



SEP 20, 2023

OPEN  ACCESS**DOI:**[dx.doi.org/10.17504/protocol  
sio.5qpvor1exv4o/v1](https://dx.doi.org/10.17504/protocol.sio.5qpvor1exv4o/v1)

**Protocol Citation:** Mrinalini Watsa, Cristian Tirapelle, Alice Poirier, Alexandra Sacco, Priscila Peralta-Aguilar, Efstathia Robakis, Ishaan Raghunandan, Gideon Erkenswick 2023. Handling and Sampling Small Nonhuman Primates - ISL Peru. **protocols.io**  
<https://dx.doi.org/10.17504/protocol.sio.5qpvor1exv4o/v1>

**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

## Handling and Sampling Small Nonhuman Primates - ISL Peru

Forked from a private protocol

Mrinalini Watsa<sup>1,2</sup>, Cristian Tirapelle<sup>2</sup>, Alice Poirier<sup>3</sup>, Alexandra Sacco<sup>2,4</sup>, Priscila Peralta-Aguilar<sup>2</sup>, Efstathia Robakis<sup>2</sup>, Ishaan Raghunandan<sup>2</sup>, Gideon Erkenswick<sup>2</sup>

<sup>1</sup>San Diego Zoo Wildlife Alliance; <sup>2</sup>Field Projects International;

<sup>3</sup>University of Calgary; <sup>4</sup>Washington University in St. Louis



Alexandra Sacco

### DISCLAIMER

This protocol is actively used by Field Projects International at the Estación Biológico Los Amigos, Madre de Dios, Peru. It is revised annually to reflect improved capture, handling, marking, and sampling methodology. It has been reviewed by the ethics committees of multiple institutions. No author nor affiliated institution takes responsibility or bears any liability for the use of this protocol by others. The protocol is listed as having sensitive content since it involves biosampling from wildlife. Note: these procedures should be carried out only by trained personnel, and are not recommended for use without first obtaining all required permissions.

**Protocol status:** Working  
We use this protocol and it's working. The protocol is reviewed and updated annually.

**Created:** Mar 25, 2023

**Last Modified:** Sep 20, 2023

**PROTOCOL integer ID:**  
79440

**Keywords:** Neotropical primates, insitulabs, tamarins, mark-recapture, biological sampling, wildlife handling

## ABSTRACT

### Program Timing:

Trap habituation begins in May. Trapping and sample collection occur annually during the rainforest dry season (June - August). Sample transport and analyses occur between September and April.

### Capture Overview:

Entire primate social groups are captured together in multi-compartment traps in the morning to ensure same day release (~ 5 - 30 minutes depending on group sizes that range between 3 and 8 animals) -> animals are administered a small dose of anesthetic for trap extraction and 1st phase processing, then transferred to quiet, dark holding cages until 2nd phase processing (maximum holding cage time of 2 hrs for largest groups) -> one by one, animals are chemically restrained once more for 2nd phase processing for morphometric measurements, photographs, sample collection, and placement of animal identification (~ 25 minutes) -> animals recover in quiet, dark recovery cages during which 3-minute checks for breathing and movement occur until fully alert (1.5-2 h/animal since last injection of anesthetic) -> when the group is fully recovered from anesthetic all are released at the site of capture (< 5 minutes) -> group membership and well-being are confirmed with radio telemetry.

### Team Composition:

This protocol is intended to be carried out by a team of no less than 4 individuals, including at least 2 experienced personnel. Roles include (1) veterinarian/senior researcher (2) designated animal handler (3) sampling assistant (4) data recorder. Refer to the PROCESSING ROLES section.

## IMAGE ATTRIBUTION

Ishaan Raghunandan, Jorge Luis Mendoza-Silva, Silvia Carboni, Alice Poirier, Thomas Parsons, Alexandra Sacco

## GUIDELINES

Refer to [neotropical-primates-22-2.pdf](#) for a detailed explanation of primate capture protocol.

## MATERIALS

### Primate processing sheet

[PrimateForm\\_hardcopy.pdf](#)

### ODK Form

All animal and sample information collected on the hardcopy datasheet is entered into an associated digital form made with Open Data Kit Software. ODK central will generate the form from this excel file: [FPI-ODK\\_2023\\_primate\\_handling.xlsx](#)

### **Stakeout Material**

- sitting tarp
- 2 large tarps for the trap
- mosquito net
- binoculars
- playback caller
- pocket knife
- alcohol spray bottle
- bucket with bananas

### **Restraining material**

- 2 Handling towels
- 2 Handling gloves
- 1 Pair of wrist protectors

### **Anaesthetic box**

- 1 Bottle of ketamine
- 10 Pre-labeled 1ml insulin syringes
- Disposable gloves
- 10 Fecal sample bags

### **Electronics (with charged batteries)**

- 2 Stopwatches
- 1 Voice recorder
- 2 Ketomojo machines + ketones and glucose strips( at least 16 of each)
- 1 Universal microchip reader
- 1 Camera
- 2 Walkie talkies
- 1 Environmental thermometer
- 2 Rectal thermometers
- Digital calliper

### **Measuring material**

- Measuring tape
- Calliper
- 2 Weight scales
- Weighting bag

### **Sampling material**

Animal Bags (x10)

- 2 Pasteur Pipettes
- 1 hair band
- Microchip needle
- 4 Buccal/vaginal swabs
- H-DNA bag
- H-Merc bag
- Dental cast collection bag
- 2x 5ml eppendorf tubes for urine
- 2x 3ml Syringes
- 3 Microbiome swabs
- 15ml Falcon tube for blood capillary sample

#### Blood smears

- Full box (~20)
- Empty box for used smears
- Bag of Kim wipes (full)

#### Processing tools

- 2 Scissors (1 curved + 1 straight)
- Nail clippers
- 2 anatomic forceps
- 2 artery forceps

#### Sample box

- 10 biopsy tubes
- 20 blood tubes
- 30 buccal tubes
- 5 vaginal tubes
- 10 ectoparasite tubes
- 10 odor vials (optional)

#### Sterilization Kit

- 1x 50ml falcon tube containing 10% bleach
- 2x 50ml falcon tube containing distilled water
- 1x 50ml falcon tube containing 70% alcohol
- 1 Spray bottle filled with water
- 2 Spray bottle filled with alcohol

#### Dental Kit

- Dental paste gun
- 2 Dental paste tubes
- 10 Dental paste tips
- 20 Squares of laminated sheets

### **Marking material**

- Microchip syringes
- GPS and radio collars + 2 pliers + bead chains bag

### Tail bleaching kit

- Bleaching powder
- Activator
- Plastic container
- Brush
- Shampoo
- Tin foil squares
- Bucket of water
- 2 Towels for drying tail

### **Miscellaneous processing material**

- N95 facemasks
- Tent
- Cot/table for the processing
- 3 Plastic trays
- Sharps container
- 3 bags of disposable gloves (size S, M, L)
- Paper towels
- 2 trash bags
- 2 Clipboards
- Pens, Markers, Pencils
- Recovery cages for 8 individuals
- 10 Laundry bags
- Lighter
- Extra strings for the trap
- Extra hair ties
- Zip ties
- Laminated sheets: injury template, bleach pattern protocol, list of microchips numbers
- duct tape
- Sheets to cover the recovery cages
- 2 Tube racks (one for larger tubes)
- Spare 3ml Syringes (23G needle) x 20
- Spare Alcohol swabs
- 3 Thermic bottles with ice packs to store samples at a cool temperature
- Cooler bag with ice packs for sample storage
- Microhaematocrit tubes (at least 30) + clay
- 8 Ice trays for urine collection
- Parafilm strips

### **Medical material**

- Antiseptic solution
- Liquid bandage
- Stethoscope
- Pulse Oxymetre
- Cotton
- Hydrogen peroxide
- Lubricant
- Antibiotic cream

### **Recovery bag**

- Recovery sheet (hand made)
- Urine tubes
- Fecal sample bags
- Forceps
- Alcohol wipes
- Gloves

### **Odor box (temporary project)**

- Pump + tubing
- Metal cone
- Tape

### **Personal Items**

- Coverall
- Drinking water and snacks (only to be consumed outside of the processing tent)
- Headlamp
- Phone
- Rain jacket

## SAFETY WARNINGS

- ! Whenever working in the presence of a primate, all personnel involved should wear:
- (1) clean, long-sleeve coverall
  - (2) disposable gloves
  - (3) N95 face mask covering nose and mouth.
  - (4) when directly handling a bat, a minimum of 2 layers of disposable gloves and protective glasses or a face shield are required.

