Capture and Collection of Biological Samples from Free-Living Neotropical Primates

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Abstract: Restraint and threat of predation are possibly the most stressful events in wild animals' lives. Management techniques should, therefore, be improved to avoid or minimize suffering in such situations. Body mass and variation in behavior influence the techniques used during containment. Automatic traps are mostly used for small primates living in the lower canopy, while remotely delivered chemical immobilization is the recommended technique for larger primates, which live in the upper canopy. For both methods, careful physical restraint after the capture of the animal is essential. The use of equipment and materials that ensure biosecurity is imperative, as is choosing the most appropriate location for the collection of biological samples. Storage and transport must also be carried out in an adequate manner so as not to impair the samples. Here, therefore, we seek to describe capture, containment, and biological sample collection techniques with the intention of minimizing risks and increase success in the capture of Neotropical primates.

Keywords: Capture, containment, morphometrics, biological sampling

Introduction

A good understanding of physical and chemical restraint techniques and sampling methods are important for professionals and researchers—veterinarians, veterinary technicians, biologists, primatologists—and others who capture and handle wild animals (Setchell and Curtis 2011; Miller and Fowler 2012).

Correct primate restraint relies on a number of factors, including knowledge of the species' behavior and habits, as well as their anatomy, physiology, and degree of vulnerability to stress (Stone *et al.* 2015). It also relies on the mastery of the techniques and equipment used during containment (Fowler and Miller 2008; Jolly *et al.* 2011) as well as a full appreciation concerning the need for adequate environmental conditions. Examples of primate-capture and/or restraint techniques can be found in the literature, ranging from morphometrics, the attachment of tracking devices (Scott *et al.* 1976; Froehlich *et al.* 1981; Lemos de Sá and Glander 1993; Setchell and Curtis 2011), translocation and reintroduction (Jolly *et al.* 2011; Kierulff *et al.* 2012) to sampling for pathogen research and clinical pathology (Milton *et al.* 2009; Barros *et al.* 2016; Martínez *et al.* 2016; Almeida *et al.* 2019; Bernal-Valle *et al.* 2020; Abreu *et al.* 2019).

The techniques employed to capture and restrain non-human primates (NHPs) vary according to the size of the individual and its behavior (Jolly *et al.* 2011; Cunningham et al. 2015). Capture techniques and success in capturing are influenced by the arboreal, and mostly diurnal, habits of Neotropical NHPs, together with surrounding environmental conditions (Helder *et al.* 2019). Wire traps can be used for small (Callitrichidae) and medium-sized (Cebidae) primates (0.5 to 5.0 kg), and Stone *et al.* (2015) also demonstrated the use of a net trap for *Saimiri* sp. In the case of medium-sized and large primates (Atelidae) (> 6.0 kg), anesthetic dart projectors are used most (Scott *et al.* 1976; Fedigan *et al.* 1988; Karesh *et al.* 1998; Teaford and Glander, 1991; Cubas *et al.* 2007). Aguiar *et al.* (2007), however, conducted a study using wire traps for the capture of *Alouatta* and found them to be successful.

Containment is possibly the most stressful non-natural moment in the life of a wild animal. It can result in the animal's death, in (1) an extremely acute manner—ventricular fibrillation caused by the release of catecholamine due to

the alarm reaction, in addition to acidosis and hypoxia, (2) an acute manner—acidosis caused by continuous muscular effort while resisting procedures, leading to high glucose consumption and lactic acid production or (3) in a delayed manner—myopathy due to capture, a degenerative muscular disease with an extremely low prognosis occurring as a result of altered pH, hypoxia and death of muscle fibers with the release of potassium, myoglobin and lactate (Minton *et al.* 1995; Morton *et al.* 1995; Thun *et al.* 1996).

It is essential to observe certain criteria when selecting the anesthetic drug to be used (Cunningham *et al.* 2015). The different ways such drugs act, the application method used, possible interactions with other drugs that present high LD50 (Lethal Dose), and the correct use of high drug concentrations and low total volumes to ensure rapid infusion (Massone 2003). Correct precautions regarding animal and handler biosecurity should be taken to avoid and/or reduce field accidents, whether physical, chemical or biological. The use of Personal Protective Equipment (PPE) is essential for maintaining hygiene levels, not only for humans but also for the animals and the environment (Brazil, Ministério da Saúde 2017; Costa and Costa 2005).

By combining information retrieved from the literature and from the results of field work, the present study sought to disseminate NHP capture information that is out of date, contribute to standardize capture methods, and contribute with data on biological sampling methods of free-living Neotropical NHPs.

