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Handling and Sampling Bats - ISL Peru

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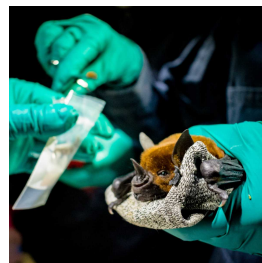
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Manuscript citation:

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Protocol status: Working

We use this protocol and it's working. This protocol is reviewed and updated annually.

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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.



Abstract

Program Timing:

Sample collection occurs annually during the rainforest dry season (June - August). Sample analyses occur between September and April.

Program Overview:

Bat mist nets are set up at multiple locations encompassing major habitat types surrounding the field station (terra firme, flood plain, swamp, bamboo, successional forest, primary forest, edge forest, etc). Mist netting occurs over 3-4 consecutive days at each location throughout the sample collection period. Dependent on resource availability and personnel, we strive for an annual sample size of 200 - 300 bats.

Team Composition

This protocol is intended to be carried out by a team of 3 individuals, including at least 2 trained personnel. Roles include (1) designated bat handler (2) sampling assistant (3) data recorder. Modification of team composition should be made according to the anticipated number of captures.

Capture Overview:

Mist nets are installed a day prior or several hours ahead of an intended capture event → nets are opened between 17:00 and 22:00 (generally), and captures occur spontaneously throughout (~ 5 - 20 animals/event) → animals are extracted from net (1-2 minutes) → transferred into a light and breathable cloth bag and kept in order-of-capture in a nearby tent until processing (0 - 30 minutes) → one-by-one animals are processed for morphometric measurement, photographs, marking (optional) and nonlethal tissue collection (~10 minutes) → animals are immediately released at the site of capture once processing is complete, but away from mist nets.

Image Attribution

Jorge Luis Mendoza Silva, Ana Priscila Peralta Aguilar, Naija Cuzma, Thomas Parsons

Guidelines

Whenever working in the presence of a bat, **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 recommended.

It is MANDATORY to be vaccinated with pre-exposure rabies prophylaxis in order to handle bats.



Materials

Bat processing sheet



BatsForm_hardcopy.pdf 159.6KB

ODK forms

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_bat_handling.xlsx 105.6KB

Miscellaneous

- Clipboard
- 30+ processing sheets
- Pens, sharpies
- Sharps container
- Trash bag
- Gloves—size (S,M,L)
- Masks (N95) x6
- Processing table/cot
- 2Processing Plastic trays
- 4 pairs of handling gloves
- Masking tape
- Paper towels
- Processing bags (35+)
- Tent

Electronics

- Voice recorder (charged)
- Environmental thermometer
- 2 Stopwatches
- Extra batteries (AA and AAA)
- Ketomojo machine+ glucose and ketones stripes (10 each)
- Camera (charged)
- Black plaque for image background

Tool bag

- 3 anatomical forceps
- 2 Scissors(one straight, one curved)
- 2Hemostatic forceps

Sampling Material



Blood

- 15 falcon tubes
- 2 tubes of micro haematocrit capillary tubes (at least 50)
- Clay
- Cotton buds (30+)
- Vaseline

Blood smears

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

Sample box (at least 25 of each):

- Blood tubes w/ 1x zymo shield
- Ecto tubes with 70% ETOH
- Biopsy tubes with RNA Later
- Buccal tubes w/ 1x zymo shield, at least 60
- Poop tubes

Biopsy bag

- 30+ small squares of cardboard
- 3 punch biopsy devices

Sample bags (25 of each)

- H-DNA bags
- H-Merc bags

General

- 10% bleach spray bottle
- 70% Alcohol spray bottle
- Hydrogen peroxide
- Cotton
- 1, 3, mL syringes (25 of each)
- Antiseptic towelettes, 10+
- 25-gauge needles, 30+
- Swabs, 60+
- Tube rack

Sterilization kit

- 1 × 10% Bleach in 50 mL falcon tube
- 2 x distilled water in 50 mL falcon tubes
- 1 × 70% alcohol in 50 mL falcon tube

Cooler for samples

- Ice pack
- Ziplock bags

Medical

- Saline solution 100ml
- Antiseptic solution
- tissue glue

- sugary water jar

Measuring material

- calliper
- Pesola weighing scales (30g, 100g, 300g)

Safety warnings

White-nose Syndrome

White-nose syndrome (WNS) is a disease found in bats caused by the fungus *Pseudogymnoascus destructans*. This disease has spread rapidly in the US and Canada and is a severe threat to bat populations across North America, especially those species who participate in communal roosts. The US Department of Agriculture outlines the following options for decontaminating equipment that may have been exposed to the WNS-causing fungus: submerging equipment in water greater than 50 degrees Celsius for at least 20 minutes (most appropriate for clothing); submerging equipment in a diluted bleach solution for at least 10 minutes; cleaning non-submersible equipment with lysol disinfecting wipes. Consult this pamphlet from the USDA for more detailed instructions, https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5378842.pdf. Even when working with bats outside of regions where WNS has been identified, these guidelines should be adhered to.