It is COMPULSORY to be vaccinated against rabies in order to handle bats and primates. If a bite or a scratch from an animal occurs, clean the affected skin thoroughly, and disinfect the area with an antiseptic solution. After washing, if bleeding is present apply some pressure and consider temporarily bandaging the wound. The PI must be notified IMMEDIATELY, and post-exposure treatment must be arranged.

## ETHICS STATEMENT

This protocol is modified annually as improvements are discovered, better technique is published, or new technologies are available. This protocol is based on prior versions that received approval by Institutional Animal Care and Use Committees (IACUC) of the University of Missouri - St. Louis, Washington University in St. Louis, and, most recently, by San Diego Zoo Wildlife Alliance.

## BEFORE START INSTRUCTIONS

Trapping events are never spontaneous. They are always planned after careful review of trap habituation data collected during the weeks leading up to trapping. The decision to capture a group is based on observation (either direct or via camera trap footage) of entire social groups reliably entering and feeding inside traps to ensure successful capture of the entire group. The PI is in charge of coordinating the primate capture activities and reviewing habituation data to determine when trapping events take place and at which trap sites.

Prior to trapping, all team members have received prior training on how to process these primate taxa. Typically, all trapping team members participate in several mock trapping sessions beforehand, simulating all steps hereinafter.

The functionality of the trap(s) is checked the day before a trapping event. Primate team personnel pack and double-check that all capture materials needed are cleaned and organized the night before a planned trapping event. Refer to the MATERIALS section.

## Processing roles

### 1 Team composition.

This protocol is intended to be carried out by a team of no less than 4 individuals, including at least 2 experienced personnel. Roles include (1) a veterinarian/ senior researcher (2) a designated handler (3) a sampling assistant and (4) a data recorder.

- 1.1 The **veterinarian/senior researcher** is experienced with every step of the primate capture process. This person is responsible for administering the anesthesia and deciding when an animal might need an additional anesthetic dose and at which point to collect the most invasive samples (biopsy, blood, dental casts).
- 1.2 The **designated handler** is responsible for handling the anesthetized animal during sample collection, checking vitals to monitor anesthesia (including taking rectal temperature), and disinfecting the sample collection tools in between animals.
- 1.3 The **sampling assistant** assists with less-invasive sample collection (hair, nails, buccal swabs), taking images listed in the processing sheet, reading aloud the serial codes that identify each sample bag and tube, and passing tools, bags, and sampling tubes to senior researcher(s).
- 1.4 The **data recorder** records the weight of the animal, morphological measurements, sample numbers, notes from senior researchers, and important timestamps, on the processing sheet. The data recorder also monitors recovering animals every 3 minutes, and stores samples in designated sample boxes as they are being collected. As the team member looking at the processing sheet, the recorder also ensures all relevant samples and information are collected from each animal before completing processing.



A capture-team member records data on the processing sheet. Note the tubes prepared on the rack and ready to be used to store samples collected. Courtesy of Silvia Carboni.

## Preparatory phase

- 2 The day prior to capture, all the materials and equipment necessary for trapping and processing primates are packed and set ready to take to the trap site. The senior researcher decides which trap site will be chosen for the trapping event.

- 3 The functionality of the trap(s) is checked the day before a trapping event. Ideally, minimal modifications to trap placement, positioning, and operation are made on the day of capture.

**MULTI-COMPARTMENT TRAPS:** A string is tied to the outside of each door of the multi-compartment trap (made of galvanized mesh and wood) and passed through a screw eye fixed directly on top of the entrance of each compartment (one for each door). The other end of each string is attached to a tree or rope just in front of a stakeout location where researchers will be seated to monitor the trap. This manual pull system avoids non-target captures and makes it possible to close one door at a time. The strings and trap are setup and tested well in advance. The interior of trap compartments is inspected for sharp points that could cause injury, which are removed or covered as necessary.



Manual trap positioning with strings. Photo by Ishaan Raghunandan

- 4 On the day of capture, the team arrives at the capture site 30 - 60 minutes before the earliest expected capture time. For these primate taxa, this is before sunrise, as groups may visit a trap first thing upon waking up. This allows for enough time to carry out a final trap inspection, assemble a processing tent, and unpack sampling materials before sunrise.
- 4.1 Two people, at least one being a senior researcher, stay at a designated stakeout location to directly monitor the trap. In some cases, the use of vocalization playbacks may be used to attract the target species to the area sooner, but the decision to use playbacks requires careful consideration of location, time of day, and known identity of the target group.



A trapping team waiting at the stakeout spot monitoring the trap. The mosquito net is a useful protection against biting insects while waiting quietly for the monkeys to come.  
Photo by Field Projects International

- 4.2** The remainder of the capture team, usually **2 or 3 personnel**, are responsible for assembling the tent and preparing the materials for processing captured animals. The tent is usually set up approximately 50 meters from the trap site, outside of direct visibility from the trap. Following set-up, these personnel remain quiet, out of sight, and ready. They also keep a lookout for any primate movements or calls in the general area in order to give advance notice of target animal(s) approaching the trap.

Communication between the stakeout location and the processing tent is facilitated by walkie-talkies.



A capture team waiting inside the processing tent. Photo by Jorge Luis Mendoza Silva.

## Trapping

- 5** As soon as the target primate(s) are at the trap, the stakeout team notifies team members in the processing tent via walkie-talkie. All sampling materials are setup on a processing table, and trays and sample collection tools are disinfected. A stopwatch is then started to allow the team to closely monitor the total duration of trapping and processing. A voice recording is also started as a safeguard for ensuring all data are recorded (i.e., morphological measurements, sample tube numbers, etc. that may be missed by the data recorder).



*The person starting the recording on the voice recorder reads out the date, the time, the weather conditions, the trap site and primate target group, as well as the team members participating.*

### Note

From this moment onwards, any relevant time reported on a processing sheet refers to the stopwatch time, which will be kept running until release.

## Safety information

Each member of the team wears a clean coverall, disposable gloves and a N95 face mask.

Whenever handling the animals or the animals' secretions, the use of a face shield or protective glasses is strongly recommended.

It is compulsory for all team members who handle wild mammals to be vaccinated against rabies.

- 6 **For manually capturing primates**, a senior researcher positions themselves ready to pull the strings that close the individual trap doors, while the other assists by observing (using binoculars), immediately notifying when a primate fully enters a trap compartment. Animals are trapped one by one in this manner, maintaining tension on the strings so that the trap doors do not fall or get pushed open.



A trapping team positioned ready to pull the strings that close the doors of the trap. Photo by Ishaan Raghunandan

## 6.1

 **For group capture**, the stakeout team waits until all the animals of the group have entered the trap. Ideally, all individuals are trapped within 20-30 minutes or less. Limiting this trapping period reduces stress and risk of injury to animals.

