BE PREP

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BEPREP STANDARD OPERATING PROCEDURE

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Disclaimer:	None of the procedures described in this SOP can be done without the appropriate national permissions.		

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1 Abbreviations and definitions

SOP - standard operating procedure
CSA - case study area
PPE - Personal Protective Equipment
BACI - Before, After, Control, Impact
DRS - DNA-RNA Shield

2 Aim and scope

This SOP describes the trapping and diverse sampling of small rodents, including sampling from both live and dead rodents, as well as data collection. These procedures are designed to:

- describe the small rodent communities in terms of species presence and (relative) abundance;
- collect samples that can be used to aid molecular identification of rodent species, pathogen detection and characterisation of host's microbial communities.

3 Health and safety information and procedures

Always have a first aid kit with you when doing fieldwork. This can be a generic first aid kit, that can be used in cases of cuts, sprains, or other injuries.

Always wear adequate field work clothing and footwear, to protect yourself against the weather, arthropods, and possible injuries.

Never go into the field alone, or without informing someone else about your whereabouts and expected time of return. Keep your phone fully charged and carry GPS device and/or a map when needed.

After being in the field, remove all clothes (put them in the sun) and carefully examine yourself for any ticks; then have a hot shower if you possibly can. Removing the tick with appropriate tweezers in a short time after a bite can significantly reduce the likelihood of acquiring *Borrelia burgdorferi* (causative agent of Lyme disease) infection and inform you of possible exposure to other tick-borne pathogens.

Make sure that all your vaccinations are up to date. While some locations may require specific vaccinations, all staff should also have up-to-date tetanus vaccination.

Before commencing any work, make sure you are familiar with the sampling procedures and the risk associated with these procedures and work with wild rodents!

3.1 Personal Protective Equipment (PPE)

Personal protective equipment MUST be worn during trapping and handling of live animals to avoid exposure to infectious zoonotic pathogens, contamination between animals and transmission of infectious agents from humans to animals!

Appropriate PPE to be used as needed:

- Sterile nitrile gloves
- Safety googles
- FFP3 (or higher rating) masks
- Protective clothing (overalls, surgical gown, aprons)

3.2 Actions in case of injuries

Notify other personnel about the injury – give exact details – what happened, when and where!

If a rodent has bitten you: clean the wound with sterile water and soap and disinfect the wound with betadine solution or a similar disinfectant as soon as possible, apply dressing as needed.

In case of blood or chemical products in the eye: rinse for 10 minutes using sterile water. Seek medical attention if needed.

In case of fever during/after fieldwork or any unusual symptoms: seek medical attention as soon as possible. Be prepared to tell the medical personnel what you have been doing, where and what animals you have been in contact with – so that the medical personnel can help you as efficiently as possible.

Remember - human health is the top priority!

4 Treatment and disposal of biological waste

Any biological waste produced during field work should be regarded as potentially infectious, therefore there is a need for traceability and security.

All biological waste (tissue paper, used gloves, carcasses etc.) must be stored in a dedicated container with the appropriate biohazard label (Fig. 1). The waste must be disposed of following the national regulations. All sharp objects (needles, scalpels) must be placed in the appropriate container (Fig. 2) and likewise disposed of according to national regulations.

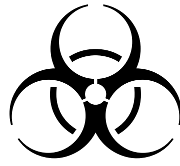

Figure 1. International biohazard symbol. Unicode U+2623.

Figure 2. Example of a sharp waste bin. These bins must also include biohazard symbol.

5 Live trapping materials and equipment

Small rodents can be trapped using live traps and if necessary, also snap traps. Live trapping is always preferred over snap trapping, as snap trapping has lower sample quality. Pitfall traps must be avoided.