Description

Our methods and protocols were approved by the institutional Ethics Committee for Animal Experimentation and by the Brazilian Ministry of the Environment (Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio, Sistema de Autorização e Informação em Biodiversidade – SISBIO). The research adhered to the American Society of Primatologists' Principles for the Ethical Treatment of Non-human Primates.

Capture of Neotropical primates

Capture and restraint techniques are diverse, and there is no single ideal method for all situations, since the success of these techniques depends on different biological (species, age, sex), ecological (topography, climate), and practical factors (costs, logistics) (Shury 2007). The purpose of the capture also influences the methods used (Aguiar *et al.* 2007; Isbell *et al.* 2019).

Locating groups

Firstly, the study area must be identified, and the target group located. There are few ways to locate and find groups of NHPs. A practice adopted by some researchers is that of playback of individual vocalizations of the same genus or species. This has been used for some primates, such as callitrichids (Bezerra and Souto, 2008). The researcher plays recorded vocalizations of the species in the target area using a portable stereo and waits for the vocal response of the wild group (Miller *et al.* 2005).

A period of baiting is recommended for such as the Callitrichidae and Cebidae, especially if they are not habituated. Baits can be placed on platforms with the traps located in a small section (10 m²) (Paim and Rabelo 2015; Rocha *et al.* 2007). Informal interviews with local people can provide information about the whereabouts of the primates in the study area, as well as their diets and behavior. (Jones Engel et al. 2011; Davis and Wagner 2003). Sharing information with key institutions and local residents has been found to optimize efforts for the capture of howler monkeys in the Atlantic Forest. Mobile message exchange apps can be used maintain contact during the search (Abreu *et al.* 2019; Andrade *et al.* 2022). It is important to be aware of how the primates are called in each region, since they may have different popular names, especially those which have large geographical distributions. All field materials required for animal management and sample collection must be carried by the team to the capture location.

Capture and containment: physical and chemical restraint

Any procedure involving the physical or chemical restraint of an animal must be conducted in an appropriate location. These locations should be quiet, away from curious onlookers, protected from the rain and direct sun, and should be convenient and comfortable for both the animal and the handler. Ideally, a field laboratory should be set up in an area of approximately 10 m² that is isolated using zebra tape, and all those entering the perimeter must be equipped with all appropriate PPE. All team members should know their role and the activities for which they are responsible right from the start, and should be familiar with the equipment, materials, and procedures used. The team can be composed of four members: a veterinarian, responsible for the containment; an assistant to support the veterinarian; a team-member for recording the collected data; and somebody for handling emergencies. All instruments necessary for handling the captured animals must be available in this area.

Capture procedures using traps

Callitrichids (*Callithrix penicillata* and *C. jacchus*, for example) are the species captured most frequently, due to their abundance and wide distribution, in addition to their ability to adapt to diverse environments, including secondary forest around gardens and small farming plots, rural villages and urban areas. These species use the lower canopy and, among houses, use roofs and walls to move about. Traps can be placed at heights of no more than three meters, even where local inhabitants may place food for the monkeys (Watsa *et al.* 2015). A good strategy is to place food on the platforms around the traps and gradually inside the traps while they remain locked open. The marmosets then get used to the traps. Monitoring the baits, it is possible to see if the marmosets are eating them and, by moving the bait gradually into the traps, getting them accustomed to enter them

as soon as they arrive on the platform or whatever trap array is being used. Of course, other animals such as opossums will also eat the bait, but through observation you can tell which are eating it by the way it has been disturbed (tooth or clawmarks). Camera traps can be effectively used at this stage. If the aim is to trap an entire group, then it is important to know the group size to place sufficient traps to catch them all at once. Garber *et al.* (2016) described a strategy for the capture of tamarin groups using a single trap with 10 compartments, which could be closed by manually pulling a string from behind a blind, approximately 5 m from the trap.

Cebids are larger than callitrichids, but the same capture techniques can be applied. For *Saimiri*, *Sapajus* and *Cebus*, automatic traps must be larger (40 × 40 × 60 cm) than those used for the callitrichids (20 × 20 × 60 cm).