In case of a bite from a wild mammal

Thoroughly rinse the site for 10 full minutes, disinfect the site, and notify the PI immediately.

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 approved 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

The PI is in charge of coordinating the bat capture activities and selecting the sites where mist nets are placed. Sites are changed every 3 - 4 days to avoid high recapture rates, or as determined by the PI.

Bat team personnel will pack and double-check that all capture materials needed are cleaned and organized before departing to the capture site. Refer to the MATERIALS section.

ROLES

1 Team Composition

This protocol is intended to be carried out by a team of 3 individuals, including at least 2 trained personnel. Modification of team composition should be made according to anticipated number of captures. Roles include, (1) designated animal handler (2) sampling assistant (3) data recorder.

1.1 Designated Handler

This is a trained and senior researcher/veterinarian that is experienced with every step of the bat capture process. They are responsible for animal mist-net removal, as well as direct handling during the processing phase and release.

1.2 Sampling Assistant

Will assist the designated handler with:

1. Passing tools, bags and tubes needed for processing
2. Reading aloud the serial codes that identify each sample bag and tube.
3. Taking all images listed in the processing sheet.

Images					
face / chin	teeth	body	profile	fur	UPAG
genital	Calcar / ankle	gland	thumb / claw	tail	Wing / PROP
cups	Injury	datasheet		NO IMAGE	



Body and dental photographs are recorded to help identify the species, age, pathology, etc. Courtesy of Zane Libke.

1.3 Data Recorder:

Will perform data recording on the animal processing sheet

 BatsForm_hardcopy.docx.pdf :

1. Write down the weight of the animal and any other measurements, sample codes, and important time stamps.
2. Recording notes from the handler
3. Storing samples in their dedicated sample boxes.

2

PREPARATORY PHASE

- 3 Bat team personnel pack and double-check all bat capture and processing materials needed before departing to the capture site. Refer to the MATERIALS section.
- 4 One day prior, the team will install 4-12 nylon mist nets (6 - 12 × 2.7 m, 4 - 5 shelves, mesh size 15 - 20 mm) in the chosen areas. The number of mist nets used should reflect with the size of the bat team and frequency of bat capture at that particular site. Nets must be closed if:
 - inadequate personnel are available to adhere to expected processing times

- weather conditions are not poor
- or to reduce inspection time when temperatures are low

Generally, nets remain activate from 17:00 to 23:00.

Note

Nets are closed by sliding all loops close together and twirling the net onto itself so that it is readily visible from a distance and hard to become entangled. **Nets are opened** by un-twirling the net and separating the loops so that each shelf is expanded and no longer easily visible from a distance.

Video on how to setup and take down a mist-net

- 5 A screened-in tent with a table or processing cot is setup within 25 - 50 meters of the mist nets, which will be used for processing animals.



IMAGE DESCRIPTION: A ready processing table during a bat capture session. Some of the visible items include: **(a)** sample tubes, bags, and sampling tools needed to process a bat are placed on clean plastic trays; **(b)** sterilization kit consists of four falcon tubes, filled respectively with 10% bleach, distill water(x2) and 70% alcohol, and is set close to the sampling tools, so that tools can easily be disinfected in-between animals; **(c)** hard-plastic container with a safety lid is used to collect sharp waste and a zip-lock plastic bag is dedicated for other trash produced during the processing; **(d)** measuring tools such as calipers and scales are also sprayed and wiped cleaned after each use and set on the table to process the next animal; **(e)** stopwatches run during the entire capture session and are used to record important time points (refer to animal processing sheet); **(f)** jar containing sugary nourishment is present on the table, a new syringe is used to offer nourishment to each bat during processing. Courtesy of Naija Cuzmar

CAPTURE

6

Safety information

Whenever working in the presence of a bat, 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 recommended.

It is **COMPULSORY** to be vaccinated against rabies in order to handle bats. 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**.