The complete list of equipment and materials for rodent live trapping can be seen in the table 1 below:

Table 1. Materials needed for rodent live trapping

Item	Purpose
Live traps	To trap animals
Bait (e.g., seeds, carrots, peanut butter)	For animal survival while in trap
Straw or hay (optional)	For bedding
GPS device	For locating traps
PPE – googles, masks, gloves, overalls	Personal protection
Scotch or masking tape	To mark trap ID on the trap
Forestry marking tape or flags	To mark trap's location
Rope	If trap needs to be tied to a tree
Water resistant permanent markers	To mark traps
Basin or large bucket	To clean used traps
Brushes and sponges	To clean used traps
Soap	To wash hands
Water	To wash hands and traps
Spray bottle with 20% Chlorhexidine solution or 1% Virkon solution	To disinfect traps
Spray bottle with water	To rinse traps
Headlamp	For light when needed
Portable freezer (e.g., solar) or dry ice in a box	For sample storage in the field

5.1 Traps

Single or multiple-capture traps can be used to capture small rodents. Table 2 includes some of the preferred live trap types, however locally made traps can be used if the commercial traps are not available.

Table 2. List of commercially available and BEPREP preferred live traps

Trap name	Link to website or supplier	Notes
Ugglan trap	Ugglan trap	Traps multiple, above-ground animals; optional hole (12mm) for shrews.
Longworth small mammal trap	Longworth	Mainly trap single animals; option with a hole for shrews also available; treadle can be set to certain masses below which it is not triggered.
Sherman traps	Sherman traps	Various sizes available. Choose the size that is appropriate for your target species. Easily transported; do not trap the larger species.
Tomahawk live trap 102	Tomahawk Live Trap Model 102	Standard trap used for Malagasy rodent biological inventory

Note! In many locations we must make sure to decrease the number of bycatch animals or accidental trapping of protected species. For example, if shrews are protected in your area of trapping and will not be sampled, please consider one of the following modifications to the trap setup:

1. Using traps that have shrew holes in them.
2. Adding shrew food to the trap to prevent death of captured shrews.
3. Avoid placing traps in places that are likely to have a lot of shrews, e.g., near streams, wetlands.

5.2 Bait

The preferred bait for rodents in European CSAs is sunflower seeds with a piece of carrot in the trap. In other CSA oats, pieces of apple, banana, dry fish or peanut butter can be used, depending on the target species.

Always write down what type of bait is used in the trapping data sheet (Appendix 1).

6 Trapping locations and setup

Trapping locations will depend on the CSA. However, if possible, replicates within “control” and “impact” areas are encouraged.

Important! The capture protocols (trap layout and number) are designed to be applicable to as many CSAs as possible, however there is no “one size fits all” solution. **It is most important to stick to the same protocol and trapping schedule within the specific CSA across the study seasons and years**, to make the data comparable within the BACI framework.

6.1 Trap layout

Traps can be set in lines, square grids, or cross grids (Fig. 3).

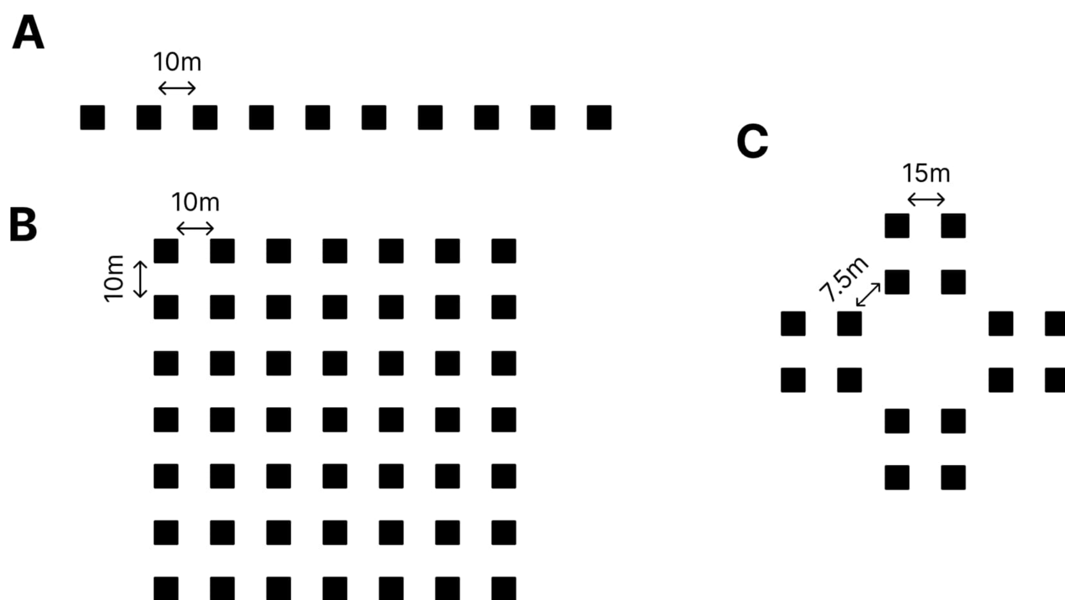

Figure 3. Acceptable trap layout. A - line of 10 traps with inter-trap distance 10m; B - square grid of traps (7x7) with inter-trap distance 10m; C – cross grid with four 2x2 squares, inter-trap distance 15m and inter-square distance 7.5m.