For callitrichids and cebids, trap locations should be selected once groups and their locations have been identified. Automatic traps with a pressure-activated trigger device are the most used (for example, Tomahawk traps). Collapsible traps, with a treadle of 40 × 20 × 20 cm are ideal. They are easy to handle and carry. These should be attached with galvanized wire (1 mm) on horizontal or slightly bent tree branches, with the entrance facing up. If traps are too acutely tilted the automatic door-closing may not be triggered. As these animals move in groups, the chance of group capture is greater if the traps are clustered (five to six traps in close proximity) (Fig. 1). Once the traps are set, they must be monitored at least once every hour, to deal with situations where an animal is uncomfortable because of stress, or sun exposure, an accident caused by the closing of the automatic door, or predators.

For cebids, large manual traps can be successful, allowing for the capture of more than one individual at a time (Rocha *et al.* 2007). For capuchin monkeys (*Cebus* and *Sapajus*), it is important that traps are well fixed and constantly checked, as they are strong and can dismantle and escape from traps even after being captured. Once the animal has been captured, the trap should be covered with a dark cloth, to calm the animal down and reduce risk of injury.

Fruits such as banana, mango, and papaya are the most common baits (Aráujo *et al.* 2000). Animals are attracted not only by the sight of these fruits, but also by their smell, and most arboreal primates tend to focus on ripe fruit (Nevo *et al.* 2016, 2018). Baiting time will vary according to how often the traps are visited, ranging from hours, days to even weeks.

In small primates, physical handling precedes chemical restraint. The first step is to remove the bag or cloth covering the trap. The trap door should be facing upwards and the opposite wall resting on the floor. The handler should wear leather scrap gloves over the latex gloves used for the procedure. The assistant should open the door and the handler should quickly hold the animal to prevent it from getting hurt or running away. At the time of containment, the animal should be at the bottom of the trap and should not be looking up. At this point, it is important for other team members to be aware of the possibility of escape. If the animal escapes from the trap, recapturing attempts can be made using a thin hand net. Another method of containing the animal is to insert a press or trap divisor of material that can be properly disinfected, such as treated wood or plastic,

Figure 1. Left – Tomahawk traps properly attached with galvanized wire on horizontal or slightly bent branches with the entrance facing up. Right – Traps are clustered in proximity (arrows are showing the traps).

toward the bottom of the trap (such as a squeeze cage) preventing the animal from reaching the door. In this case, the animal is lightly pressed against the back of the trap using a plunger, so that the chemical containment can be carried out (Fig. 2). Following the physical restraint, the chemical restraint is carried out by intramuscular injection or with a mask for inhalation anesthesia. Anesthetic protocols for small, medium, and large primates are described below.

Capture procedures using an anesthetic dart

For the large neotropical monkeys—howler monkeys (*Alouatta*), spider monkeys (*Ateles*), woolly monkeys (*Lagothrix*) and muriquis (*Brachyteles*) of the family Atelidae—capture techniques require an anesthetic dart projector to administer the drugs and deeply sedate the animals (Scott *et al.* 1976; Cunningham *et al.* 2015; Almeida *et al.* 2019). Chemical restraint precedes physical handling of the animals and it is fundamental to know the species characteristics, such as weight, group conformation, breeding season, and behavior. Anesthetic doses should be calculated for the mean weight of males and females, and in particular cases, the darts must be prepared before entering the capture area (Brazil, Ministério da Saúde 2017). A blow pipe or rifle using compressed air or carbon dioxide can be used to deliver the darts (Sleeman 2014). Since dart pressure is controlled by butane gas, it must be replenished at the end of the day if the dart is not used as, over time, the gas can escape from the device. Darts have volumes ranging from 1.0 mL to 10 mL, depending on the drop volume and the target. Most delivery dart systems have a manometer connected to a switch, which regulates the shot pressure. The pressure for the shot be adjusted according to the distance from the animal, direction of the shot and the target's body mass. Other variables, such wind, and those involving the shooter's characteristics (calm, concentrated, confident), can influence the success of a shot. Depending on the projector, a dart can be projected up to 70 m, but the accuracy of the shot declines significantly after 20 m. The distance, direction and training of the shooter are critical for successful captures (Cunningham *et al*. 2015).

Figure 2. Restraining the animal inside the trap through the insertion of a wooden press to conduct the chemical containment.

Once the animal is identified, the shooter must wait for the ideal moment to shoot. When primates notice that they are being observed they usually leave the area, especially if they are not accustomed to human presence. If the animal flees, the whole team should follow it until it stops to rest, which will provide a good opportunity to shoot at the animal. Darts should never be fired if the animal is looking at the shooter, as chances of an accident increases. The safest situation is when it has its back turned to the shooter, with part of the hind limbs visible. The best part to shoot at is where the most voluminous muscles are located—the lateral faces of the quadriceps and biceps femoris (Cunningham *et al.* 2015; Glander *et al.* 1991). The risks associated with darting should be carefully considered: sciatic nerve injury, or perforation of the thorax or abdomen, which can occur if the animal changes position when the dart is fired. This procedure must be performed by an expert, and the team needs to be prepared to deal with emergency situations.