7 The nets are opened from sunset, typically around 17:30 hours, until 22:00 hours and checked according to the following schedule:

- First 2 hours - every 20 minutes
- After 2 hours - every 30 minutes, if the rate of capture is low
- Cold temperature - every 15 minutes if air temperature is below 20 degrees Celsius

8 Start the STOPWATCH and VOICE RECORDER, which should be running during the entire session. Say aloud: (1) the full date and time; (2) the weather and temperature; (3) the capture site; (4) the team members participating; (5) total number of nets; (6) the times on the stopwatch and time elapsed on the recording.

Important times recorded on the processing sheet should refer to the time indicated on the stopwatch.

TIMES

IN NET	IN BAG	OUT BAG	RELEASE
--------	--------	---------	---------

List of important times as they appear on the BAT PROCESSING SHEET (see MATERIALS section).

Note

Whenever a sample or measurement is collected say it aloud clearly for the VOICE RECORDER and DATA RECORDER

9 NET INSPECTION:

The person(s) inspecting the nets carry a walkie-talkie to communicate with the rest of the team waiting inside the tent. As soon as a captured bat is discovered the team is informed so that DISCOVERY TIME, NET NUMBER and PANEL NUMBER may be recorded.

Note

The NETS are numbered sequentially with 1 closest to the processing tent

PANELS are numbered sequentially with 1 being the top panel

10 BAT REMOVAL:

Only designated and experienced personnel may remove the bats from the net:

1. Place a clean thick leather glove in between the ventrum of the bat and the palm of the non-dominant hand. The bat is restrained between the palm and the thumb. The thumb is positioned on the bat's dorsum and pushes the bat gently against the glove. CAREFUL NOT TO COVER THE HEAD OF THE BAT, WHICH COULD CAUSE SUFFOCATION. See image below.
2. Using the dominant hand, gently pull the mesh of the net away from the feet and legs, untangle the wings, and lastly the head.
3. Once removed, the bat is placed inside a clean cloth-bag and brought to the processing tent. The BAG NUMBER should be written on a piece of masking tape attached to the bag, and recorded on the ANIMAL SHEET.

Record the time that the animal is placed inside a cloth-bag (IN BAG TIME)



Image of a trained personnel removing a bat from the mist net using a thick leather glove in between the ventrum of the bat and the palm of their hand. Courtesy of Jorge Mendoza.

- 11 An animal is processed straight away or left on a line and processed in order of net extraction, however, always give first priority to bats that appear depressed. The waiting line must be in a DRY place that is not exposed to wind.

Bats waiting in bags must be checked for signs of movement/life every 10 minutes. If an animal appears unwell the team leader must be notified immediately.

PROCESSING

12

Note

A picture of the animal sheet showing the BAT NUMBER, should be taken before beginning PROCESSING. This will serve as a place marker on the camera for sorting bat images later

Record the weight of the cloth holding bag + bat inside

- 13 **Designated personnel** will remove the bat from the bag and handle the animal for the entirety of processing.

- 13.1 Carefully open the bag where the bat is kept, identify the head of the animal, and position it so that the head is facing away from you.
- 13.2 Grab the animal by putting the dorsal aspect of the index finger in contact with the dorsum of the animal, and by holding the folded wings from the humerus. Use the thumb on one humerus and the middle finger on the other one.



Image showing how to hold a bat when removing it from the cloth-bag.
Courtesy of Jorge Luis Mendoza Silva.

- 13.3 Pull the animal out from the bag and place it with its ventrum on a thick leather glove to prevent your hand from being bitten (instead of a glove, the holding bag may be used if it has been folded onto itself to be sufficiently thick). With the thumb, hold the animal gently pressed against the glove, which is positioned on the palm of your hand.



Image showing how to handle a bat by restraining it against a glove. Courtesy of Jorge Luis Mendoza Silva.

- 13.4 Use the dominant hand to grab the tools needed for the sampling or to move the wings of the animal.
- 13.5 Inspect the wings for possible biopsy-punch holes (usually on the right wing). If you find a punch hole that looks fresh, look along the dorsum for a patch of cut hair, which means that the animal is a recapture from the same trapping season. Taking pictures can help determine which individual it is.

In the case of recapture, the animal can be released straight away without undergoing SAMPLING.

SAMPLING

13.6

FRUIT

Although rare, if a bat is found captured in the net with any fruit in its mouth, collect the sample into labeled zip-lock bag and record as an additional sample on the animal sheet



Image showing a bat captured next to a fruit that it was carrying in its mouth. Courtesy of Jorge Mendoza.

FECES

Check for FECES inside the holding bag and always collect feces opportunistically during the PROCESSING phase. Use clean forceps or a sterile swab to transfer feces into designated pre-labelled tubes or plastic zip-lock bags

Note

As a convention, only feces collected directly from the anus (with clean tweezers) or the rectum (via rectal inspection or a swab), and transferred straight away into a dedicated bag/tube, can be considered "uncontaminated".