6.1.1 Line of traps

One line consists of 10 traps set in a roughly straight line with approximately 10m between traps (Fig. 3A).

Where applicable (e.g., different sized rodent communities (rats, voles, mice)) a different type of trap from the one used every 10m, should be added every 10-20m to increase the likelihood of capturing higher number of different species.

When appropriate, different distance between the traps can be used – in this case the distance should be recorded in data collection sheets and kept the same within the trapping site across years.

6.1.2 Square grid

A square grid consists of 7x7 traps, depending on the home range of the target species. The distance between traps should be 10m. The placement of the traps can be seen in Fig. 3B.

6.1.3 Cross grid

A cross grid consists of total of 16 traps, which are arranged in four squares with four traps per square (Johnsen et al., 2017). The inter-trap distance is 15m, and inter-square distance is 7.5m (Fig. 3C).

6.2 Definitions of trap setup and scale

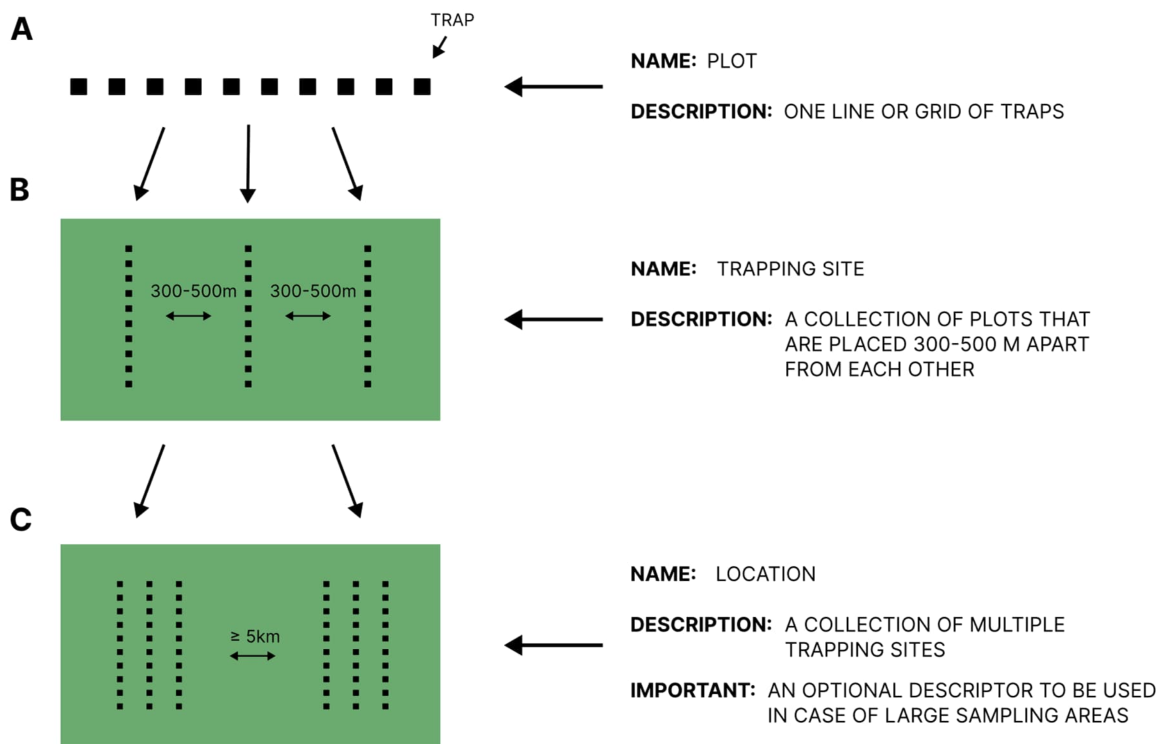

Figure 4. Definitions used for describing sampling scale. A – a plot is one line or one grid of traps. B- multiple plots that are placed 300-500 m apart form a trapping site. C – multiple trapping sites separated from other trapping sites by a few kilometres form a location.