Depending on the anesthetic used, after being shot, the animal may try to escape before falling from the tree. Atelids can sometimes remain stuck hanging by their prehensile tails, and the team should be prepared to remove the animal as safely as possible through one of two ways. The first using tree climbing equipment to reach it, the second option is the use of a slingshot with a rope to gently shake the branch. In all circumstances a safety net (5 m × 5 m) must be positioned and held under the individual, to soften its fall and avoid it hitting the ground (Houle *et al.* 2004). Once the animal is in the safety net, the area should be set up for sample collection. Darting should be carried out with extreme caution. At least four people, using PPE, in addition to the shooter, are required to participate.

Monitoring vital signs during the physical and chemical restraint

Each individual must undergo an initial assessment of airways, breathing, and circulation (ABC), as well as its state of consciousness, verifying or ruling out at the same time, bleeding, bone deformities and/or instabilities, or other traumas, lesions or evident signs of disease. Subsequently, a physical examination must be carried out systematically (Varela 2006), in conjunction with monitoring of vital parameters every 5–10 minutes. While the animal is anesthetized, the veterinarian should check and record at least the following health aspects: mucous membrane color and humidity, hydration status, skin and coat condition, peripheral lymph node condition, eyes, nostrils, oral cavity, ear canal, and weight (Varela 2006), and score body condition (Clingerman and Summers 2005).

Monitoring of physiological variables is essential during the anesthetic procedure. The main parameters that should be taken are temperature (T), heart rate (HR),

and respiratory rate (RR). For callitrichids, the baseline values are T (38.5–39.5°C), HR (>200 beats per minute), and RR (60–70 breaths per minute). For cebids the values are T (37.2–40.2°C), HR (165–240 beats per minute), and RR (20–50 breaths per minute). For atelids, the values are T (36–39.4°C), HR (160–210 beats per minute), and RR (18–30 breaths per minute) (Thompson *et al.* 2014, Verona and Pissinatti 2014; Varela 2006).

Anesthetic Protocols

Capture, handling for examination, and the collection of biological samples requires chemical restraint techniques and protocols to guarantee complete and safe immobilization. It also minimizes stress and the risk of injury, ensuring the safety of the animal as well as the team involved in the procedure (Longley 2008; Murphy *et al.* 2012). Frequently, a pre-anesthetic health status evaluation is performed just by observation, without an adequate hands-on evaluation. The aforementioned protocols ensure greater levels of safety for both the animal and the veterinarian.

There are many possibilities of drug associations and delivery systems of an ideal protocol, depending on the species, individual responses, capture technique, equipment viability, the anesthetist's experience, and atmospheric conditions. Assessing the success of anesthesia involves maintaining the physiological variables within normal limits and a quick and smooth return to consciousness (Fasano 2010; Murphy *et al.* 2012).

Injectable anesthesia

In non-human primates, most injectable protocols for short-term chemical restraint contain a dissociative anesthetic, such as ketamine (10–15 mg/kg), or tiletamine (5–10 mg/kg) associated with an alpha-2 agonist, such as xylazine 0.5–2mg/kg), medetomidine (0.04–0.06 mg/kg), and more recently, dexmedetomidine (0.005–0.05 mg/kg) (Murphy *et al.* 2012; Theriault *et al.* 2008; Vasconcellos *et al.* 2000). Alpha-2 agonist-ketamine combinations to ensure immobility are excellent analgesics, have good myorelaxant effects, and reduce the drug doses used, decreasing the occurrence of undesirable side effects and shortening anesthetic recovery time (Hess 2004; Carpenter and Brunson 2007).

The inclusion of opioids, such as tramadol or fentanyl, is recommended for procedures that require more intense analgesia. These drugs inhibit pain modulation and alter nociception and are the basis of analgesic therapy in patients with moderate to severe pain and can be included in chemical containment protocols for procedures with associated pain potential. When combined with sedatives and tranquilizers, opioids act synergistically and intensify their effects (Fantoni and Garofalo 2012; DiVicentini Júnior, 2013). However, as in humans, respiratory depression has been observed in non-human primates receiving relatively low doses of opioids, especially when administered in conjunction with benzodiazepines. (Fasano 2010; DiVicentini Júnior 2013).