Conversely, feces collected from a surface or the perineal region are to be considered contaminated, and this should be specified on the form.

13.7 BUCCAL

Collect 2 buccal swabs by inserting a small plastic swab in the buccal cavity and rotating it approx. 5 times.



Image showing a buccal swab gently being inserted into the buccal cavity.
Courtesy of Jorge Mendoza.

- 13.8 **Before collecting HAIR**, take a picture of the dorsum of the bat if there is a morphological character present that could help identify the species.



Picture documenting the hair pattern on the dorsum of the bat. Note the correct way to handle a bat using a handling glove to keep your hand away from its mouth, and the thumb to gently press on its shoulder area to restrain it for processing. Courtesy of Ana Priscila Peralta Aguilar.

13.9 **PLUCKED HAIR**

From the dorsum, hold a few hairs with hemostatic forceps and pull gently in the direction of the hair, repeat the action several times to have a good amount with bulb collected. Place it inside a plastic bag labelled as: "H-DNA" . Read clearly the serial number identifying the H-DNA bag for the voice recorder and for the data recorder.



The image shows how to remove hair for DNA analysis. Courtesy of Jorge Luis Mendoza Silva.

Safety information

USDA Pain/Distress Category: C

Risk(s): If you hold a lot of hairs at the same time and pull aggressively, the action will cause a lot of pain to the animal and part of the skin may tear.

13.10 **CUT/SHAVED HAIR**

Before cutting, identify the length of the hair on the dorsum by pulling it back with the scissors closed. This action will help to show the maximum length that can be cut to avoid cutting skin. Then, with a fine scissor held parallel to the dorsum cut HAIR and place it in a H-merc bag.





Picture showing how to cut hair for mercury study. A team member is assisting by having the sample bag open and ready. Courtesy of Jorge Luis Mendoza Silva.

Safety information

USDA Pain/Distress Category: C

13.11 **WING BIOPSY**

Unfold one of the wings (usually the right one), and place it against a light source (we recommend a light pad such as [this one](#)). Mark a small dot at a non-vascularized area of the dactylopatagium with a black marker (sharpie).

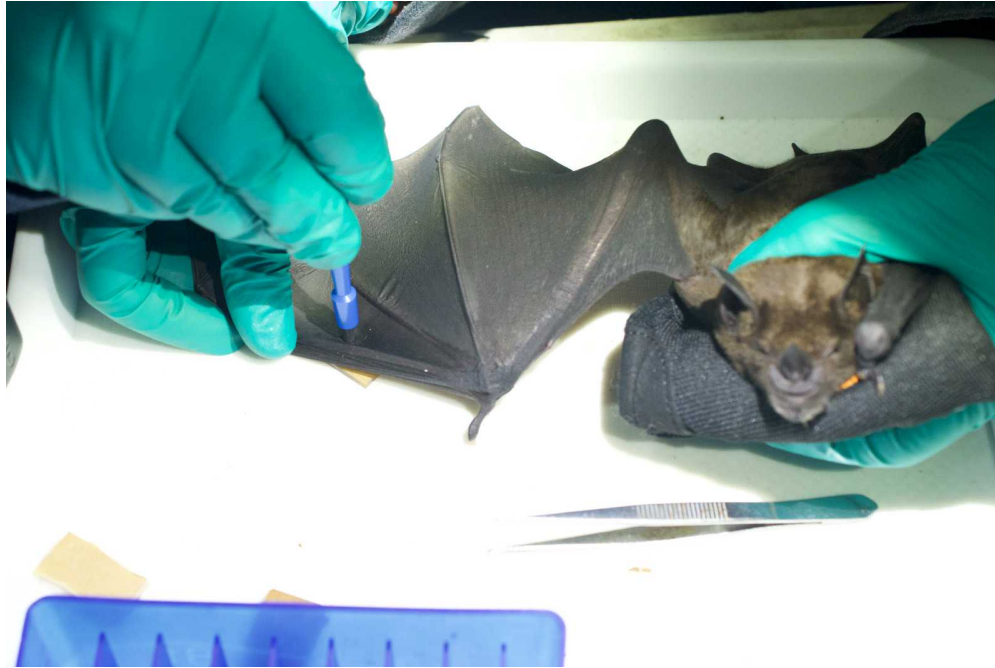
Move the wing against a sterile piece of cardboard that provides a firm surface for extracting a clean biopsy.

Disinfect the area with an antiseptic wipe.