One trapping line or grid or cross constitutes one **plot** (Fig. 4A). Multiple plots that are placed 300-500 m apart and form a group is called a **trapping site** (Fig. 4B). Multiple trapping sites that group together but are separated from other trapping sites by at least few km (depending on the species) constitute one **location** (Fig.4C shows two locations).

There will be multiple (1 to *n*) trapping sites per each CSA and "treatment" (BACI). This number will vary between the CSAs but should be kept the same within CSA. The location is an optional term, that can be used for bigger CSAs. If not applicable, should be marked with a 0 in data collection sheets and sample labels.

6.3 Setting the traps

Traps are prepared in advance to setting of the traps. Each trap is baited with sunflower seeds and carrots or other available bait. If needed, e.g., very cold weather or potential rainfall, bedding consisting of a hay or wood strip ball can be added to the trap to avoid hypothermia. If bedding is added, one must make sure that it does not prevent rodents from entering the trap.

As rodents will be trapped multiple years, use sticks and forestry marking tape to mark the location of each plot, e.g., first and last trap of the line, to make the trapping easier the following trapping season.

Each trap should be labelled using marking tape and water-resistant marker with a unique trap number. Trap ID consists of "location - trapping site - plot number - trap number". For example, in a line trap layout, trap ID *1-2-1-1* indicates the first trap from location 1, trapping site 2 and plot 1.

Note! Please remember to record what type of bait is used.

The exact timing of trap setting will depend on the species, CSA and national regulations. Traps should be placed under some cover (rock, shrubs) so that the captured animals are protected from weather and predators. If possible, the GPS coordinates of all traps should be added to the GPS device (**using WGS84 system**). If this is too labour intensive, the first and last trap **must** be marked for lines, 5 traps should be marked for the square grid (each corner and centre) and the cross grid (centre of each square and middle of the cross-grid).

Whenever possible, photographs should be taken from each trap line or grid. One in each direction (four in total) as to capture the whole plot. Photographs can immediately be sent to a dedicated chat (e.g., WhatsApp) to ensure no data are lost.

All traps should be checked regularly, e.g., if the traps are set in the late afternoon, they are checked the following morning, early enough to decrease the time animals are exposed to the elements, especially heat. The number of hours between checking however depends on the respective national regulations and permits, which always must be followed. **PPE must be worn to prevent exposure to potential pathogens while collecting the traps.**

The minimum trapping period at each trapping site is three consecutive nights in all CSAs.

The minimum number of sampling sessions (as agreed in project proposal) is two per year.

The number of sampling sessions can be increased when possible or needed. The number of plots and traps used, and the length of trapping must be marked every time to record **trapping effort**!

6.4 Cleaning of the traps

A trap should be cleaned if it had an animal (irrespective of the animal species). Washing the traps in a basin filled with Virkon solution is preferrable, however spray cleaning using Virkon solution can be used. Other cleaning agents can be used, however the chemical used must neutralize viral, fungal and bacterial pathogens. **Please note the necessary exposure time needed for the cleaning agent to work.** For example, if 20% chlorhexidine is used, leave it on for 5 minutes. Rinse the traps with water after washing them and dry with paper towels or under direct sunlight.

Take care not to use dirty traps in new environments to avoid introducing new pathogens to the specific area and cross contaminating any potential samples taken from the trap.

6.5 Recording the trapping data

When checking the traps, record the state of the trap in the datasheet:

0 –trap is closed and empty,

1 –trap is closed and has the target species/genus inside,

2 –trap is closed and has bycatch (not sampled in BEPREP or CSA) inside,

3 – trap is missing or has been damaged/destroyed

All animals caught in traps must be noted, even if they are not target species in the CSA (e.g., reptiles, amphibians or shrews). Non target species can be released immediately.

All information on each trap checking must be recorded, for full details please see Appendix 2.

