et al.* 2009). In addition to mature forest, groups range in secondary forest and cacao and rubber tree agroforests (Raboy et *al*. 2004; Oliveira *et al.* 2011; Catenacci *et al.* 2016; Oliveira and De Vleeschouwer 2017). One limiting feature for this species is the need for large trees with tree-hollows for shelter and an abundance of arboreal epiphytes (Rylands 1989; Raboy *et al.* 2004). From a health perspective, they are hosts to several pathogens, including intestinal endoparasites (Monteiro *et al.* 2007, 2010; Catenacci *et al.* 2016b; Costa *et al.* 2020), as well as protozoans (Monteiro *et al.* 2007, 2010; Aitken *et al.* 2016; Molina *et al.* 2017), bacteria (Molina *et al.* 2019; Santos *et al.* 2018), microfilariae and viruses (Catenacci *et al.* 2018; Miranda *et al.* 2019; Molina *et al.* 2019).

Most of the field studies described above used radiotelemetry as a tool to facilitate access to groups. Animals that receive such monitoring equipment are chemically immobilized to ensure safe and efficient placement of the telemetry collars. Anesthetizing these animals has allowed individual identification of the group members by tattooing and/or hair-dying on specific parts of their body (Dietz *et al.* 1996). In addition to placing (or replacing) radiotelemetry and marking the animals, field coordinators take advantage of the animals in hand to obtain biometric data and collect biological samples such as blood, hairs and feces.

In this paper we present details of capture techniques we have used to study populations of *L. chrysomelas in situ* in southern Bahia. We offer our long-term experience as a guide to inform other studies of small Neotropical primates. We emphasize that the techniques described here are a result of our particular experience, and that other techniques can be used, according to the context and objective of each field project and the animal involved.

Planning and Preparations

Permits

Before starting the field activities, official permission to capture these or any other nonhuman primates must be obtained from the Brazilian Environmental Agency (ICMBio) or similar body in other countries. In our case, activities related to research and/or teaching also required the approval of the Animal Welfare Committee of the *Commissão de Ética no Uso de Animais (CEUA)* and the Brazilian Genetic Heritage System (SisGen).

Pre-capture preparation

A well-trained multidisciplinary team is required to meet the basic requirements for the confinement of any wild species, *in situ* or *ex situ* (Mangini and Silva 2006). Holding pre-and post-capture meetings is a crucial step in the process. The planning of a capture involves training and organization of all team members (Brazil, MSD, SVS 2005). Each team member must know why the capture is occurring and what tasks are to be carried out during the capture, with consideration of ethical and legal issues, animal welfare (Fedigan 2010), and team safety (Brazil, MSD, SVS, DVDT 2014). In general, a capture team involves the project coordinator, biologists, veterinarians, and field assistants (Brazil, MS, SVS, DVDT 2014). A general coordinator should be appointed for the capture, and if the team has more than one veterinarian, a veterinary coordinator should be designated for anesthesia and monitoring. The person in charge of the team should discuss the schedule of activities, including

considerations for any possibility of failure, in order to minimize the risks. During chemical containment, the number of people present should be kept to a minimum to minimize stress for the animal.

Biosafety and biosecurity measures

The COVID-19 pandemic resulted in a series of recommendations and a reassessment of conduct in biosafety along with fieldwork with wild animals, including primates. Field team members should have updated vaccinations (including yellow fever, tetanus, hepatitis, influenza, rabies and COVID-19), with antibody titers periodically checked. Personal protective equipment must be available prior to handling animals, and may include goggles, disposable gloves, and masks with a PFF2 (N95) or PFF3 (N99) filter, leather gloves, closed-toed shoes, and coats (Brazil, MS, SVS, DVDT 2014). Other recommendations include those of the OIE (2020):

- Hand-washing with soap and water and/or application of hand sanitizer (>60% alcohol applied to clean hands) before and after handling wild mammals.
- Wearing disposable or clean, re-usable gloves, and change gloves between sampling events or handling individuals of species that are solitary.• Avoiding blowing on mammal's fur to see anatomical features or ectoparasites.
- Separating captured animals from each other to the extent possible when capturing and handling.
- Avoiding touching your own face or mask, and if contact occurs, changing/disinfecting your hands/gloves.
- Properly disposing of used materials and biological and hazardous waste.
- Cleaning and disinfecting all re-usable field gear and equipment that may be exposed to wild mammals prior to starting the work and after each fieldwork shift or between handling individuals of species that are solitary.

As concerns the COVID-19 pandemic, it is also important to assess the field team:

- If someone on the team tests positive for SARS-CoV-2 or has COVID-19 symptoms, they should follow public health advice on quarantine protocols and avoid working with wild mammals.
- If someone on the team has had contact with a confirmed or suspected case in the past two weeks, they should follow public health advice.
- No one who is currently showing symptoms of SARS-CoV-2 (fever of 38°C [100.4 °F] or higher, cough, etc.) should work with wild mammals.