If one or more animals are not entering the trap, a decision is made whether it is safe to leave the animal outside on its own for the duration of processing. For example, if a juvenile is not entering the trap, the team will either release one of the other adult animals or a trapping event may be aborted. In this case, simply releasing tension on the strings allows the animals to exit the trap by themselves. Capture can then be attempted again at a later date.

### Note

As a general rule: trapping will not be carried out if it will cause juveniles to be separated from a group, and is aborted if this case seems likely.

## 6.2

**For single animal captures**, once the trap door is shut, the team allows the animal to finish eating the bait while checking to make sure that no other conspecifics are watching before approaching the trap. A single animal may be captured if it is a lone dispersing individual.

- 7 Once all group members are believed to have been trapped, the stakeout team scans the surrounding area to ensure no straggling animals are around and watching before approaching to lower the trap. While wearing a face mask, the observer who is not holding the strings quickly approaches the trap and secures all trap doors closed, either using a gloved hand, a rope, or string to hold the front doors closed, as needed. The person holding the strings maintains tension until all doors are secured, and their partner has indicated that they have stabilized the trap. If not stabilized, the trap may tip or fall due to the release of tension.
- 8 The person holding the strings then approaches the trap while maintaining string tension, bringing two tarps from the stakeout spot. The long door strings are cut approximately 40 - 60cm from the doors using a pocket knife to avoid the strings getting tangled in the vegetation when the trap is moved to the processing tent. While ensuring the doors remain secured and the trap is stabilized, one person unties the rope tethering the trap to the platform. The trapped animals usually stay in the back of their compartment, away from the person holding the doors.
- 9 The trap is then swiftly lowered down to the ground on top of a tarp, with doors facing upwards. The other tarp is used to cover the top of the trap, preventing animals from seeing outside. The tarps ensure that the animals remain calm, and prevent fecal samples from dropping to the forest floor as the trap is transported closer to the processing tent.



The image shows two monkeys entering and feeding inside the individual capture trap. Photo by Jorge Luis Mendoza Silva.

## 1st Phase - Processing

- 10 The **veterinarian or senior researcher** loads syringes with a fixed amount of anesthetic for each animal. Syringes are pre-labeled with the animal capture number and all contain the same volume of anesthetic (around 3 times the estimated volume for an adult individual of that species). Pre-labeling syringes 1-n in accordance with the animal processing order eliminates any confusion about which syringe belongs to which animal.





The image shows the veterinarian or principal investigator refilling anesthetic syringe. Photo by Jorge Luis Mendoza Silva.

#### Note

Despite the fixed volume of anesthetic loaded in the syringes, only a selected dose, based on the size of each individual, is delivered when needed, and may vary across animals. The remaining volume of anesthetic is kept in the syringe for possible later usage, and disposed of at the end of processing of each animal.

In the case of tamarins and marmosets, the dosage is 10-30 mg/kg (0.1-0.3 ml/kg), giving approx. 2 - 4 mg per time, as needed.

11

A **veterinarian or senior researcher** injects anesthetic through the mesh trap intramuscularly, while the animal is physically restrained with a towel and a leather glove by another team member. Animals are retrained and injected with the anesthetic one at a time.



To restrain an animal, a senior researcher opens the compartment door just enough to quickly

slip in a towel while the animal is on the bottom of the trap. While the animal is physically blinded or distracted by the towel, the restrainer pushes the animal toward the side of the trap using a gloved arm. While the restrainer holds the animal in place, the other **veterinarian or senior researcher** maneuvers into position to inject the anesthetic.

### Safety information

USDA Pain/Distress Category: C



An animal is pushed towards the mesh of the trap to allow intramuscular injection of ketamine hcl.

Photo by Jorge Luis Mendoza Silva.



An animal is pushed towards the mesh of the trap to allow intramuscular injection of ketamine hcl.

Photo by Ishaan Raghunandan

- 11.1** After injection, the team allows the animal approximately two minutes in the covered trap to allow for the anesthetic to take effect. The veterinarian or senior researcher periodically glances at the animal to ensure there are no complicating drug reactions. If an animal is not reacting at all to the first dose after 5 minutes, a second dose may be administered, as it is likely the animal did not receive the full dose during the first attempt.
- 11.2** If the dose was effective, a person calls out the time of pass-out and the animal is then moved into the tent to undergo the first phase of processing. The animal may not be completely immobilized, but the level of sedation is enough to allow for safe handling.



The correct handling of a tamarin: holding the animal from underneath the armpits prevents biting. Photo by Jorge Luis Mendoza Silva.

- 11.3** While one person carries the animal into the processing tent, another inspects the empty trap compartment for any feces that might be present. If present, they are collected and placed in a clean, labeled sample bag. The serial code of the sample bag is recorded on the animal's processing sheet, and the fecal sample is stored in a cooler filled with ice packs.

The tarp placed under the trap also allows for easy retrieval of any feces dropped by the animals, minimizing contamination with the ground.

- 12** As soon as the animal is moved inside the processing tent, it is placed inside a cotton bag and weighed using a digital handheld scale. Note: the weight of the empty bag is always recorded by the processing team before placing an animal inside of it.

**Note**

The weight and any other measurements or relevant information are recited clearly so that they can be recorded on the processing sheet by the person in charge of recording, and also detected by the voice recorder.



Research assistants weighing the animal inside a cloth bag. \**This is an old picture, the coverall dress-code was not yet in use.* Photo by Ishaan Raghunandan.

13



The animal is then moved onto a clean plastic tray that is disinfected between animals and allows for opportunistic collection of urine or fecal sample collection. A first assessment of the vitals is performed, including rectal temperature, respiratory rate, the color of the mucous membranes and palpebral reflex.

A	B	C	D	E
Time				
Resp Rate				
Temperature				
Color of mucus membrane				
Palpebral response	Y / N	Y / N	Y / N	Y / N

The above table is an example of the one found in the primate processing sheet.



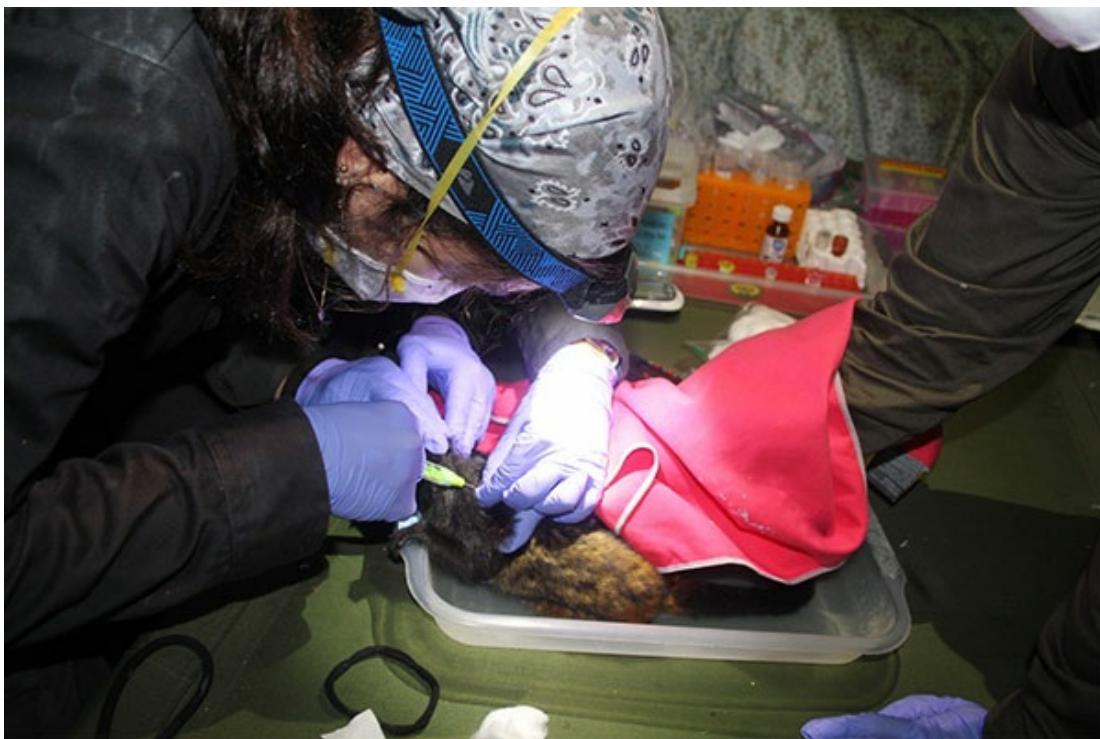
Rectal temperature reading. Photo by Jorge Luis Mendoza Silva.