If the trap is empty, it is left in the field, bait is added if needed. If relevant in the CSA: close those traps (e.g., Sherman traps) or open them (e.g., Ugglan traps), to prevent diurnal species from entering the traps and dying from heat exposure.

Traps containing animals are collected while wearing appropriate PPE, and are replaced with clean and empty traps. Traps with animals are then taken to the animal processing point either at the trapping point or a specifically set processing point. It is important for the animals to be released back into or adjacent to the trapping plot.

If the number of animals that can be sampled has been reached, continue trapping until the minimum of three trap nights is completed. Release the trapped animals after marking them with an animal pen or a fur clipping (e.g. on the back or thigh), making note of the individual in trapping data sheets.

Please see Appendix 1 for information on trap data that needs to be collected the day the traps are set and Appendix 2 for an example forms for continuous trap checking.

6.6 Snap trap setup

Snap trapping can be used if live trapping is not possible. However, it will limit the different samples that can be taken, e.g., samples from these animals cannot be used in gut microbiota analyses.

All snap trapping is done similarly as live trapping. Same trap layout and definitions apply also to the snap trapping. The trap model and size will depend on the target species.

As in other trapping, please fill in the trapping data forms, noting all trapping information the first time, and trapping success each check.

7 Animal handling & sample collection

Animal processing point should be protected from the elements yet ventilated.

7.1 Materials and equipment

PPE:

- FFP3 masks (those that prevent particle spread in both directions are preferred)
- Googles
- Disposable nitrile gloves
- Coveralls (e.g., Tyvek suits)

Animal processing site:

- Worktable and at least two benches
- Tissue paper or paper towels
- Disinfectant (Virkon 1% solution) in a spray bottle (surface cleaning)
- Ethanol 70% in a spray bottle (surface cleaning)
- Falcon tubes or beakers with bleach (20% v/v) or Virkon (1%) and water for tool disinfection
- Forms for data collection
- Pencils and pens
- Sharp waste bin
- Biohazardous waste bin
- Sample labels
- Sample tube holders
- Permanent markers
- Sample tube boxes

Animal sampling materials:

- Sample tubes (O-ring screw-cap or safe-lock lid tubes, 2ml)
- Cryotubes (for organs that might need bigger tube diameter, 2ml)
- Ruler

- Digital scale, or a spring scale (e.g., Pesola)
- DRS
- Small comb and magnifying glass to find ectoparasites (and a basin for water if possible)
- Tweezers (for removing ectoparasites)
- Sterile oral and rectal swabs.
  For example, cotton, polyester or flocked swabs (Bruijns et al., 2018). The diameter will depend on the size of the sampled species.
- Container with dry ice (if used)
- PIT tags and syringes for applying the PIT tags (if used)
- PIT tag reader (if used)
- Syringes and needles for blood sampling (the needle gauge will depend on the target species size)
- Disinfection wipes for ears
- Ear biopsy punch

Animal dissection materials:

- Inhalant anaesthetic (e.g., enflurane, isoflurane, sevoflurane)
- Dissection surface (a polystyrene, cork or plastic board)
- Hermetic container or Ziplock bag for anesthetizing the animals or a falcon tube with cotton ball
- Dissection tools (forceps, blunt-edged scissors)
- Three beakers for washing the dissection tools (water → Bleach/Virkon → ethanol)
- Spray bottle with 70% ethanol

Animal carcass preservation (if required by the local legislation):

- Plastic jars
- Formalin or alcohol for museum collections
- Tags for museum specimens

7.2 Sample list and storage

For **most** samples, the best storage option is putting the tube with the sample on dry ice (−80 °C) in the field, followed by continuous storage at −80 °C or at maximum −20 °C. The second-best option is DRS, which should be used when cooling chain cannot be maintained.

For rectal and oral swabs – in addition to the swab stored in DRS, a second swab should be put in bacterial lysis buffer (provided by the Knauf lab) whenever possible.

For untreated blood samples – in addition to a sample in DRS, collect an additional sample on FTA card whenever possible.

Please note that freezing samples does not inactivate the pathogens, so samples without any buffer and just frozen are considered infectious.

Full list of rodent samples that **can** be taken their amount/volume can be seen in table 3. The type of samples collected will depend on the trapping protocol (Section 7.4).

Table 3. List of rodent samples.