These biosafety measures aim to minimize the risk of accidents to people during the research process as well as to reduce the risk of pathogen spread to the animals (Fedigan 2010).

Organize material: use a checklist

To begin field activities, all materials must be purchased, tested, and ready to use prior to animal handling activities (Brazil, MS, SVS, DVDT 2014). Ideally, the team will have a list of all equipment that will be used in the capture, and the name of the person responsible for procuring the material and the quantity of each item to be used to ensure that nothing is forgotten (Brazil, MS, SVS 2005). In addition, the field team can make a diagram/flowchart summarizing all the field activities. We recommend having checklists for the following items, considering that the choice of these materials depends on the management objective and costs:

A. Materials needed to monitor animals in traps: binoculars, pliers of different sizes, wire, bait, trap covers, leather gloves (highly recommended), and personal material (field notebook, pencil, water bottle, raincoat, flashlight and watch), and radio-telemetry receivers and antennas if the group is to be monitored.

B. Veterinary supplies, materials and equipment for containment and collection of biological materials: anesthetic and reverse drugs, stethoscope, 1 ml and 3 ml syringes, 22G and 26G gauge needles, 70% alcohol, cotton wool, gauze, tape, disposable gloves of various sizes, leather gloves, scales, calculator, PFF3 disposable mask, watch, doppler, cuffs, oximeter, thermometer and clinical and anesthetic monitoring sheet (highly recommended). Also useful, depending on the procedures and material to be collected, might be blood collection tubes with and without EDTA, iodized alcohol, trichotome, a razor blade, scalpel, microscope slides, filter paper, clipboard, permanent marking pen, pencil, 2 mL cryotubes, a feces collector, sterile pots, 10% formaldehyde, panoptic dye, methanol and Giemsa dye, a centrifuge, pipettes 10, 20, 200 and 1000 µL tips, liquid nitrogen canister, a styrofoam box, recyclable ice, small batteries, hemostatic forceps, net and cage, an apron, goggles, flashlight, candle, and lighter.

C. Veterinary emergency materials, equipment and drugs: ambu bag resuscitator, catheter, saline solution, 24 gauge catheter, tubing, scalp vein set #21, surgical kit, surgical sutures, thermal bag, laryngoscope, Adrenaline (dose: 1 ampoule/5 kg every 5 minutes), Aminophylline (dose: 0.04 mL/100 g, IV, IM or SC), Atropine (dose: 0.05 mg/kg IV or 0.02–0.04 mg/kg IM), Ketoprofen (dose: 5 mg/kg IM), Dexamethasone (dose: 0.25–1 mg/kg SC, IM), Doxapram (dose: 2 mg/kg IV), Epinephrine (dose: 0.1 mL/animal IV, IM, SC), Benzathine Penicillin G (20,000 IU/kg IM) (Valverde, 2010; Verona *et al*. 2014).

D. Materials and equipment for biometrics, radio collar placement, and marking of individuals: ruler, measuring calipers, pliers, tape measure, plastic bags, newspaper, camera, clock, clipboard, biometrics sheet, radio collar,

tattoo machine, Nyanzol dye, toothbrush to apply the mixture of Nyanzol and Hydrogen Peroxide, paper towels, permanent marking pen, Hydrogen Peroxide, 70% alcohol.

Reference values for the species

A literature search and contact with experienced field personnel greatly increase a team's knowledge and the safety of the work to be performed. When working with wildlife, especially *in situ*, it is essential to have a good understanding of the behavior and physiology of the species to be captured (Mangini and Silva 2006; Brazil, MS, SVS, DVDT 2014). The reference data for the genus *Leontopithecus* and for the golden-headed lion tamarins (Santos *et al.* 2019) are shown in Table 1.

Table 1. Reference values for the genus *Leontopithecus* (Source: Verona *et al*. 2014).

Parameters	Value
Adult male/female weight (in grams)	600–800 grams
Heart rate (beats per minute)	180–260 bpm
Respiratory rate (breaths per minute)	20–50 bpm
Rectal temperature (°C)	37.2–39.6 °C

Choosing where to set up the feeding platforms and baited traps

Procedures for assembling platforms, using traps and marking the animals were adapted from methods already used with *L. rosalia* in the state of Rio de Janeiro (Kleiman *et al.* 1986). The platforms should be set up in an area regularly used by the animals. If this area is not known, platforms should be set where a group has been sighted. It is important to verify, if it is known, that the location of the platform does not overlap with the home ranges of other groups of lion tamarins, so as not to risk capturing individuals belonging to different groups on a single platform (Brazil, MS, SVS, DVDT 2014). Prior to capture, the chosen site can be observed at different times to ensure, to the extent possible, that only one group is using the area.