- 13.1** If the body temperature is above 101 degrees Fahrenheit, water or alcohol is sprayed on the hands and feet of the animal, and a team member begins fanning the animal to cool its temperature more quickly. Vital assessments are carried out one or two more times during the first phase of processing until temperature and breathing appear normal.
- 14** Meanwhile, another person scans the animal to look for previously placed microchip ID tags.



Scanning for previous microchips. Photo by Ishaan Raghunandan

- 14.1** If no microchip is present, the animal is placed in a sternal position and a new microchip is inserted subcutaneously in the area between the shoulder blades.



Placing a microchip ID tag subcutaneously at the level of the shoulder blades of a tamarin

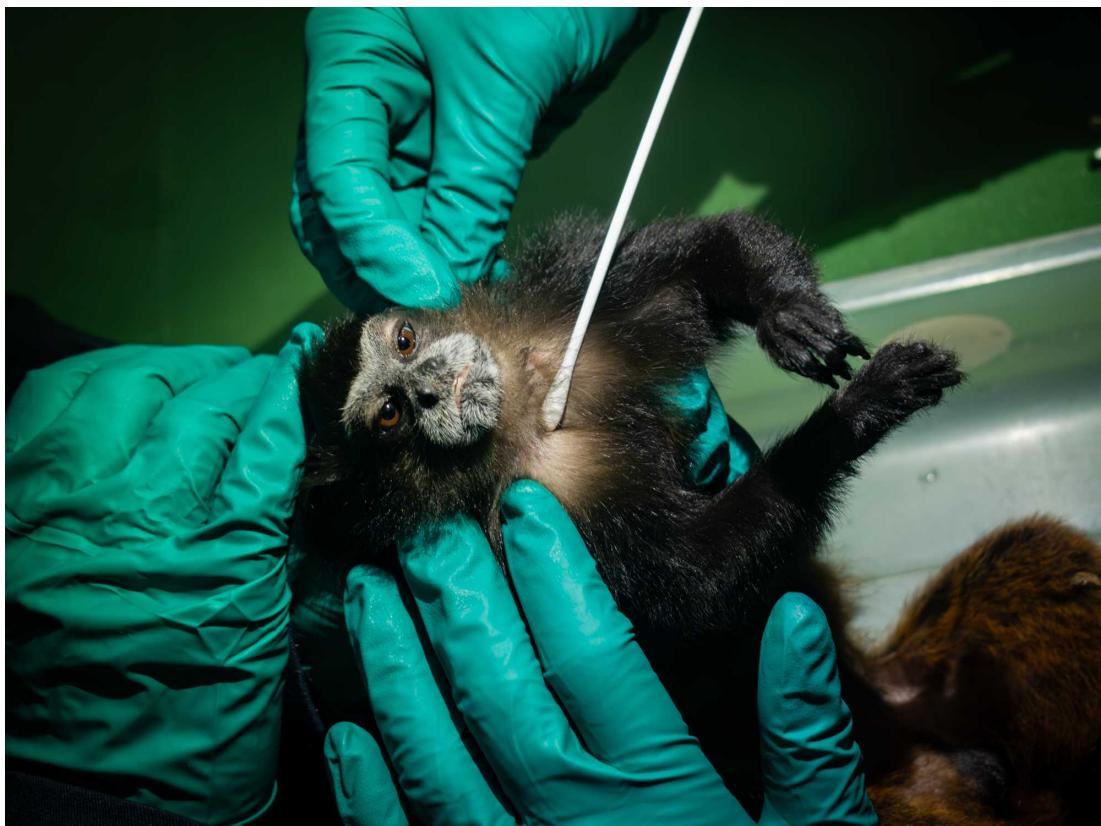
positioned on a sternal recumbency. Courtesy of Silvia Carboni.

Once inserted, the microchip number is read out loud, and the corresponding sticker with the identification number is attached to the animal's processing sheet.

## 1st Phase - Sampling

### 15 GLAND SWABS

A small cotton swab is rotated 10 times on the sternal and suprapubic glands and stored in a thermos filled with ice packs, for a microbiome study.



The image shows the swab process of the sternal gland. Photo by Jorge Luis Mendoza Silva.



The image shows the suprapubic swabbing process. Photo by Jorge Luis Mendoza Silva.

## 16 HAIR

A small amount of hair is cut and stored in one sample bag for use in an ecotoxicology project. Additional hair is also plucked from the root and stored in a separate sample bag for use in a DNA project. As a standard rule, all hair is collected from the left flank.



A sufficient amount of hair collected inside the dedicated sample bag.

Photo courtesy of Jorge Luis Mendoza Silva

## 17 NAILS

The tip of one nail from each hand and foot (4 in total) are collected on a small piece of cotton and stored with the hair DNA sample, in the same sample bag.



Cutting the tip of a nail of a tamarin. Photo by Jorge Luis Mendoza Silva.

## 18 VAGINAL SWAB

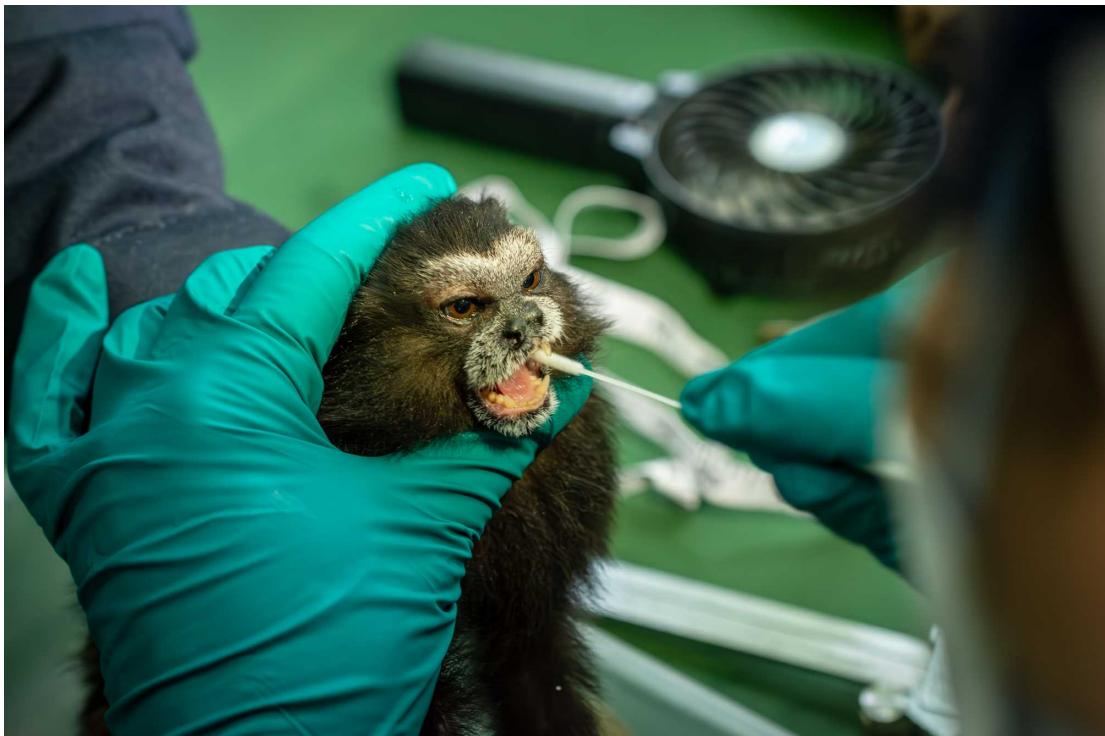
If an adult female is still under a deeper plane of anesthesia, a vaginal swab is gently rotated in the vaginal canal 2-3 times. The tip of the swab is then cut inside a 1.5mL tube filled with preservative. This sample may be collected during second phase processing if the animal is starting to wake up during the first phase of sampling.