Sample	Priority sample	Number of samples and amount
Blood (untreated/no additives)	yes	1 - as much as possible in 2ml tube 2* - FTA card
Ear pinna or biopsy	yes	1 – 2-3 mm ⌀
Faeces – parasites	yes	1 - min 1 pellet
Faeces – microbiome	yes	1 – min 1-2 pellets
Oral swab	no	1 swab 2* - if not frozen - in 200 µl of lysis buffer
Rectal swab	no	1 swab 2* - if not frozen - in 200 µl of lysis buffer
Lungs	yes	1 – fill up 2ml tube**
Kidneys	yes	1 – both in 2ml tube if fit**
Spleen	yes	1 – whole in 2ml tube if fits**
Liver	no	1 – fill up 2ml tube**
Head (including brain and septum)	no	1 – whole head in 1 tube

Every sample should be kept in a single tube!

When using DNA-RNA Shield please remember to keep the ratio of sample to buffer!

DRS ratio:

- biological liquids → add 3 volumes of DRS to 1 volume of sample (e.g., 100 µl blood + 300 µl DRS)
- tissue and environmental sample → submerge (e.g., 30 mg of sample + 300 µl DRS)
- swabs → enough to submerge the tip in DRS
- unsure → 9 volumes to 1 volume of sample

All samples should be stored in **sample boxes**, that are appropriate for the tube size and the number of samples. All sample boxes should be clearly labelled with UPPERCASE letters for easy identification. The minimum required information:

Project: BEPREP
Year:
CSA:
Samples: *E.g., rodent whole blood, samples 1-81*
Responsible person: *Name, Surname, phone number with country code*

7.3 Data recording and storage practices

All data must be recorded in the individual's data sheet by a staff member who does not process any animals, i.e., is "clean" and protected from infection hazard.

All data collected during the day should be regarded as confidential and stored safely.

After each day, take a photograph of the individual's data sheet and share it in the local WhatsApp group. At the nearest opportunity, the data should be transferred to metadata spreadsheet and saved. **The entered data should be cross-checked by a person other than the entree.**

After the fieldwork, scan the original data forms and add these to the CSA folder in Teams. Store the originals in a dedicated folder. **Keep a copy of the files in a location other the MS Teams!**

The aim is to eventually make the collected data available to the broader scientific community (within ethical and privacy limits).

Rodent samples should be labelled the following way:

BP-CSA-R-running number-sample type-sample type running number

For example, *BP-01-R-001-F-1* is sample taken from CSA 1, from a reservoir (the "R"), animal number one, and it is a faecal sample number 1. The first 4 fields also identify the animal ID (in the previous example, animal ID is BP-01-R-001)

The **sample type running number** should be used if there are multiple samples of the same type from the same individual, for example, faecal sample. For more details, and sample name "generator" please refer to the "Sample-name.xlsx" file in SOP folder in Teams.

The final animal metadata file (information from each CSA on all rodents) should have all the information listed in table 4.

Table 4. Information to be recorded for each animal:

Information	**Details**
CSA number	Format: 01-11
Animal ID	Format: BP-CSA-R-running number *e.g., BP-01-R-001* (please zero pad!)
PIT tag ID	If applicable
Trap ID	Format: location-trapping site-plot number-trap number e.g., *1-2-1-1*
Date of trapping	Format: YYYY-MM-DD (this will keep Excel and other software happy)
Weather	When applicable, an approximation, e.g., rainy, cloudy, sunny. Temperature and humidity (if possible, as will be done for tick collection).
Sampling time	Format: 24-hour At least approximate, but exact is preferred
Species	Latin (abbreviations are acceptable if explained) Genus if uncertain
Body length, head to tail base	Format: cm Done when possible; animal dorsal recumbency, see Figure 6
Tail length, tail base to tip	Format: cm Done when possible; animal dorsal recumbency, see Figure 6
Body weight	Format: grams
Sex	M or F (please don't use numbers to be consistent)
Age group	Adult, subadult, juvenile
Reproductive traits	Females: • Gestation (yes, no, unsure) • Lactation; nipples visible (visible, not visible) • Vagina perforation (perforate/imperforate) • Number of uterine scars (if applicable) Males: • Testes size (small, medium, large) • Seminal vesicle size (if applicable; developed/undeveloped)
Sexual maturity	Immature/Mature
Ectoparasites	• Ticks (presence and count if possible) • Fleas (presence and count if possible) • Mites (presence and count if possible)

For the full data collection spreadsheet, please see file xx in Teams. For an example of data collection form, please see Appendix 3. All metadata must be transferred daily to a dedicated spreadsheet.