Assembling the platform sites

The platforms should be set up about 1.5 m above the ground, with a wide central wooden base supported by strong wood crossbeams that are secured to adjacent trees (Kierulff *et al.* 2004; Tivosec *et al.* 2014). The central base can be assembled from tree trunks and interwoven with vines to resemble a table. It must be strong enough to support bait (e.g., several bunches of bananas), the traps and the monkeys themselves, in addition to the force of monkeys jumping on it (Fig. 1A). The platform should be set up in a wooded area with the trees and the crossbeams stable enough for the tamarins to descend and, by using the crossbeams as trails, access the bait. The supporting trees, crossbeams and the platform itself will all be options for subsequent trap placement.

Preparing the bait

After assembling the platforms, the bait (in this case, bunches of bananas) is placed on the base of the platform and attached with vines to prevent animals removing them from the platform. It is important to constantly check the baited platforms to ensure that there are always enough bananas to attract the lion tamarins. The bait should be replaced every 2 or 3 days and should contain both green and ripe bananas. It is recommended that the handling of bananas and bait should be done taking care to avoid contamination of microorganisms. Good handling practices should be discussed with the team and as recommended by the OIE and the ICMBio biosafety manuals (OIE, 2020; Brazil, MMA, ICMBio 2020). Bananas are the bait of choice because they are attractive (visually and aromatically), last a long time, are inexpensive (in our study locations), and useful to identify bite marks that indicate when the group has visited the platform. Camera traps can also be placed at the platform to check if the animals are visiting (Kierulff *et al.* 2004, 2005). If bananas fail to attract the lion tamarins, other fruits may be used such as grapes (which are similar to forest fruits; C. E. Guidorizzi and B. E. Raboy, pers. obs.).

Baiting duration and setting up traps

The lead-in time for baiting will vary depending on the habituation level of the group being handled. Habituated groups require a baiting time of about three weeks, depending on the specific environment. During the first week, the platform is built, and bananas are placed only on its central base, without traps. In the second week, a small number of traps (e.g., for a group of eight individuals, up to six traps) are set up locked in an open position (the trigger is deactivated), with bait at the base of the platform, at the top, and inside the traps. If the group is habituated, all the traps needed for capture can be placed at once (Fig. 1A). We use Tomahawk® live traps (49×17×17 cm), with their weights previously recorded in order to better estimate the lion tamarins' weights prior to anesthesia. The traps can be placed on the crossbeams that form the trail to the base of the platform and lean against the adjacent trees so the lion tamarins can descend easily and safely to the trap. They should be fastened with strong wire, both at the base (next to the beams), and on the edge (next to the tree trunk). Some traps can be placed on the base itself. When not being used for capture, they should remain locked open with a wire, so as not to be triggered. Special attention should be taken to make sure that the tips of the wires used on the traps will not prick or scratch the tamarins.

In the third week, the rest of the traps are opened and locked. If there are other primate species in the area, such as *Callithrix kuhlii* abundant in our study areas, it is advisable to place twice the number of traps than the number of tamarins in the group to be captured. It is known that *C. kuhlii* associate with the golden-headed lion tamarins (Rylands 1989; Raboy *et al.* 2008; Oliveira and Dietz 2011) and may unintentionally be captured with or instead of them. It is also important to set up extra traps so that the animals have

Figure 1. Images of the capture procedures for *Leontopithecus chrysomelas*. A: Platform for habituation to the traps (Photograph by B. E. Raboy); B: Trap open to start the capture campaign (Photograph by B. E. Raboy); C: Captured lion tamarin (Source: Projeto BioBrasil); D: Traps with covers for the field-laboratory transfer (Source: Projeto BioBrasil); E: Physical-chemical containment (Source: Projeto BioBrasil); F: Bead radio collar (Source: Projeto BioBrasil); G: Biometry; H: Releasing the animals in the field.

choices of which to use in the event that one location feels safer to them for descent than another. At the end of the third week, bait should only be placed inside the traps.

When capturing unhabituated groups, the process of baiting and setting traps can be carried out more slowly, to gradually accustom hesitant animals and reduce anxiety to the novel situation. The duration of baiting will depend on the visitation success of the relevant species. If after two weeks the animals only visit the platform occasionally, another location for the platform can be considered. For groups whose living area is unknown, it is advisable to set two platforms and abandon one as soon as the group begins to visit the other.

Monitoring visits of animals to the platform

Once the bait is in place, the platforms should be monitored every 2 or 3 days to check if they were visited by the lion tamarins, and to see if more bananas are needed. Checking to confirm no lion tamarins are in the vicinity, the researcher should approach the platform and look for evidence that the group had visited it. One method to confirm this is to check whether there are teeth and nail marks left on the bananas. Experience is needed to be confident that the bites are from lion tamarins as other marmosets or opossums, for example, may also be visiting the platforms. It is important to collect the remains of bananas that are partially eaten or have marks on them, so that there is no doubt in follow-up checks regarding the timing of subsequent markings.