Collect a 3mm biopsy-punch sample using a clean biopsy punch.

Store the biopsy in a buffer that will not lyse the sample. Avoid Zymoshield, longmires, etc.

Take the hole punch in the same location for all bats you work with to ensure easier recognition of recaptured animals at the same site in the next few days.



Picture showing how to collect a punch biopsy from bats. Courtesy of Jorge Luis Mendoza Silva.

Safety information

USDA Pain/Distress Category: C

13.12 BLOOD

1. The bat is manually restrained between the thumb and palm of the non-dominant hand and its wing extended until its fore and upper arm forms a 90° angle. The medial aspect of the wing should be facing the handler.
2. The venipuncture site is prepared with an antiseptic swab and by applying a thin layer of vaseline with a clean cotton swab, in order to create a hydrophobic surface that allows the formation of a neat blood droplet.
3. A 25 g needle is used to puncture the antebrachial vein.
4. Touch the blood drop with the tip of a microhematocrit capillary tube. Bats must be bled with caution to maintain a ratio no greater than 6 μ L of blood per gram. Up to 0.6% body mass of volume of blood can be collected using microhematocrit capillary tubes*. (NOTE: some capillary tubes can hold up to 70 μ L of blood). From the capillary tube, blood is transferred into a 1.5mL tube containing lysis solution. If needed the capillary tube can be broken into the sample tube. Close the sample tube and shake to homogenize the blood sample.



5. If the animal is sufficiently large, additional capillary tubes are collected for serum. Fill to $\frac{3}{4}$ and seal with clay on both sides so that it can be spun for serum collection. Keep sample cool in a labelled 15mL tube.
6. Additional drops of blood are used for glucose and ketone reading and to create 2 blood smears.
7. Use a cotton ball to put pressure on the venipuncture site and fold back the wing to hold it in place for about 2 minutes.
8. Reopen the wing and check that the venipuncture site is no longer bleeding. If not bleeding, remove the cotton ball, otherwise apply light pressure until bleeding stops.

Venipuncture site description



The image shows taking a blood sample. Courtesy of Jorge Luis Mendoza Silva.



Safety information

Total blood collected is < 0.6% 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.

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 light pressure to the puncture site once blood is collected.

Post procedure monitoring

None except confirming that the puncture site is not bleeding before animal is released

Citation

Jonathan Epstein, EcoHealth Alliance Matthew LeBreton, Metabiota Melinda K Rostal, EcoHealth Alliance Leticia Gutiérrez Jiménez, EcoHealth Alliance and the PREDICT One Health Consortium

(2016). Bat Sampling Methods.

PREDICT Consortium. PREDICT Standard Operating Procedures for One Health Surveillance..

https://ohi.vetmed.ucdavis.edu/sites/g/files/dgvnsk5251/files/inline-files/PREDICT%202%20SOP%20Book_v3.%20Aug2020.pdf

LINK

13.13 NOURISHMENT

Offer the bat 1 mL of nourishment (sugary water) slowly with a 1 mL syringe and record if it is consumed or not.

Remember to use a new syringe for each different animal.





The image shows the process of giving sugar water to the bat for its recovery. Courtesy of Jorge Luis Mendoza Silva.

MEASUREMENTS

- 13.14 Measure all the body parts indicated in the processing sheet using a clean caliper.

Protocol



NAME

Bat Measurements - ISL Peru

CREATED BY

Cristian Tirapelle

Preview



The image shows how to measure the Forearm length of the right wing. Courtesy of Jorge Luis Mendoza Silva.

13.15 Inspect the wings and the body for injuries, measure, take pictures and record on the animal sheet.

13.16 **ECTOPARASITE**

Collect any ectoparasites that you might see, if necessary, cut some barbs where the ectoparasites might be hiding in between, and place them inside a 1.7 mL tube containing minimum of 70% ETOH.



The image shows an ectoparasite (fly) found on the bat's wings. Courtesy of Jorge Luis Mendoza Silva.

Tagging and Photos

14 zTracking & Life Tags

Prior to release and photos a subset of animals will be fitted with a UHF tracking tags ([Cellular Tracking Technologies](#)). Tags are temporarily glued to the dorsum (duration ~ 20days). Animals will only be considered for a tracking tag if their weight is $\geq 60\text{g}$.

Transmitter attachment:

- Transmitters should be attached to the area between the shoulder blades so that the bats cannot use their hind feet to pull off the tag.
- For bats with shorter hair, transmitters will be attached without clipping hair, but for long haired bats its best to clip hair close to the skin. Do NOT full shave hair, some hair is provides a good matrix for glue binding.
- We choose Permatype Adhesive Surgical Cement as the adhesive of choice, based on tests on adhesion strength and time to tackiness (3-4mins).