Where species identification based on morphological traits is needed, please measure the length of hind feet and ear length!

7.4 Rodent sampling protocols

There are three possible sampling protocols, determined by the specific CSA and sampling permits:

Protocol 1 (P1) – live capture followed by release;

Protocol 2 (P2) – live capture followed by euthanasia or snap trapping;

Overview of samples collected from small rodents in each sampling protocol. For details on subsamples and storage, please refer to the appropriate protocol and Table x.

7.4.1 Preparing the animal processing site

Bring traps to "animal processing site" and place them in a place safe from exposure to the elements and out of view of bystanders.

Animals should be processed in a random order, to avoid any biases. **However, all traps containing multiple animals should be processed first!** All animals should be processed on the day of capture.

Processing sites handling table/station should be fully prepared/assembled **before** handling of any animals. All tubes and reagents should be ready – filled with appropriate buffers and labelled.

A sampling table/station should have the following:

- Tissue paper or paper towels
- Disinfectant (Virkon 1% solution) in a spray bottle
- Ethanol 70% in a spray bottle
- Forms for data collection
- Pencils and pens
- Sharp waste bin
- Biohazardous waste bin/bag
- Sample labels
- Sample tube holders
- Permanent markers
- Sample tube boxes
- Box(es) of disposable gloves
- Sample tubes (O-ring, screw-cap or safe-lock lid tubes, cryotubes, 2ml)
- Ruler and a (digital electronic) calliper
- Digital scale, or a spring scale (e.g., Pesola)
- DRS
- Small comb and magnifying glass to find and collect ectoparasites
- Container with dry ice or ice packs and a cold box

If animals are released after sampling, the station should also have:

- PIT tags and syringes
- PIT tag reader
- Some other materials for marking the animals, e.g., scissors for fur clipping or animal marker

In case of dissections in the field, the sampling station should also have:

- Inhalant anaesthetic (e.g., enflurane, isoflurane, sevoflurane)
- Dissection surface (a polystyrene, cork or plastic board, silicone pad)
- Hermetic container or ziplock bag
- Dissection tools (forceps, blunt-edged scissors)
- Three beakers for washing the dissection tools
- Virkon 1% or bleach 20% v/v, ethanol and water for washing the tools
- Wash bottle with 70% ethanol
- Cotton balls

7.4.2 Protocol 1 – live captured animals released after sampling

1. Remove the animal from the trap

   There are multiple ways to remove the animal – placing the trap in a clear bag and coaxing animal into the bag. Opening the trap and allowing the animal to move into a bucket (if species is not able to jump out).

2. Examine the animal for ectoparasites.

   If possible, examine the animal over a tub of water to collect escaping parasites, such as fleas. Write down the presence/absence of each ectoparasite group and if possible, count the numbers. Place each ectoparasite in its own tube.

3. Collect the rectal swab by gently inserting the swab into the rectum of the animal. Store the swab on dry ice or in DRS.

4. Collect the faeces

   After collecting the rectal swabs, the animal might defecate, which makes collecting faecal samples easier. If that doesn't happen - place the animal in a sterile/cleaned box for maximum of 10 minutes or until defecation occurs. A source of water (e.g., carrot piece) should be added to the box.

   Two samples should be collected:

   - minimum of two pellets collected directly from the animal on dry ice or in DRS for microbiome analysis

   - minimum of one directly from the animal or from the trap (if animal was alone in the trap) on dry ice or in DRS for parasite analysis.

Only fresh samples can be used for microbiota analyses! Do not use pellets from the trap!

5. Record the animal's species.

   If uncertain, take pictures of the animal that may help species identification afterwards.

6. Weigh the animal

   This can be done by putting animal in a sturdy and jump/escape-proof container or in a Ziplock bag and weighing them on a digital scale, or by using a spring scale (e.g., Pesola). Do not forget to remove the weight of the container from the final weight!