Cleaning the field laboratory before use

The laboratory areas where the animals will be anesthetized and the "quarantine" areas where they are housed overnight should be disinfected prior to bringing animals in. The most frequently used protocol for cleaning these areas is to first sweep, then wash the area with soap and water, and finally disinfect the floor and benches with Sodium Hypochlorite 2%. Once the area is dry, a blowtorch should be used in places where the traps will be lodged and, on the benches, where the physical restraint and anesthesia will be conducted. The laboratory should have a place to store newspapers, as well as multiple disposal container options (common trash, hospital waste and sharp items). The cleaning protocol must be repeated between each group.

Cleaning the laboratory equipment before use

All material/equipment must be disinfected prior to use. When selecting a disinfectant, its efficacy against SARS-CoV-2 (EPA) and other pathogens that the animal being sampled may carry should be considered in addition to its potential effect on the equipment that will be used and its environmental impact. The 70% isopropyl alcohol or a 10% solution of household bleach are recommended. For both disinfectants, the surface must be cleaned before they are applied, and a working solution of bleach must be made fresh every day (OIE 2020). The same procedure must also be performed when manipulation of different groups will be performed.

Capture

We refer to the time during which the team will be actively capturing the lion tamarins as the capture period.

Opening the traps for the capture period

The capture period can begin when there is proof the group is regularly visiting the platform. A single banana is placed in the back of each trap and anchored by a thin wooden stick (Fig. 1B). Then, the wire previously affixing the trap in an open-locked position is removed to arm the trigger device. It is important to test the trigger device several times making small adjustments to minimize errors such as the trap triggering from too light a touch or not triggering despite a heavy touch. Other bananas on the platform must be cleared away leaving only the single banana in each trap.

Monitoring the traps during the capture campaign

Monitoring should be done about every two hours, depending on the weather conditions (rainy season, intense heat) and the composition of the group (nursing females, young animals or subadults). With unhabituated groups, the interval may be longer (but not more than three hours) if the team is sure that the platform was set up in a place where there are no overlapping groups. On rainy days, the interval between visits to the traps should be shorter in order to avoid hypothermia caused by the animal remaining wet for an extended period of time. In the case of heavy rain, the traps must be closed, and the capture stopped until weather conditions improve. Platform visits should be carried out with as few people, and as quietly, as possible, because the animals may be close, and the presence of researchers may drive them away. If when on a visit the tamarins are in the traps, note the time on the field sheet (Fig. 1C). If the group was not entirely captured, cover the trapped individuals' cages with large leaves to provide shade/cover and thermal comfort and retreat to let the rest of the group enter traps. The time between visits should be shortened to one hour, to ensure that trapped animals are not preyed upon.

If infants or lactating females are present in the group, infants less than one-month old should not be kept separate from the female for more than four hours. If necessary, the nursing female and the adult with the infant must be released to ensure that the infant is breastfed. Another option is to take all the animals to the field laboratory and anesthetize the nursing female first, followed by the animal with the infant. As soon as the animal carrying the infant is sedated, transfer the infant to the trap where the nursing female is located. If the female with the infant is out of the trap, another adult should be released so that it can support the female for the rest of the day, and so that the female and infant do not spend the night alone. If other species

or pregnant females are trapped, they must be immediately released.

Each capture situation is unique, and the team is responsible for deciding the best time to intervene, but animal welfare must always prevail in the decisions that the team has to make. After the entire group is captured, researchers should approach the trap set, reinforce the latch that holds the trap door closed with a wire, release the trap from the platform, and then put a sanitized cover over each trap to minimize stress (Fig. 1D). The traps with the animals should be taken to the field laboratory, where the other procedures will be performed. The animals should be transported to the field laboratory taking in account welfare measures, such as avoiding noise, covering cages and assessing distance and weather (with less exposure to sun and rain).

Processing

Quarantine and the sequence of activities to be conducted in the laboratory

Upon arrival at the field lab, immediately place the captured lion tamarins in a quarantine area, covered by newspaper or other paper sheets for better acclimatization. The order of housing the animals is important and should correspond with the order of processing to remove confusion when individuals are later taken in and out of the quarantine room. Usually, the order goes from those that seem to be larger and presumably weigh more to the smaller individuals. There are occasions, however, when the order may be changed. For example, if it is known that a certain animal will receive a collar regardless of their size (such as choosing a full-sized adult male to wear the collar even if it is slightly smaller than another because it does not carry infants as much) you will want to start with it first. Beginning with the lion tamarins that will receive the radio collars leaves flexibility in the event they are discovered upon processing to have problems that would contraindicate radio-collar placement, another animal may be chosen to wear the collar instead that has not yet been anesthetized. If an intermediate-sized animal already has a radio collar, it is suggested that the collar be removed and placed on a larger animal. Juveniles should be placed close to their mothers, when known, to keep them calm. The order may also be changed if there is any individual requiring immediate clinical examination or there is an animal that is carrying infants that's not the mother. They should be processed first, so as to get the infants off and move them to the mother.