Image shows the tag after being installed. Courtesy of Jorge Luis Mendoza Silva.

Citation

Carter, T.C., Sichmeller, T.J. and Hohmann, M.G., (2009)
. A field-and laboratory-based comparison of adhesives for attaching radiotransmitters to small insectivorous bats. .
Bat Research News, 50, pp.81-85..

<https://www.holohil.com/wp-content/uploads/2015/07/carter2009.pdf>

LINK

- Apply a very thin layer of adhesive on the transmitter and on the bat. Let it stand for 5 mins until the glue bubbles
- Then press the transmitter to the bat applying a steady pressure for five more minutes
- To be certain the tag is fully fixed, do not release the bat immediately, but hold for 10 more minutes at a minimum.
- While holding, the handler and research assistant can begin to take photographs of the bat during that 10 minute time span.
- It is important to choose a large bat, unstressed by handling, and to provide nourishment (see above) during this process.

Permanent identification:

- Additionally, we intend that all bats will eventually receive a P-chip identification tag (<https://p-chip.com/>) which has been successfully used by SDZWA on the Pacific Pocket Mouse.
- P-Chips are micro transponder tags (500 µm x 500 µm x 100 µm) with photocells that are powered by a handheld laser wand connected to a computer or tablet and read through PharmaSeq's p-Chip Reader software to emit a unique 9-digit signal (PharmaSeq Inc., Princeton, NJ).
- P-Chips are small, lightweight, and provide individual identification
- Tags come in an individually packaged sterile injector and are inserted subdermally in the uropatagium such that they are visible under the skin as a tiny black dot.
- Each p-Chip should be scanned with the handheld laser after tagging to record tag number.

P-Chip Placement

Clean the dorsal aspect of the right forearm of the bat using an alcohol swab. With the p-chip applicator, direct your needle with the bevel up parallel to the forearm. Insert the bevel subcutaneously from proximal to distal and press the plunger to deliver the chip and safely remove the needle.

Check the insertion site for any superficial bleeding. Confirm the readability of the p-chip using the scanner.

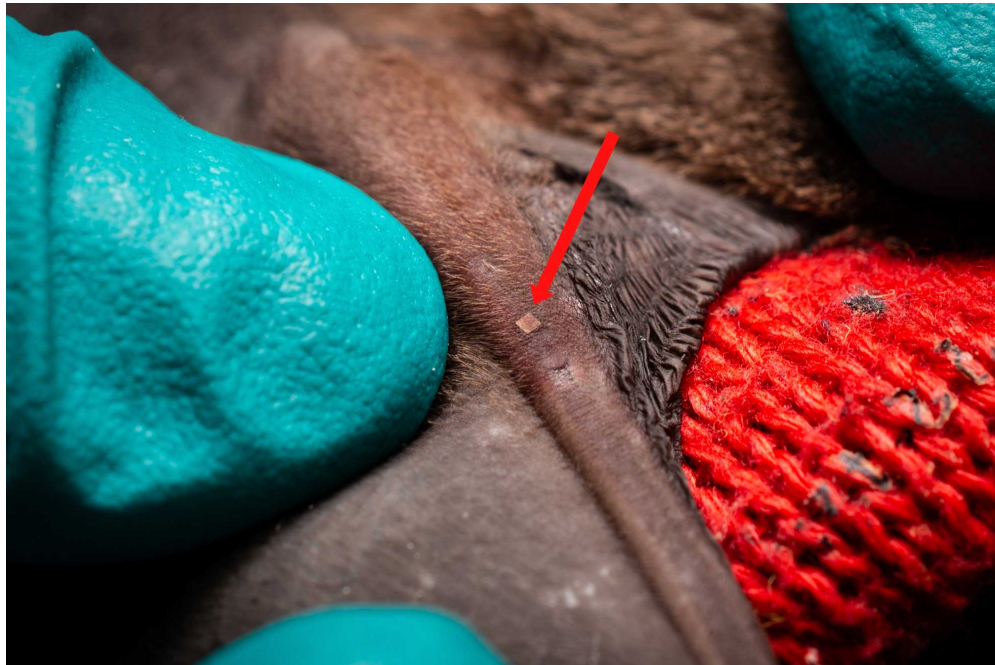


Image showing the p-chip after being inserted subcutaneously in the right forearm of the bat.

Courtesy of Jorge Luis Mendoza Silva.



The image shows the number reading of the P-chip installed in the bat. Courtesy of Jorge Luis Mendoza Silva.