The main disadvantages of injectable anesthetics are related to their variable effects, which are conditioned to the different physiological characteristics of each species. Once sedated or anaesthetized, it is extremely important to keep monitoring at least the main physiological parameters of the animal, such as consciousness, heart rate, respiratory rate, and temperature. Prolonged recovery times demand more attention from the team and impair the rapid reintroduction of these patients to their groups (Olberg and Sinclair 2014). Even in low doses, hypothermia and the prolongation of anesthetic effects are common in some individuals, which can cause an animal to sleep for hours, thus making their release on the same day impossible. In these cases, it is necessary to keep the animal trapped until the next day, leaving the trap covered with a piece of cloth somewhere quiet. In general, it is recommended that the animal be kept under surveillance and not be released without complete recovery. If recovery is incomplete, it is vulnerable to predation and can lose its group or its social position.

Inhalation anesthesia

The use of general anesthesia for short procedures that do not require analgesia, but for procedures where there is a need for long-term chemical restraint, has become common and necessary in non-human primate medicine, especially due to the low health risks of inhalational anesthetics, such as isoflurane. This type of anesthesia guarantees unconsciousness for longer periods of time, provides oxygen supplementation to the patient, and enables better muscle relaxation. Furthermore, inhalation-anesthetic recovery is faster than with dissociative drugs, due to the inexistence of cumulative effects, and because the inhalation agents do not depend on hepatic biotransformation (Longley 2008). The most used inhalation agents, isoflurane, and sevoflurane, have a minimum alveolar concentration of 1.2% and 2%, respectively, in primates (Olberg and Sinclair 2014; Coleman *et al.* 2017).

In smaller primates such as callitrichids and cebids that can be manually contained with the aid of leather gloves or a press in the trap (Fig. 2), anesthetic induction can be performed using facial masks or induction boxes, and on average it occurs with a latency of two to three minutes. With the animal relaxed and under anesthesia, handling for biological data, sample collection and medical procedures can begin. This can also be performed using facial masks (Shury 2007) (Fig. 3). In the past, the use of inhalation anesthesia was not considered feasible under field conditions due to the weight of the equipment, especially the anesthesia machine and oxygen cylinders. This method of anesthesia application has become a common practice in the field, however, keeping in mind that the portability of the anesthesia machine is a prerequisite for application in remote conditions or locations. Equipment portability should be maximized by acquiring lighter components, such as oxygen cylinders made of aluminum, small vaporizers, and non-rebreathing anesthesia circuits with reduced dead space, such as Mapleson breathing systems.

Figure 3. Inhalation anesthesia administered to a callitrichid through a face mask connected to the VetBag®. The device has an anesthetic vaporiser and a complete anesthetic delivery system. Photograph: Danilo Simonini Teixeira.

Researchers from the universities of Brasília (UnB) and Santa Cruz State (UESC) have developed a portable inhalation anesthesia machine for use in the field, called VetBag® (Fig. 3). This device is recommended for the application of anesthesia to animals of up to 10 kg because it has a non-rebreathing breathing circuit; however, it may be used for larger animals in situations where it is needed to maintain the anesthesia for a longer period without using injectable anesthetics. The recovery from an inhalation anesthesia is faster and allows a timelier release (Mosley 2015).

Collection of Biological Data

Biometric data

Handling primates during physical restraint must be performed while wearing leather gloves, and animals should be quickly taken to the field laboratory following their successful restraint. It is extremely important to follow a specific method during data collection, and to organize the materials necessary for collection and measurement, which must be within reach of the team. Whether using a caliper or a measuring tape, the same tool should be used to measure the same variables for all the animals and, if possible, by the same person.

We suggest the following biometric data as a base line, using a measuring tape (Fig. 4). Head and body length: place the animal in the prone position and take measurements from the top part of the parietal bone in the skull, along the entire vertebral column to the first coccygeal vertebra. Tail: with the animal in the prone position, measure from the first caudal vertebra to the last, disregarding fur. Skull circumference: pass the measuring tape around the head, passing over the eyes and ears. Neck circumference: pass the measuring tape around the neck. Pectoral circumference: the measuring tape should be positioned around the thorax, in line with the nipples. Right pinna: better assessed with the use of a pachymeter, measuring from the Super auricle to the Sub auricle. Right femur length: from the greater trochanter to the lateral epicondyle at the knee. Right tibia length: from the lateral condyle on the knee to the fibular notch on the ankle. Right foot: from the calcaneus to the tip of the central toe. Right hand: on the palmar side, measure the length from the carpus to the tip of the central finger.