Careful attention should be made as to when to feed (or not to feed) the captive animals. The lion tamarins should fast (no solid or liquid foods) for three to four hours, starting from the time the animal stops eating the banana used as bait in the trap before being processed. Depending on their recovery time, the time spent on procedures or weather conditions, the animals might be released on the same day or returned to the quarantine area where they will remain until the next morning for release. In the overnight stay in quarantine, each animal will be kept in its own cage, placed side by side, allowing visual and touch contact. The animals should be fed with a piece of banana during the quarantine.

The order of the procedures in the laboratory is shown in Table 2. Table 3 shows the methods and analyses, which, however, depend on the protocols, purpose, and capacities of each field team.

Physical confinement

On a bench, transfer the lion tamarin to a confinement cage and corral using a movable "door" with a range of motion sufficient to immobilize the animal and allow for the injection of the anesthetic (Fig. 1E). In the case of captured infants or juveniles under 3 months of age, it is not advisable to use chemical restraint. In these cases, leather gloves are used, and the animal is quickly weighed using a cloth bag and a scale. In addition to this procedure, sex determination is done, and the animal is marked with dye before being returned to the trap. Professionals experienced in restraint should perform the physical containment when possible.

Table 2. Order of procedures.

1. Weigh the trap with the animal inside (the empty trap is weighed before the capture). This is the most accurate way to estimate the animal's weight for calculating the dosage of the anesthetic.
2. Physical containment using the physical confinement cage.
3. Chemical containment and initial anesthesia monitoring.
4. Weigh the animal to adjust the dose of the anesthetic, if necessary.
5. Blood collection.
6. Mark the individual (tattoo or microchip).
7. Collection of hair.
8. Removal of radio collar.
9. Collection of biometric data.
10. Radio collar placement (usually one or two individuals per group).
11. Collection of other biological materials, such as feces and ectoparasites.
12. Mark individuals through dyeing
13. Monitor anesthetic recovery.

Chemical containment and anesthesia monitoring

Once the animal is trapped in the containment cage, the intramuscular anesthetic (Fig. 1E) is injected. The anesthetic protocol that has been used since 2006 is a combination of Ketamine Hydrochloride (dose 8–10 mg/kg) and Midazolam (dose 0.25–0.5 mg/kg) (Savage *et al.* 1993; Branson *et al.* 2003; Catenacci *et al.* 2016; Molina *et al.* 2019). The individual's induction and recumbency times are recorded

Table 3. Examples of collection, field processing, and storage of biological material for the *Leontopithecus chrysomelas* and other non-human primates.

Biological material	Purpose/Objective	Collection	Field processing	Storage
Blood	Blood counts	EDTA tube	6–10° C and processed in 24hrs	-20°C or -70°C for further purposes (as molecular testing)
	Chemistry and serological tests	Tube without EDTA	Centrifuge at 2500 rpm for 15 minutes	-20° C after centrifuge
	Molecular tests	EDTA tube or w/ EDTA Filter paper Diluted with RNA/DNA stabilization solution	Frozen immediately (dry ice, nitrogen liquid) Room temperature (15°–25°C) 7 days at 15–25°C, for 4 weeks at 2–8°C	-20°C or -70°C * Air dry at room temperature -20°C or -80°C
	Hemoparasites	Blood Smears	Methanol and Giemsa dye staining	Air dry at room temperature
Hair	Genetic	50 strands of hair	Remove the hair with the root	Air dry at room temperature
	Ectoparasites Dermatophytes	Scarification of the skin with hairs, but no cutting	Wrap the content between two microscopy slides	Air dry at room temperature
Stool	Endoparasites	Feces	Under refrigeration or stored in sterile pots containing 4 oz 10% formaldehyde. For molecular tests, frozen immediately or diluted with nucleic acid stabilizing solutions	Same as the field processing
Ectoparasites	Identification	Manually removed	Stored in 70% alcohol solution at room temperature For molecular purposes, store in cryotubes and frozen immediately.	Same as field processing
Vaginal Cytology	Reproductive status	Plastic sterile urogenital cotton-swab introduced into the dorsal commissure of the vulva	Vaginal smear and stain with Panoptic Staining Kit	At room temperature
Oral, auricular and rectal secretions	Characterization of the microbiota	Sterile cotton or rayon swabs AMIES swabs	Add the swab into the PBS or other transport solutions under refrigeration Add the swab into AMIES solution at the room temperature	Under refrigeration and frozen for long periods Same as field processing