Safety information

GENERAL ANIMAL MARKING GUIDELINES

As a general rule of thumb, tags must weigh $< 5\%$ of animal body weight. 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.

Tags are placed with the following guidelines in mind:

- In this program all animals are habituated to traps for annual health monitoring, and to replace/remove tracking devices. We rely on longitudinal data to demonstrate that the tags used do not effect the survival or reproduction of study subjects.
- If recapture is not anticipated, tags are damaged in a way that will cause it to fall off over time due to normal wear and tear. Alternatively, tags are attached with a material that is temporary and will fall off over time (e.g. skin glue and thin-stretch plastics)

MONITORING

According to the tag 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.

PHOTOS

While waiting for the tag to be secured on the bat, the handler and a research assistant can begin to photograph the pictures listed on the processing sheet.

After taking all the photos, the handler can check the tag and make sure it is secure.

Release

15 **RELEASE**

When PROCESSING is complete, the bat is taken outside and released by supporting it in an open hand with a protective glove, allowing the bat to push off and take flight.

The release can be recorded with a video and the RELEASE TIME should be reported on the processing sheet.

Record unusual behaviour of the animal at the time of the release and define it as IMMEDIATE or PROLONGED release.



Image shows the release of a bat. Courtesy of Jorge Luis Mendoza Silva.

Cleaning

16 **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, as follows: leave submerged in 10% bleach for 5 minutes → 1st rinse in distilled water → 2nd rinse in distilled water → submerge in 70% ethanol (begin this process immediately after using a tool, rather than at the end of processing each bat, to not lose time)
3. Dry all tools and surfaces with clean paper towels or sterile cotton.



Sterilization solutions prepared for use in a 50 mL tube rack. Courtesy of Thomas Parsons.

- 17 Repeat this protocol for each consecutive bat following the capture order, unless instructed otherwise by the PI or team leader.

➡ go to step #12 To process the next animal

END SESSION

18 FIELD WRAP UP

Once all animals have been released the team should:

- End the Voice Recorder after stating the following: full date, location, number of bats processed, names of each bat team member.
- Securely close the nets. If nets accidentally open during the day or later in the night it will lead to animal injury or death

- Record "NET CLOSE" time
- Clean and pack materials used for processing
- Check, arrange and pack samples
- Return to base camp

.

18.1 **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 clear
2. Identify a coplin jar with methanol that has not expired
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 evaporation.
4. Leave the smears in solution for 5 minutes
5. Wearing a pair of gloves, remove each slide and place in an open slide box to dry overnight. Make sure to close the coplin jar as quickly as possible to preserve to the methanol

18.2 **TEMPORARY SAMPLE STORAGE**

Unless otherwise indicated by the PI, all other samples should be stored in the freezer until sample intake procedure the next day. NOTE, serum samples must be spun and extracted to a serum storage tube the same night and stored frozen.

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.

18.3 **END TASKS**

Deposit all other materials in the laboratory prep area until morning noting the following instructions:

- Items that may be wet should be removed and placed somewhere to dry
- Materials that are dirty or came into contact with an animal should be in a secure bag or container, and marked as such.
- The voice recorder and data sheets should be stored in a safe place where they cannot be confused with that of other teams.

DAY AFTER TASKS



19 The following morning the team will:

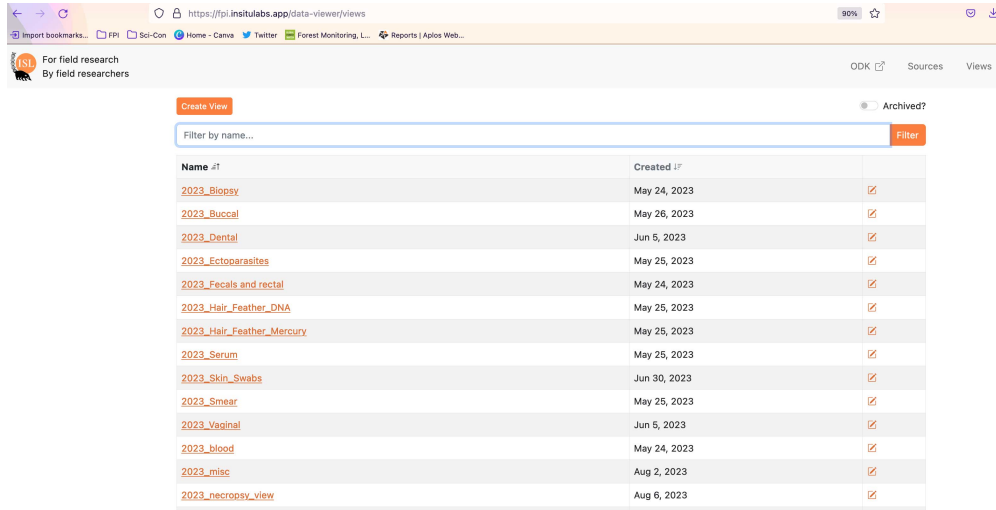
19.1 Check any information on the processing sheet, and listen to the voice recording to fill in missing information on the data sheet with a differently colored ink pen (usually red).