Biological samples

Obtaining some biological samples may requires invasive procedures and the application of anesthesia; the presence of a veterinarian is essential. The most common samples to be collected are feces, ectoparasites, hair, oral and/or genital swabs, and blood (Gillespie *et al.* 2008). As capture/collection is an extremely stressful event, it is important to take advantage of the opportunity by sharing the sample with several research groups and to perform as many analyses as possible. Obviously, collection will follow norms according to study locations and will depend on the procedures used, as well as the aims of the studies. These factors influence choices ranging from the culture medium used for the storage method, including whether the temperature needs to be controlled (Table 1).

Feces can be sampled from the trap or at the time of containment, i.e., spontaneous defecation, by introducing a "fecal loop" in the rectum or by inducing defecation through abdominal massages in a cranial-caudal direction on the caudal abdominal region, to stimulate defecation (Fei Fan *et al.* 2015). The volume collected depends on the research aims. Three to five grams is usually sufficient for conducting the main exams (de Assis *et al.* 2016). Aspects such as coloring, odor and physical appearance may be indicative of health. It is important to observe if whole fruits are present in feces (which indicates malabsorption), if the odor is characteristic of the species, and if there are parasites which can be seen with the naked eye. Fecal samples can also be used in genetic analysis or for the detection of pathogens (de Assis *et al.* 2016). Some authors (Chaves *et al.* 2011; Oklander *et al.* 2004; Surridge *et al.* 2002) have used feces for DNA extraction and sequencing, while others (Raminelli *et al.* 2010) have them for hormonal assessments, such as that of basal cortisol levels. Feces can also be used to identify the types of food consumed and exposure to pesticides (Table 1).

It is usual also to collect tissue samples, especially for genetic analyses and the detection of pathogens. These should be stored in collecting pipes or cryotubes, depending on the size of the sample. For primates, the most appropriate

region for tissue collection is in the external ear, or pinna. Sampling should be carried out with the aid of rat-tooth forceps and sharp-point scissors, or a punch devise. The skin sample can be as small as 4–6 mm (Kirmaier *et al.* 2009) and if it is a tissue sample obtained from a necropsy, for histopathology, genetics, or pathogen detection, 2 cm² should suffice (Brazil, Ministério da Saúde 2017). Several solutions can be used for tissue preservation, such as 70% alcohol, absolute alcohol, and RNAlater. Cold preservation may also be an option, when available. For immunological analyses, such as immunohistochemistry—widely used for diagnosing yellow fever in dead NHPs—preservation in 10% formalin is used (Passos *et al.* 2022). However, formaldehyde impairs genetic analysis (Table 1).

Ectoparasites such as fleas, ticks, and dipteran larvae are less common due to social grooming, but they do occur (Gilbert 1997). Ectoparasites can be stored in 70% ethanol for identification purposes (Martins *et al.* 2021) and, in 90% ethanol or isopropyl alcohol for DNA identification of vector-borne pathogens (Magalhães-Matos *et al.* 2017) (Table 1).

Blood is more difficult to collect. It relies on the technical skills of the collector, and it is an invasive procedure that can inflict pain, which should be avoided when possible (Dysko and Hoskins 1995). It is even more complicated for small animals since, in addition to the challenge of calculating the correct volume of blood to be sampled, the blood vessels have small diameters, hampering collection. Blood sample collection should be carried out in the femoral plexus, preferably in the vein (Brazil, Ministério da Saúde 2017) (Fig. 5). Although arterial pulsation may be felt in the collection area, needle insertion should be medial and parallel to the pulse, i.e., where the vein is located. There are no major issues in collecting blood from arteries, except that hemostasis (local coagulation) may take longer due to the physiological pulsation of the arterial blood vessel. To ensure that complete hemostasis has been achieved, move the leg from which the blood was taken and observe if there is any overflow. The materials that should be used are syringes (3–5 ml), needles (0.55 × 20 mm), iodized alcohol and cotton wool (Brazil, Ministério da Saúde 2017). The volume of blood collected will depend on the size of the animal, and the collection of no more than 10% of the calculated normal blood volume, roughly estimated to be 10% of body weight (Wolf and White 2012), is recommended. Since callitrichids weigh between 300–400g, it is possible to collect about 3–4 ml of whole blood. Once collected, the blood should be stored in the appropriate tubes, depending on the types of analyses to be performed.