after drug administration. The animal is then removed from the containment cage, and anesthesia monitoring is started. Heart rate, respiratory rate, rectal temperature, salivation, muscle tone and caudal, anal, atrial, intra-auricular, dermal and interdigital reflexes are measured at three time points (as the animal goes into recumbency, 15 and 30 minutes later). Reflexes are measured with hemostatic forceps wrapped in rubber (to not injure the animal). The forceps should be used up to the first locking point to standardize the intensity of the stimulus. The quality and duration of the latency period and anesthetic recovery should be recorded on the anesthesia-monitoring sheet. After anesthetic recovery, the animal should be returned to quarantine. Clinical examinations of the animals are performed in addition to anesthetic monitoring. They include body evaluation, mucosal color assessment, capillary perfusion time, lymph node size, the existence of old fractures, skin lesions.

Weighing the animals

Each animal should be weighed with a one-gram digital precision scale soon after beginning anesthetic monitoring to assess the need to increase the anesthetic dose. If the animal has an old radio collar, it should be removed before weighing. For anesthesia supplementation, we consider not only the weight, but also the clinical condition of the animal and the stage of sedation. If it is necessary to supplement anesthesia, use Ketamine Hydrochloride at a dose of 5 mg/kg IM.

Blood collection

It is important that the person responsible for blood collection has previous experience in performing this procedure; we suggest training with captive animals, preferably of the same species or genus. Blood can be collected by puncturing the medial inguinal vein or femoral vein (Catenacci *et al.* 2018; Santos *et al.* 2018; Molina *et al.* 2017, 2019) using a 24G gauge needle and a 3 ml syringe (Brazil, MS, SVS, DVDT. 2014; Monteiro *et al.* 2010; Santos *et al.* 2018). The volume harvested can be up to 1% of body weight, which usually ranges from 2 to 3 mL (Santos *et al.* 2018). Prior to blood collection, the site should be shaved and cleaned with antiseptic. The blood should be aliquoted

and stored according to the tests to be performed. For blood counts, blood should be stored in EDTA tubes and immediately refrigerated (6–10°C), and the test should be performed as soon as possible (within 24 hours). For chemistry and serological tests, blood aliquots should be stored in tubes without anticoagulant agents and, if possible, should be centrifuged at 2500 rpm for 15 minutes in the field laboratory, to obtain serum which is then frozen. In the absence of a field centrifuge, refrigerated blood may be stored until centrifugation in the laboratory (Brazil, MMA, ICMBio 2012). For molecular testing, an aliquot of blood should be stored in cryogenic tubes and deposited in a liquid nitrogen canister (Brazil, MS, SVS, DVDT 2014). Molecular testing may be done by depositing the blood remaining in the syringe plunger and needle on filter paper and allowing it to air dry at room temperature. An aliquot of blood for blood counts also might be used for molecular tests or another aliquot might be collected and mixed with RNA/DNA stabilization and storage solutions during the field.

To investigate possible hemoparasite infection, we recommend making two blood smears in the field, followed by methanol and Giemsa dye staining (Brazil, MMA, ICMBio 2012). Blood smears should be stored at room temperature. All the techniques used must be adapted to work with the minimum blood aliquot to obtain the results. It is essential to record the correct identification of the tubes and slides with labels and using a pencil. The collected blood can be used to perform blood counts, chemistry testing, hematozoan testing, molecular, and serological profiles for various infectious agents, which will depend on the primate species, the area studied, and the objectives of the research (Brazil, MS, SVS 2005). The veterinarian in charge of the team should be familiar with the zoonotic pathogens present in the study region, so that they may help ensure data collected from the animals may be of value for public health recommendations.

Tattooing and microchips

The animals are tattooed by adapting tools initially designed for humans. It is recommended to shave the fur to see clearly and clean the area with antiseptic solution before tattooing (Brazil, MS, SVS, DVDT 2014). The marking for definitive identification of each individual is done with numbers on the medial region of the thigh. The tattoo helps in the future identification of the animals in case of recapture. In general, this procedure only needs to be performed on the first capture of the animal, but in all recaptures, the marking should be checked and, if necessary, repeated. Between animals, the tattoo needle must be disinfected with alcohol. Microchips might be used instead of the tattoo and should be injected subcutaneously on the dorsum of the animal after cleaning with antiseptic solution.

Collection of hair

After making the first anesthesia recording (when the rectal temperature is being measured), hair collection can begin. For optimal results, we recommend pulling a pencil thickness of hair (25–40 follicles) from each animal making sure the root bulbs are clearly visible. Between animals, the forceps used for collection should be heated to eliminate remnants of genetic material from other individuals. The hair should be stored in paper envelopes or in sealed plastic bags and held at room temperature (Brazil, MMA, ICMBio 2012).