### Safety information

USDA Pain/Distress Category: C

## 19 BUCCAL SWABS

Two buccal swabs are collected by inserting a small plastic swab in the buccal cavity and rotating it approx. 5 times. The tip of the swab is then cut inside a 1.5mL tube filled with preservative.



Collection of a buccal swab from a tamarin. Photo by Jorge Luis Mendoza Silva.



Collection of a buccal swab from a tamarin. Photo by Jorge Luis Mendoza Silva.

## Safety information

USDA Pain/Distress Category: C

- 20 When the animal is awakening or within 15 minutes from the anesthetic injection, the animal is placed inside a cotton bag and moved to an individual compartment in a recovery cage, which is kept inside the processing tent. The cage is covered by sheets to maintain a quiet, dark area for recovery from anesthesia.



The animals are placed inside a mesh bag and transferred temporarily into a recovery cage while waiting for the second phase of processing. This allows for a safer handling of the animal while reducing stimuli. *\*This is an old picture, the coverall dress-code was not in use.* Photo by Ishaan Raghunandan

### Note

For the last animal out of the trap (or in the case of a single individual capture), first phase and second phase processing are completed continuously without any pause or placement in a mesh bag.

Therefore, in the case of a single individual capture, the calculated dose of anesthetic drugs is administered as a single injection.

- 21 Everything that has been in contact with the animal is then cleaned with (1) 10% bleach, (2) water and then (3) alcohol. Gloves are always changed in between animals.



Surfaces that have been in contact with an animal are decontaminated between animals. \**This is an old picture, the coverall dress-code was not in use.* Photo by Ishaan Raghunandan



Sterilization kit prepared with a rack and four 50ml falcon tubes. The tools used to collect samples are decontaminated in 10% bleach for 5 minutes, then rinsed with water twice and finally rinsed in alcohol, using the sterilization kit, organized as shown in this picture. Courtesy of Thomas Parsons.

- 22 The first phase processing is repeated with each animal. [ED go to step #10](#)

## 2nd Phase - Processing

- 23 The animal, still inside its mesh bag, is removed from the recovery cage, placed on the plastic tray, covered by a light towel, and then injected intramuscularly with the anesthetic, in the thigh. Usually, 2/3 of the total dose (calculated based on the measured body weight of the individual) is enough to allow safe handling of the animal for the second processing.
- 24 After about 1-2 minutes, or when induction is achieved, the animal is removed from the mesh bag and vital assessments are recorded (see **step #13**) in the processing sheet.
- 25 A team member then measures the tail with a measuring tape before applying a bleach mix to

the hair on the tail for a temporary form of identification.

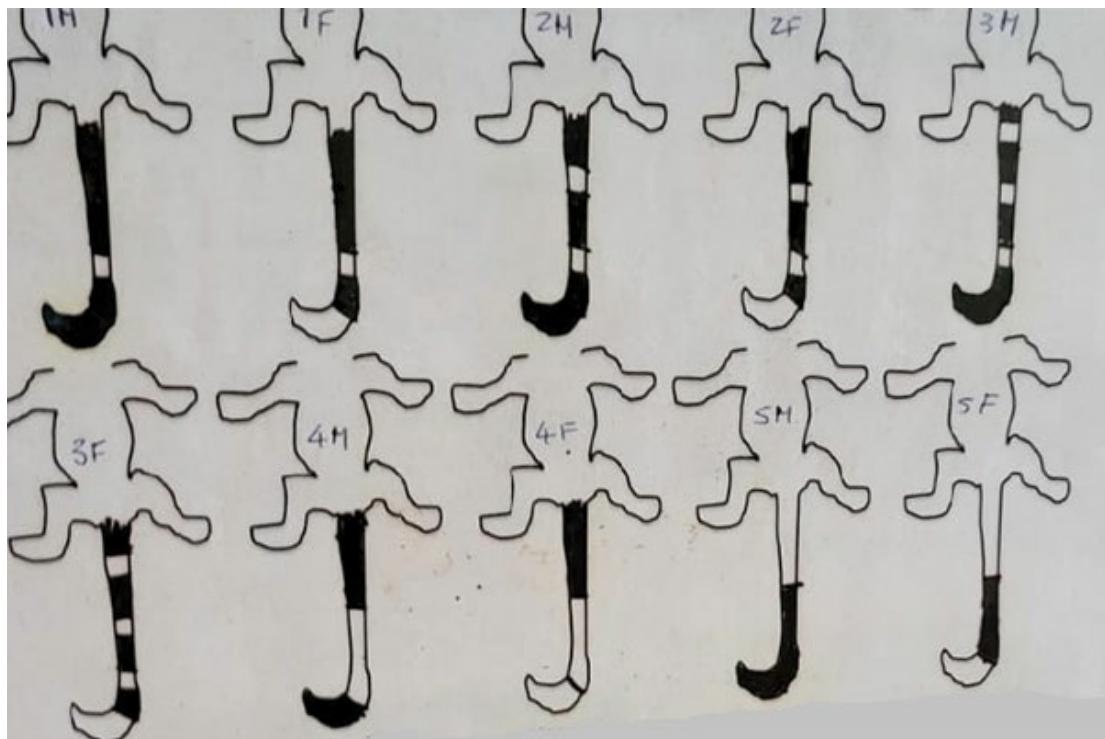


Image showing different bleaching patterns used for the tail. The letters "M" and "F" refer to the sex of the animal, while the number refers to the individual pattern.



To bleach the tail, the hair-bleaching powder is mixed with liquid activator. The mixture is then applied to the fur on the tail in a specific pattern and covered with aluminum foil. These foils are then left in place for at least 10 minutes to allow decoloration.



The process of tail bleaching. \*This is an old picture, the overall dress-code was not yet in use.  
Photo by Ishaan Raghunandan

## 2nd Phase - Sampling

**26** Once the vitals are assessed and the bleach is in place, second phase sampling proceeds as follows:



### BIOPSY

1. The animal is placed on its dorsum, while another team member is covering its head with a light towel.
2. Using an antiseptic wipe, the bald skin on the armpit is disinfected.
3. Using forceps, a small piece of skin (~ 0.3 mm) is isolated and cut around the tip using fine scissors. The 0.3 mm diameter skin biopsy is then placed in a 1.5mL tube filled with preservative (ideally not a lysis solution).
4. A drop of liquid bandage is immediately placed on biopsy wound and allowed to dry.