Briefly, blood can be collected in tubes with or without an anticoagulant (Abreu *et al.* 2019). If the goal is to obtain whole blood, it is important to use an anticoagulant. EDTA and heparin are the most frequently used anticoagulant reagents for hematological and molecular analyses. The

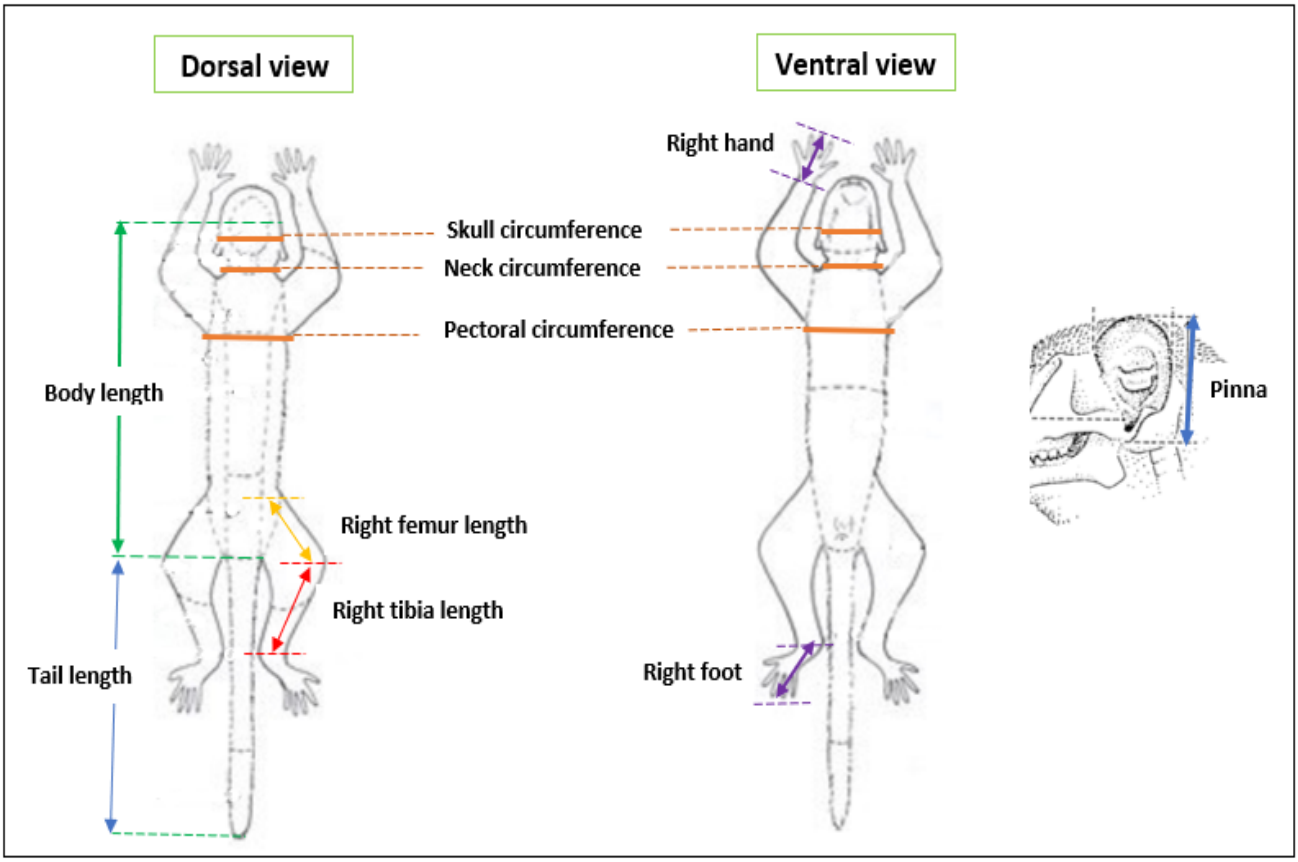

Figure 4. Baseline biometric data for primates. Courtesy of the Centro Nacional de Pesquisa e Conservação de Primatas Brasileiros (CPB/ICMBio).

Table 1. Biological samples by study aim, collection, storage and preservation methods.