Placement of the radio collar

The radio collar used for the genus *Leontopithecus* should weigh no more than 5% of the body weight of the animal. Adult dominant animals should be selected because the animal's growth is already stabilized. This is also better to ensure group monitoring, since the probability of a dominant adult migrating to other groups is lower. If an individual's age is not known, a weight of 510 g is used as the minimum for an individual to be able to receive a collar, always taking into account the physical condition of the animal at the time of processing. The collar is beaded in order to reduce the possibility of friction-related injury to the skin (Hollohil, model RI-2D) (Fig. 1F). Frequencies should have at least 20 units between collars for animals in the same groups, or for different groups in the same region. This is essential to minimize interference of the frequencies during field monitoring. The functioning of a radio collar must be tested before fixing it on the animal.

When placing the radio collar, there should be a slack of approximately 1.0–1.5 cm between the beaded collar and the animal's neck. This allows the radio to move freely around the neck but prevents the animal's arm from passing between the collar and the neck or from slipping and being stuck in the animal's mouth. It is important to move the radio around the animal's neck several times to check for the best fit before finalizing the closure of the collar. This includes testing to check if the collar can be moved over the head. Always test one to two beads tighter and looser than the one believed to be ideal to ensure you have chosen the best length. For final closure, clip excess beads away and firmly tighten the clasp with pliers to avoid overlap. Make sure there are no sharp edges that could injure the animal's neck. From our experience, the duration of the radio-collar battery we used is about six months. Scheduling recaptures should allow time to re-find the animal to replace the collar or take it off before the battery on the collar dies.

Collection of biometric data

Biometry methods in this manual were adapted from those previously developed by researchers monitoring *L. rosalia*. In general, measurements of the entire body of the animal are taken, including the tail, head and limbs (Fig. 1G). In addition, specific body measurements such as skull, dentition, sternal marking glands and circumgenital measurements are also recorded. Further details can be found in the supplemental material (Fig. S1).

As various manipulations of the animal are performed during biometrics, it is advisable to keep the animal's head upright whenever possible to reduce the chance of food reflux complications. The use of precision instruments, such as digital calipers, improves data accuracy.

Collection of other biological materials

A. STOOL: To investigate intestinal parasite infection, feces should be collected by manual extraction from the rectum or by collecting samples off the ground (Molina *et al*. 2019; Costa *et al*. 2020). Samples should be stored depending on the parasite to be investigated. In general, helminths and protozoans should be stored under refrigeration in previously identified containers prior to being transported on ice to the researcher's parasitology laboratory. In the absence of refrigeration, feces should be stored in sterile pots containing 4 oz of 10% formaldehyde (Brazil MMA, ICMBio 2012; Monteiro *et al.* 2007; Costa *et al.* 2020). If there is no electricity available, samples can be kept refrigerated in a Styrofoam box with ice for 24 hours. For molecular tests, a fecal aliquot should be stored in a cryotube and frozen immediately, although different nucleic acid stabilizing solutions can be used in the absence of cryogen.

B. ECTOPARASITES: Ticks and other ectoparasites (such as fleas, lice and mites) should be manually removed, stored in 70% alcohol solution and kept at room temperature. For molecular purposes, store in cryotubes without solution and freeze immediately (Santos and Cubas 2014).

C. HAIR WITH SKIN SCARIFICATION: It is also necessary to evaluate possible parasites (mites) present on the fur and skin of these animals. For this purpose, the skin should be scraped using a scalpel blade, maintaining a 45^{o} angle, so that there is scarification of the skin but no cutting. In addition to the parasitological identification of the skin samples, the hairs should be collected for isolation and identification of dermatophytes (fungi belonging mainly to the genera *Trichophyton*, *Microsporum* and *Epidermophyton*) by fungal culture. Once hairs are collected, wrap them between two microscopy slides and send the material at room temperature to a specialized laboratory (Santos 1999).

D. VAGINAL CYTOLOGY: To identify the vaginal cell structures of the species at the time of collection to infer the reproductive status of the individual, a vaginal smear should be performed in all adult females. For the smear, the labia should be gently separated with one hand. Using the other hand, a plastic sterile urogenital cotton swab should be advanced through the dorsal commissure of the vulva. The swab should be inserted until it reaches the pelvic canal. At this point the swab should be rotated in all directions. This whole procedure only takes a few seconds and is painless (Snoeck *et al.* 2011). The swab should then be gently rolled over a slide, usually making three linear impressions. This smear is stained immediately after collection using a Panoptic Staining Kit (PubChem CID: 13735) and then sent at room temperature to the specialized laboratory.

E. SWAB SAMPLES: Using sterile cotton swabs with a plastic shaft, samples can be collected from the oral, auricular and rectal cavity and then placed into a sterile tube containing Phosphate-buffered saline (PBS) or other transport solutions depending on the goal of the research. The samples should be kept under refrigeration until processing (Miranda *et al.* 2019).