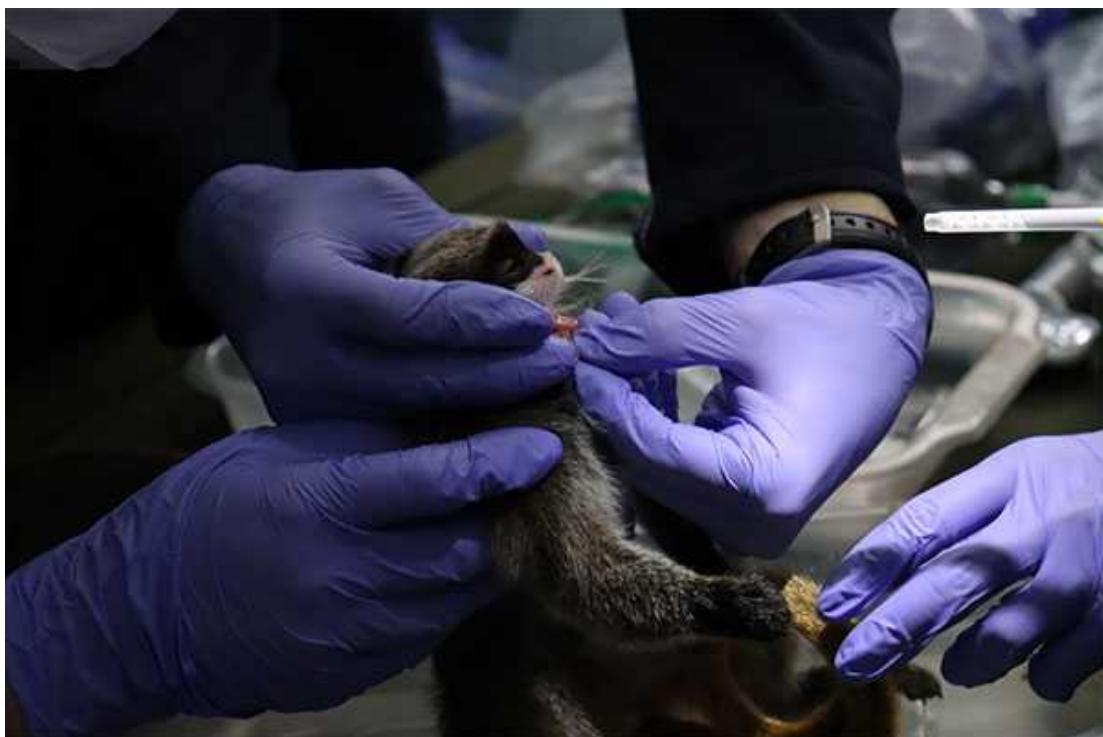
**USDA Pain/Distress Category: C**

27

**DENTAL MOLD**



1. A new tip is placed on the dental gun and filled with dental impression paste by pressing repeatedly on the gun's trigger.
2. The designated handler holds the animal with one hand, from underneath the armpits.
3. A senior researcher holds the mouth open by holding the top jaw with one hand and holding the thumb of the other hand on the lower incisors, gently pulling down, keeping the animal's mouth open in a stable position.



Picture showing how to hold the mouth open to collect a dental cast on a tamarin.  
Photo by Jorge Luis Mendoza Silva.

4. The other experienced team member then uses the dental gun to place the dental paste in a "U" shape layer covering the occlusal aspects of the teeth of the lower jaw.



Use of the dental gun to apply the resin on the lower jaw. Photo by Ishaan Raghunandan

5. A square of laminated paper shaped to fit inside the mouth of the animal is then placed on top of  
the first layer of the dental paste. This allows for the upper and lower jaw dental molds to be  
collected simultaneously and then easily be separated for later analysis.
6. The entire surface of the laminated square (facing the palate) is covered with dental paste to  
get  
obtain a dental mold of the upper jaw.



The laminated square is placed on top of the resin covering the lower teeth and then covered with more resin. Photo by Ishaan Raghunandan

7. The animal's mouth is then shut closed and kept stable until the resin solidifies, which takes about one minute.
8. Once hardened, the dental mold is ready to be removed and stored inside a dedicated sample bag.

#### Safety information

USDA Pain/Distress Category: C

28

#### BLOOD



1. The designated handler holds the animal with one hand, from underneath the armpits.
2. **The veterinarian or senior researcher responsible for drawing blood** holds the leg of the animal from the knee area (using the non-dominant hand), and holds the syringe with the dominant hand.
3. After disinfecting the skin in the femoral area, it is possible to visualize/palpate the femoral artery which is pulsating. To minimize bleeding following venipuncture, we opt to avoid the artery and collect blood from the vein. Parallel to the skin, a 23G needle on a syringe enters

around 0.5 cm caudal to the vein, pointing towards the vein at a 90 degrees angle with its longitudinal axis.

4. Once the needle enters the blood vessel, there is typically a "flash" of blood that appears in the plastic neck of the needle. Maintaining this position, the plunger of the syringe is gently pulled back until ~0.5 mL of blood has been drawn.
5. After the syringe is carefully removed, the person holding the animal puts strong pressure on the venipuncture site with their thumb to create hemostasis.
6. A drop of blood is used for blood glucose and ketone concentration measurements, as well as 2 blood smear slides. A 0.3mL aliquot of blood is also placed in an tube containing lysis buffer. The remainder of the sample is placed in capillary tubes.



A photo showing proper handling and collection of a blood sample from a tamarin. Photo by Jorge Luis Mendoza Silva.

## Safety information

Total blood collected is < 1% of animal body weight, in accordance with the 2016 Guidelines of the American Society of Mammalogists for the use of wild animals in research and education (<https://doi.org/10.1093/jmammal/gyw078>).

### **USDA Pain/Distress Category: C**

#### **Risks**

Loss of excessive blood: venipuncture does not cause excessive blood loss, and left alone blood rapidly coagulates and stops bleeding. However, we hasten this process by applying pressure to the puncture site once blood is collected.

#### **Post-procedure monitoring**

Confirming that the puncture site is not bleeding before the animal is placed in the recovery cage.

## **2nd Phase - Measurements and Collar placement**

- 29** Morphometric measurements are then collected using a digital caliper and a measuring tape. All measurements collected are listed in the table from the animal processing sheet below.



Measurement of the upper right canine using a digital caliper. Photo by Jorge Luis Mendoza Silva

#### MORPHOMETRICS

	L	R		L	R	Inj. Loc.	Injury Description
Up. Canine			Foot nail				
Low. Canine			Hand nail				
Nipple			Thigh Circ				
Testes/Vulva	L	W	Calf Circ				
Sp Gland	L	W	Arm Circ				
ST Gland	L	W	Forearm Circ.				
Circum.	Chest	Waist	Head L.				
Body L			Tail L.				

Table showing the measurements that are performed on tamarins, as it appears on the primate processing sheet.

- 30 Using a camera calibrated with a color card, photographs are also taken of body parts listed in the table found on the processing sheet.