19.2 Upload the information gathered on the PROCESSING FORMS to ODK.

The screenshot shows a mobile application interface for '2022 Bat Capture'. At the top, there is a title bar with a back arrow, the title '2022 Bat Capture', and icons for eye, document, share, and a menu. Below the title bar is a section labeled 'Capture Info'. The first field is 'Your name' with a subtitle 'the person digitizing the data' and an empty text input box. The second field is 'Bat # (write on bag!)' with an empty text input box. The third field is 'Bag # (if different)' with an empty text input box. Below these is a 'Recapture:' section with three radio button options: 'yes, this year', 'yes, prior year', and 'no, not recapture'. At the bottom, there is a red asterisk followed by the label '* Date:'.

First page of the bat capture form, as it appears from the tablet.

19.3 Finish sample intake procedure using the fpi.insitulabs.app.



The screenshot shows a web browser window with the URL <https://fpi.insitulabs.app/data-viewer/views>. The page has a header with navigation links: "For field research", "By field researchers", "ODK", "Sources", and "Views". Below the header is a "Create View" button and a "Filter by name..." search bar. The main content is a table with two columns: "Name" and "Created". The table lists 15 sample intake records, each with a unique name and a creation date. A "Filter" button is located to the right of the search bar.

Name	Created
2023_Biopsy	May 24, 2023
2023_Buccal	May 26, 2023
2023_Dental	Jun 5, 2023
2023_Ectoparasites	May 25, 2023
2023_Fecals and rectal	May 24, 2023
2023_Hair_Feather_DNA	May 25, 2023
2023_Hair_Feather_Mercury	May 25, 2023
2023_Serum	May 25, 2023
2023_Skin_Swabs	Jun 30, 2023
2023_Smear	May 25, 2023
2023_Vaginal	Jun 5, 2023
2023_blood	May 24, 2023
2023_misc	Aug 2, 2023
2023_necropsy_view	Aug 6, 2023

Image of the sample intake menu

- 19.4 Write a detailed narrative report of the capture session using the "trapping report template."
- 19.5 Gather pictures and videos from the session and sort them into designated folders on the project hard drive.
- 19.6 Scan the processing sheets and convert them into PDF files, named by the serial bat capture number. Attach them to the source record in fpi.insitulabs.app
- 19.7 Retrieve the recording from the voice recorder. Name the file using the following convention "YYYY-MM-DD_BATsession"
- 19.8 Group all the files from point 17.3 to 17.6 into a unique folder named by the date and range of capture numbers used [yyyy-mm-dd_captures##-##]
- 19.9 Wash all the animal bags used during the capture with detergent and a disinfectant product, such as quaternary ammonium based solutions.
- 19.10 Check that the laboratory team has all information they need for long-term sample storage
- 19.11 Re-supply and pack materials for the next capture session



Protocol references

YouTube: [Video on how to setup and take down a mist-net](#), Bat Conservation and Management (@batconservationandmanageme4343)

PREDICT, STANDARD OPERATING PROCEDURES FOR ONE HEALTH SURVEILLANCE; https://ohi.vetmed.ucdavis.edu/sites/g/files/dgvnsk5251/files/inline-files/PREDICT%202%20SOP%20Book_v3.%20Aug2020.pdf

Citations

Step 13.12

Jonathan Epstein, EcoHealth Alliance Matthew LeBreton, Metabiota Melinda K Rostal, EcoHealth Alliance Leticia Gutiérrez Jiménez, EcoHealth Alliance and the PREDICT One Health Consortium. Bat Sampling Methods https://ohi.vetmed.ucdavis.edu/sites/g/files/dgvnsk5251/files/inline-files/PREDICT%202%20SOP%20Book_v3.%20Aug2020.pdf

Step 14

Carter, T.C., Sichmeller, T.J. and Hohmann, M.G.,. A field-and laboratory-based comparison of adhesives for attaching radiotransmitters to small insectivorous bats. <https://www.holohil.com/wp-content/uploads/2015/07/carter2009.pdf>