Marking of individuals using dyes

To assist in the identification of individuals in the field and to facilitate individual behavior data collection, the hair of captured animals may be dyed on different parts of the body. This marking is not permanent and is usually re-applied at each recapture The ink used to dye animal hair is a natural dye, Nyanzol®. The ink powder should be mixed with water and 30-volume Hydrogen Peroxide until an almost liquid paste is obtained, in the approximate ratio of 1:1:1. Using a toothbrush, this mixture is applied to the animal in the region pre-established by the researcher. Excess dye is patted off with a paper towel or gauze and paper moistened with water. Another possibility includes bleaching black hair with Hydrogen Peroxide to differentiate the group, for example. In general, markings can be done on the tails, heads or limbs, but head coloring should be avoided if it is thought to interfere with possible individual recognition processes among the animals.

Monitoring anesthetic recovery

Before the animal is returned to the trap, a final check of the physiological parameters previously described (heart rate, respiratory rate, temperature) should be carried out. In anesthesia recovery, two moments should be recorded: when the animal first raises its head and when it stays in a normal upright posture.

Release

On the day after the capture, approximately two hours before the animals are taken out of quarantine, a half banana is offered to each animal to ensure it has sufficient energy and hydration for its return to the field. The condition of the animal is also observed, and the collar is checked to ensure it is still in place. Before returning to the field, the traps are covered by a cloth or plastic trap cover.

About an hour after sunrise, the animals should be released in the same place where they were caught. This is particularly important in the case of partial group captures, so that captured and non-captured group members may more easily reunite. During the release, the traps should be placed on the ground, side by side, in a sufficiently open area to give the animals a clear runway to find a nearby tree to climb (Fig. 1H). Younger animals should be released first so they have time to get their bearings and follow the adults who tend to take off faster as soon as they are released. Another option is to first release an adult animal and then the juveniles before the rest of the adults. In this way, juveniles

may follow the first adult while the other adults are being released. It is best to remain on site for approximately 15 minutes to ensure that the group has successfully gone off together. After release, the group is not monitored on that day, unless there is a reason for this emergency monitoring. It is recommended to search for the group within 2–3 days after capture to see if all the animals are together, in good condition, and verify that the collars are still properly placed.

Conclusion

This study reports our capture experiences over the last two decades with the species *L. chrysomelas*. Capture and handling of free-living animals is a common practice in wild animal management and an important research component (Pitt *et al*. 2006). Unfortunately, there are few detailed reports of the experience with *in situ*-capture methods in small Neotropical primates (Stone *et al.* 1985; Savage *et al.* 1993; Wasa *et al.* 2015). Such information is essential because of the increasing number of projects being implemented for population monitoring programs, whether for ecological, genetic or health research. The authors hope this study will help guide future fieldwork efforts. It is important that the capture objective, environment, and species to be captured are always considered. Activities before, during and after the capture must be carefully planned, prioritizing the welfare of the captured animal and the safety of the professionals involved in this activity.

Please contact the corresponding author for a Portuguese version of this article.

Acknowledgments

The authors are grateful for the important collaboration of ICMBIO, the NGO Instituto de Estudos Socio-ambientais do Sul da Bahia, to Gabriel dos Santos and the veterinarian Mariângela Cruz for their aid during the captures. We are also grateful to all field assistants involved in the projects with golden-headed lion tamarins. Finally, we thank our sponsors: Saint Louis Zoo WildCare Institute (USA), The Wild Animal Health Fund of the American Association of Zoological Veterinarians (USA), CNPq (Brazil), Center for Research and Conservation of the Royal Zoological Society of Antwerp (Belgium), the Lion Tamarins of Brazil Fund, the National Lottery of Belgium, the Primate Action Fund, the Zoological Society of London, Conservation International, the International Foundation of Science (IFS), the Wildlife Conservation Society (WCS), the University of Maryland Grants, Idea wild, World Wildlife Fund, the Durrell Wildlife Conservation Trust, the Margot Marsh Biodiversity Foundation, and the Tulsa Zoo. We acknowledge James Dietz for his invaluable training and assistance in the capture and monitoring of lion tamarins, as well as the University of Maryland, the Instituto de Estudos Sócio-ambientais do Sul da Bahia (IESB), and the Smithsonian Conservation Research Center for providing logistical support for early research whilst these protocols were first being developed and implemented on golden-headed lion tamarins.

Supplemental Information On-Line

Figure S1. Biometrics data sheet used for captures of the species *Leontopithecus chrysomelas.*
URL:<http://www.primate-sg.org/storage/pdf/PC36_Catenacci_et_al_Suppl_mat_Fig_S1>

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Received for publication: 30 March 2021
Revised: 8 September 2021