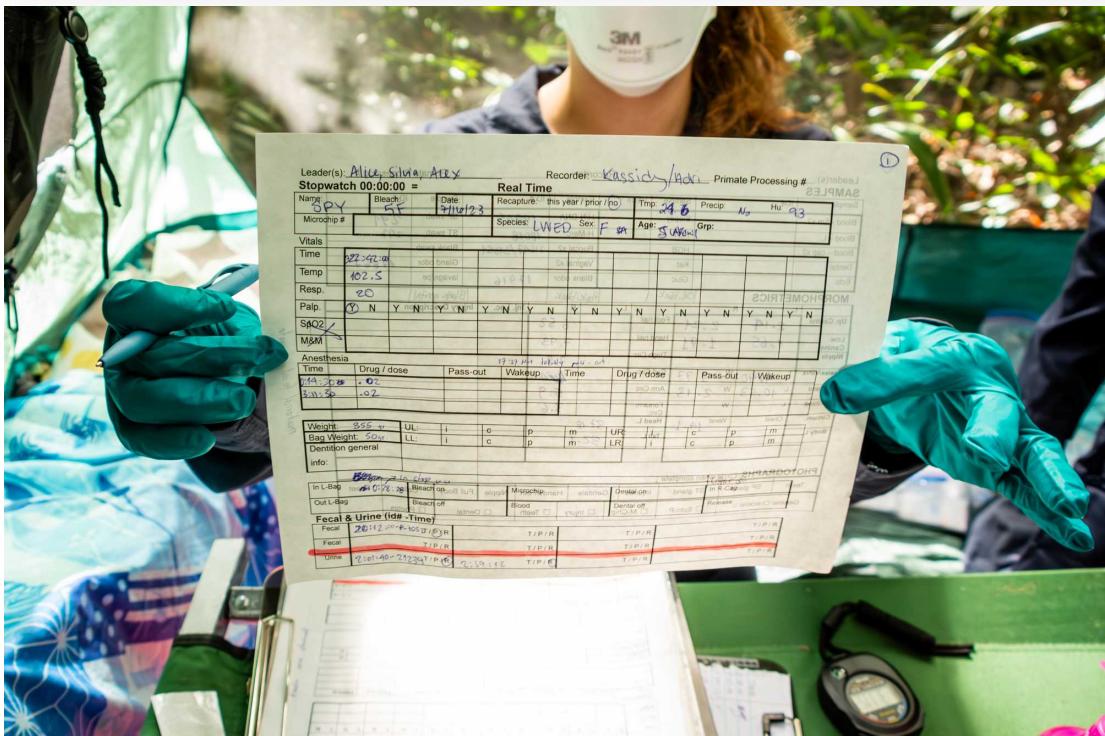
**PHOTOGRAPHS:** Circle when complete ↓

Teeth	SP gland	ST gland	Injuries	Vulva	Scrotum	Nipple	Collar	Full body
-------	----------	----------	----------	-------	---------	--------	--------	-----------

List of relevant pictures from the primate processing sheet.

**Note**

The first picture taken is always the animal's processing sheet indicating the animal processing number. This precaution allows sorting the pictures to the correct individuals after the trapping event.



The image shows the first photograph that needs to be taken to then continue with the descriptive photo session of the animal. Phoyo by Jorge Luis Mendoza Silva.

## 31 RADIO COLLAR PLACEMENT

#### Note

A subset of adults in each trapped group are given a tracking device on a collar that weighs no more than 2% of the average weight of an adult, well within the specified limits of 5-10% of body weight for mammals (Sikes, 2011). These can be either very-high frequency (VHF) radio collars or ultra-high frequency (UHF) tags.

If a tracking collar will be placed, its proper functioning is confirmed the day before capture. The collar is placed around the neck, ensuring that it is not putting too much pressure on the throat (too tight) and that if the animal was to try to put its arm through the collar, the elbow cannot get stuck in it (too loose).



Positioning of a radio collar on a tamarin's neck. *\*This is an old picture, the coverall dress-code was not yet in use.* Photo by Ishaan Raghunandan

#### Note

At least two experienced team members assess the sizing of the collar, before definitively securing it on the animal.

As a general rule of thumb, collars weigh < 5% of animal body weight. Collars and tags are used on select taxa to study their use of heterogeneous habitats, to understand territorial behavior, migration or dispersal, to visualize overlap between species in space and time, to monitor animal survival and well-being, and to conduct follow-up research.

Collars/tags are placed with the following guidelines in mind:

- In this program all animals are habituated to traps for annual health monitoring, so it is possible to replace/remove tracking devices. We rely on longitudinal data to demonstrate that the collars used do not effect the survival or reproduction of the study subjects.
- In cases in which recapture is not anticipated, collars are voluntarily damaged in a way that will cause them to fall off over time due to normal wear and tear. Alternatively, collars are attached with a temporary material that will fall off over time (e.g. thin chain, skin glue and thin-stretch plastics).
- If recapture is not anticipated and when weight is not a factor, pre-programmed drop-off mechanisms are considered. However, this feature is not a certainty, and thus the above considerations always apply.

### RISKS

Poor sizing of animal collars will result in collars falling off prematurely, or constraining an animal's normal growth and causing excessive abrasion, injury, or worst case, death.

Permanent, non-expanding radio collars are NEVER to be placed on juveniles or sub-adults with as yet unknown growth potential.

### MONITORING

According to the collar type, monitor the animal for normal movement in the days following capture, and then develop a schedule for checking on the animal regularly until the 1 year mark. After 1 year, attempt to recapture the animal to check health status, and sizing and integrity of the tracking device.

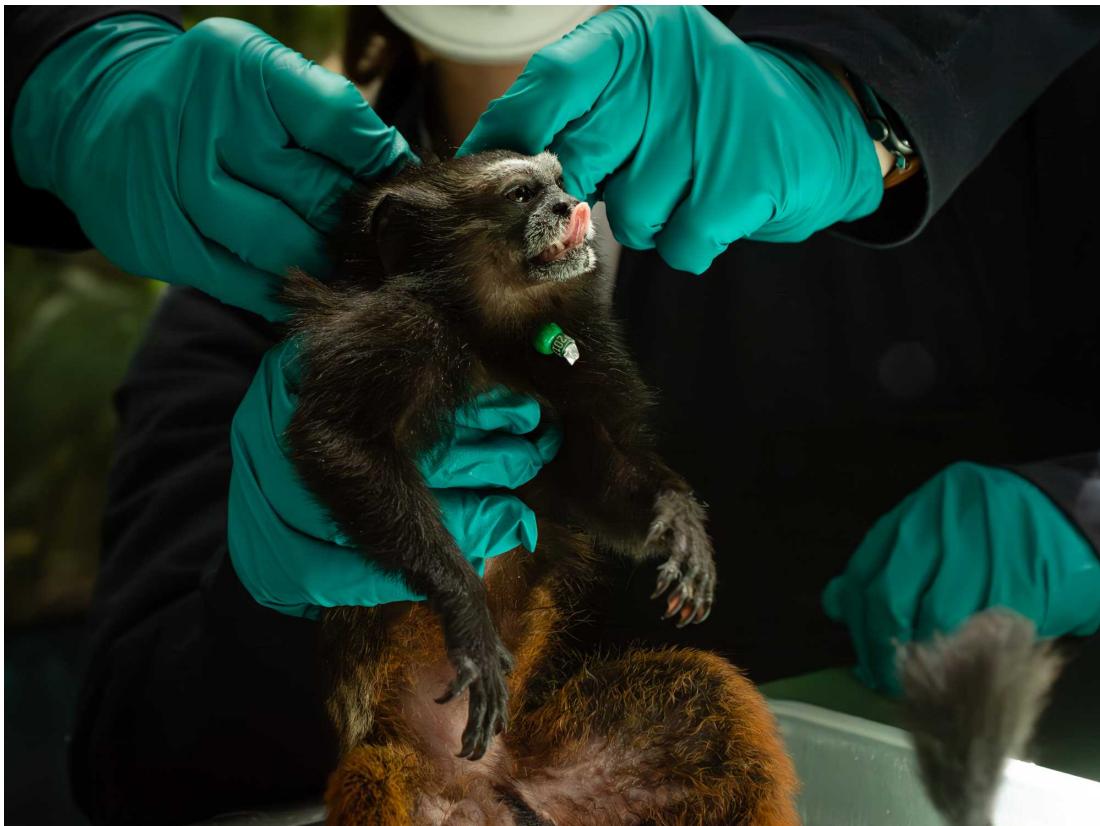
## 31.1

### BEADED COLLAR PLACEMENT

A beaded collar will be placed around the neck of other group members to allow for individual identification on behavioral follows. Beaded collars are made of rust-resistant aluminum ball-chain string that naturally wears and breaks within 6-12 months; a smaller and weaker version of the material used for army identification tags. Pliers are used to close the aluminum clasp that connects the two ends of the bead chain forming the collar.