Biological sample	Collection method		Study aim	Preservation method									Storage temperature		
	Non-invasive	Invasive		Without any extra substance	Alcohol	10% buffered formalin	MIF	AFA	Solid NaCl	RNA later	Specific Culture medium	Specific Transport medium	Room temp	Refrigerated (4ºC)	Frozen (-20, -80, -96ºC)
Fecal material	**X**	**X**	Genetics	**X**	**70%**									**X**	**X**
			Microbiota	**X**										**X**	
			Gastrointestinal parasites (oocysts, cysts, eggs, larvas, adults)		**70%**	**X**	**X**	**X**	**X**				**X**	**X**	
			Pathogens		**70%**					**X**					**X**
			Diet		**70%**								**X**	**X**	
			Cortisol	**X**											**X**
			Pesticides	**X**											**X**
Whole blood (EDTA or Heparin)		**X**	Genetics	**X**	**70%**										**X**
			Pathogens	**X**	**70%**										**X**
			Hemogram	**X**										**X**	
Whole blood in dry filter paper		**X**	Genetics	**X**									**X**		
			Pathogens	**X**									**X**		
Plasma		**X**	Blood Biochemistry	**X**										**X**	**X**
			Hormones	**X**											**X**
			Pesticides	**X**											**X**
Serum		**X**	Blood Biochemistry	**X**										**X**	**X**
			Antibodies	**X**											**X**
			Hormones	**X**											**X**
			Pesticides	**X**											**X**
Blood clot		**X**	Genetics	**X**											**X**
			Pathogens	**X**											**X**
Tissue samples		**X**	Genetics	**X**	**70%**										**X**
			Pathogens	**X**	**Absolut**										**X**
			Histopathology	**X**		**X**							**X**		
			Immunohistochemistry	**X**		**X**							**X**		**X**

Table 1. *Cont'd.*

Biological sample	Collection method		Study aim	Preservation method									Storage temperature		
	Non-invasive	Invasive		Without any extra substance	Alcohol	10% buffered formalin	MIF	AFA	Solid NaCl	RNA later	Specific Culture medium	Specific Transport medium	Room temp	Refrigerated (4ºC)	Frozen (-20, -80, -96ºC)
Ectoparasites	X	X	Identification		70%								X		
			Genetics		90%								X		X
			Pathogens		90%								X		X

tube containing the whole blood mixed with an anticoagulant may also be centrifuged to obtain plasma (supernatant), white blood cells and platelets (middle layer) and red blood cells (in the bottom). If the aim is to obtain serum, the blood can be allowed to clot naturally or a tube containing a clot activator and/or clot separating gel can be used. After centrifugation, the serum will be suspended, and the clot will sink to the bottom. The separation of blood components will require a 10-minute centrifugation with a speed of at least 5.000 rpm (Abreu *et al.* 2019). Serum, plasma, and whole blood are widely used for viral diagnoses, red blood cells for blood parasites such as *Plasmodium* and *Trypanosoma*, and serum and plasma can be used in immunological assays (Assis *et al.* 2016). Notably, the clot, which is usually discarded, can be used for the detection of several parasites, and for non-human primate (NHP) DNA it extractions should also be stored in order to optimize the sample (Abreu *et al.* 2018; Garg *et al.* 1996; Rodrigues *et al.* 2019) (Table 1).

Figure 5. Proper blood sampling from the femoral vein in a marmoset.

Once the material is in the tube, it should be stored in a styrofoam box with frozen icepacks and then as soon as possible, preferably on the same day, the samples should be stored at the ideal temperature (cold, frozen, ambient) in tubes suitable for freezing at temperatures below -80°C. As some fieldwork neither has nor requires the use of nitrogen during procedures, samples can be stored at -20°C and then taken to the laboratory, where they can be processed and stored in a freezer at -80°C until they are ready to be used (Abreu *et al.* 2019) (Table 1).

Alternatively, and depending on field collection conditions, dried blood spot samples, as well as thin and thick blood smears can be used as alternative samples, especially when working in remote areas. Genetic material from NHPs and parasites, especially DNA, can be easily obtained from dried blood collected in this way (Tan *et al*. 1997; Tran *et al*. 2014 (Table 1).

Final Remarks

Capturing Neotropical primates and collecting biological samples must be carried out with extreme care, as it involves a number of risks. In this sense, this article seeks to consolidate techniques used for this purpose, reflecting studies conducted for more than 15 years. The main points are: 1) The application of modern techniques such as the use of different anesthetic protocols and innovative equipment in the practice of inhalation anesthesia in field work; 2) The compilation of morphometric measurements in order to seek a standardization of the data to be collected; 3) The strategies described here for capturing with anesthetic darts; and 4) The implementation of biosecurity and mitigation of the possible risks involved in this type of work. Finally, procedures for the capture of primates and the collection of primates is in constant evolution, and keeping up to date with the latest techniques is strongly advised.

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