The image shows the installation of the bead necklace, which must be done very carefully and the installation should not be too tight to avoid damage to the animal. Photo by Jorge Luis Mendoza Silva.



Beaded collar placement. Great care is given to ensure the collar is neither too tight, nor loose, around the animal's neck. Photo by Jorge Luis Mendoza Silva.

#### Note

The typical three-bead colour system (top bead denoting group membership, middle bead denoting sex and third bead denoting unique individual ID) includes two sets of beads, one hanging in front and one behind, so that individuals are recognizable when their backs are facing the observer (Watsa et al., 2015).

Typically, collars naturally fall off the animals before the start of the next capture season. In subsequent trapping seasons collars are replaced until the research program ends, at which point all that remain will be removed.



The three-bead colour system denoting the group, the sex and the individual inside the group allows each animal to be individually recognized. Photo by Jorge Luis Mendoza Silva.

- 32 The fur of the tail is washed with non-irritating baby shampoo to remove any residual bleach material. The tail is dried with a towel and a full-body picture that includes the bleached tail pattern is taken.



It is important to take a final full-body picture showing the marking on the tail so that the research team can easily recognize each individual. Photo courtesy of Jorge Luis Mendoza Silva.

- 33** The animal is then left to recover from the anesthetic in a covered recovery cage with individual compartments for each animal.

**Note**

Checking the status of each animal every 3-minutes during the FINAL recovery period MUST persist until it is confirmed that an individual is fully alert on multiple consecutive checks. 3-minute checks continue for any individuals that do not meet this criteria. Individuals that are fully alert may be checked less frequently, not exceeding 10 minutes apart.



The recovery cage is covered with a sheet to reduce stress for the animals. Separate plastic trays are positioned underneath each individual compartment of the recovery cage to collect any feces or urine dropped by the animals while recovering. Using this precaution it is possible to determine which individual the sample belongs to, and to prevent contamination. Photo courtesy of Silvia Carboni.

**34 CLEANING**

Clean the surfaces that have been in contact with the animal or its secretions by:

1. Spraying 10% bleach and then alcohol;
2. Disinfect all the tools used during the processing with the sterilization solutions, accordingly to the protocol: leave submerged in 10% bleach for 3 minutes -> 1st rinse in distilled water -> 2nd rinse in distilled water -> submerge in 70% ethanol.
3. Dry all tools and surfaces with clean paper towels or sterile cotton.

- 35 The second phase processing is then carried out for the next animal, following the same order used in the first processing  go to step #23

## Recovery & Finalization

- 36 When all the captured animals have gone through the second processing, they are left to recover inside the recovery cages for about 1.5 h from the time the last injection of anesthetic was received by the last animal processed.

- 37 Most of the team members help sterilize equipment and materials, clean the tent, organize samples, and pack up most of the equipment for transportation back to the field station. The voice recording is ended after repeating the following: full date, location, primate group processed, and the names of each team member present.

- 38 While most of the team departs the trap site, a **senior researcher** stays in the tent with the animals until the time of the release: checking them every 10 minutes, making sure they are reactive to stimuli and recovering well from the sedation.

Fecal and urine samples are collected opportunistically from the trays placed underneath each individual compartment of the recovery cage. They are stored in sterile tubes and bags and placed in a thermos container filled with ice packs. Sample numbers are recorded on a data sheet.

- 39 Before the release, all the animals are offered banana pieces through the mesh of the recovery cage, which, along with movement and vocalization, helps to assess their readiness for the release and provides them with sugar and hydration.

## Release

- 40 At least two people must be present and help record the release event. Usually one member of the trapping team comes back to the site at release time, or a different researcher joins the senior researcher.

- 40.1** The recovery cage is carefully moved outside of the tent, still covered with the sheet, and placed on the ground in a suitable release spot. The senior researcher kneels down near the cage, ready to open the compartments, while the second person is positioned five meters behind, holding binoculars or a camera, ready to observe the release. A video recording is started to document the release.
- 40.2** Each compartment is opened consecutively so that the animals exit the recovery cage one at the time. This allows taking individual pictures that can be used by researchers to learn to recognize the group members.
- 40.3** The observers remain in the area for a few minutes until they can ensure that all the animals are moving normally. In the case in which one or several animals were left out during the trapping event, the observers ensure they witness the reunification of these animals with the rest of the group. Usually the animals that are not trapped will remain close by for the duration of the trapping event, often heard long-calling in search of their group mates, and reunite with them soon after release. If group reunification does not witness after release, the group is followed in days following the trapping event in order to ensure the group is complete.

## Sample sorting

- 41** After release, the rest of the equipment is packed up and brought back to the station, where sample sorting and data entry then take place.

**41.1 SAMPLE SORTING**

Organize all samples according to the laboratories sample storage protocol.

**IMPORTANT:** serum samples must be spun and extracted to a serum storage tube the same day and then stored frozen. Do NOT freeze serum tubes until they have been spun down.

**Note**

Immediate freezing will cause tissue to break and cells to lyse, and is important for nucleic acid based research. However, samples designated for morphological analysis will be ruined by freezing. Cell isolation by centrifugation will also be ruined if freezing occurs prematurely.

If sample sorting is delayed for any reason, samples should be stored in the freezer, unless otherwise indicated by a senior scientist.

## 41.2

### FIX BLOOD SMEARS

1. Arrange slides face-up on a clean surface and confirm that sample code is clearly visible.  
If not, trace over to make a clear labeling
2. Identify a coplin jar with methanol that has not expired and is full.
3. Open the jar and place smears inside (pairs can be placed back-to-back, MAKE SURE THAT SMEAR IS FACING OUTWARD). Quickly close jar again to prevent the methanol from oxidizing.
4. Leave the smears in solution for 5 minutes
5. Wearing a pair of gloves, remove each slide, gently tap the edge on an absorbent paper to remove the excess solution, and place flat on an absorbent paper to dry for a few minutes.  
Make sure to close the coplin jar as quickly as possible to preserve to the methanol.
6. Place in the storage box.

## Data Organization and Entry

42 For the remainder of the day, the team will:

### 42.1

Check that all the information on the processing sheet has been entered correctly and that the sample numbers match the data, and listen to the voice recording to fill in missing information on the data sheet with a differently colored ink pen (usually red).

#### Note

You can look at the relevant times written on the animal's processing sheet, to guide you on which time frame of the voice recording you need to listen to.

### 42.2

Upload the information gathered on the PROCESSING SHEETS to the online ODK form.

◀ 2022 Primate Capture

BLOOD

Blood Tube (4 of 4):

CAPILLARY TUBES

capillary tube codes

BLOOD SMEARS

Smear (1 of 2):

Smear (2 of 2):

Blood parameters

Hematocrit (HCT)

The Primate Capture ODK form as it appears on the tablet or computer screen.

- 42.3** Write a detailed narrative report of the capture session. This is the **TRAPPING REPORT**.

Note

Previous trapping reports may be used as a template.

- 42.4** Gather pictures and videos and sort them into designated folders on the project hard drive.

- 42.5** Convert the processing sheets of each individual into PDF files, and save them into each animal's dedicated folder.

- 42.6** Group all the files into a unique folder named by date of capture and by animal number [yyyymmdd\_group\_animalname].
- 42.7** Save the **VOICE RECORDER** file into the folder.
- 42.8** Wash all the linens used during the capture (i.e. coveralls, animal bags, towels, sheets used to cover the recovery cage) with detergent and a disinfectant product, such as quaternary ammonium based solutions.
- 42.9** Put all batteries to charge. We are using rechargeable batteries which should preferably be charged during daylight, when the solar panels that provide electricity to the station are working.
- 42.10** Replenish and repack supplies for the next trapping event. Usually part of the trapping team prepares and pre-pack the supplies, then the rest of the team double-checks the packing, to ensure nothing is missing.