7. Determine the animal's sex, and write down the reproductive traits

8. Collect an oral swab

   Gently insert a sterile swab into the rodent's mouth. Put the sample on dry ice or in DRS.

9. Take a urine sample (optional)

   This can be done simultaneously with faecal sample collection or beforehand by gently pressing the bladder of the rodent. Urine should be collected on FTA card.

10. Take a blood sample

    This will be done only in few of the CSAs. The collection site depends on the species of rodent, but are usually, saphenous vein, dorsal pedal vein, base of the tail, retroorbital sinus. Untreated blood should be collected in a 2ml tube and put on dry ice, or added to DRS. Additionally, a sample can be placed on an FTA card.

11. Take a punch biopsy from the centre of the ear (diameter 2-3 mm). Put the sample on dry ice or in DRS.

12. Mark the animal using a PIT tag, hair clipping or other methods that are the least invasive.

13. Cross-check that all measurements and samples have been taken.

14. Return animal to the trap and after processing animals, return the cleaned and sterilized traps to the locations where they were trapped.

7.4.3 Protocol 2 – sampling of animals that are euthanized or snap trapped

For snap-trapped animals, take the samples as appropriate.

1. Remove the animal from the trap

   There are multiple ways to remove the animal – placing the trap in a clear bag and coaxing animal into the bag. Opening the trap and allowing the animal to move into a bucket (if species is not able to jump out).

2. Examine the animal for ectoparasites.

If possible, examine the animal over a tub of water to collect escaping parasites, such as fleas. Write down the presence/absence of each ectoparasite group and if possible, count the numbers. Place each ectoparasite in its own tube.

3. Collect the rectal swab by gently inserting the swab into the rectum of the animal. Put the swab into DRS or on dry ice.

4. Collect the faeces

   After collecting the rectal swabs, the animal might defecate, which makes collecting faecal samples easier. If that doesn't happen - place the animal in a sterile/cleaned box for maximum of 10 minutes or until defecation occurs. A source of water (e.g., carrot piece) should be added to the box.

   Two samples should be collected – a pellet for parasite analysis and at least two pellets for microbiota analyses. Sample for parasite analysis should be placed in xx and the sample for microbiota analysis in DRS.

   The samples from the trap (if animal was alone in the trap) can be used for parasite screening. **Only fresh samples can be used for microbiota analyses! Do not use pellets from the trap!**

5. Euthanize the animal using an overdose of inhalant anaesthetic.

   Euthanasia should be conducted outside and away from the dissection place. Preferred method is an overdose of inhalant anaesthetics.

   Using a Ziplock bag:

   1. Put a cotton ball soaked with isoflurane in an airtight (e.g., Ziplock) bag.
   2. Place the animal into the bag with anaesthetic.
   3. Wait until the animal falls asleep and dies. **VERIFY** that the animal is dead – check for lack of breathing and heartbeat.

   Using a falcon tube with a cotton ball of anaesthetic (Fig. 5):

   1. Put a cotton ball soaked with isoflurane in a sample tube.
   2. Attach the sample tube to a pre-cut Falcon tube.
   3. Hold the animal by the scruff and put its nose into the falcon tube opening.
   4. Wait until the animal falls asleep and dies. **VERIFY** that the animal is dead – check for lack of breathing and heartbeat.

Figure 5. Using a Falcon tube to anesthetize small rodents. The addition of a sample tube enables addition of isoflurane to the cotton ball, and make sure the animal does not come into a direct contact with the irritating inhalant anaesthetic. Additionally, it allows processing the animal as soon as it dies.

Alternative method is cervical dislocation, however, **it can be only done on very small rodents, and it must be done by an experienced handler**, to avoid animal suffering from wrongfully performed procedure.

6. Weigh the animal using a digital scale, or by using a spring scale (e.g., Pesola). Do not forget to remove the weight of the container/bag from the final weight!

7. Collect the blood samples

   Using direct cardiac puncture. This must be done as soon as possible after death, to prevent clotting. For details on the procedure please refer to Herbreteau et al., 2011.

8. Record the animal's species.
   If uncertain, take pictures of the animal that may help species identification afterwards.

9. Place the animal in dorsal recumbency (on its back) and measure the animal's body length and tail length (Fig.6).

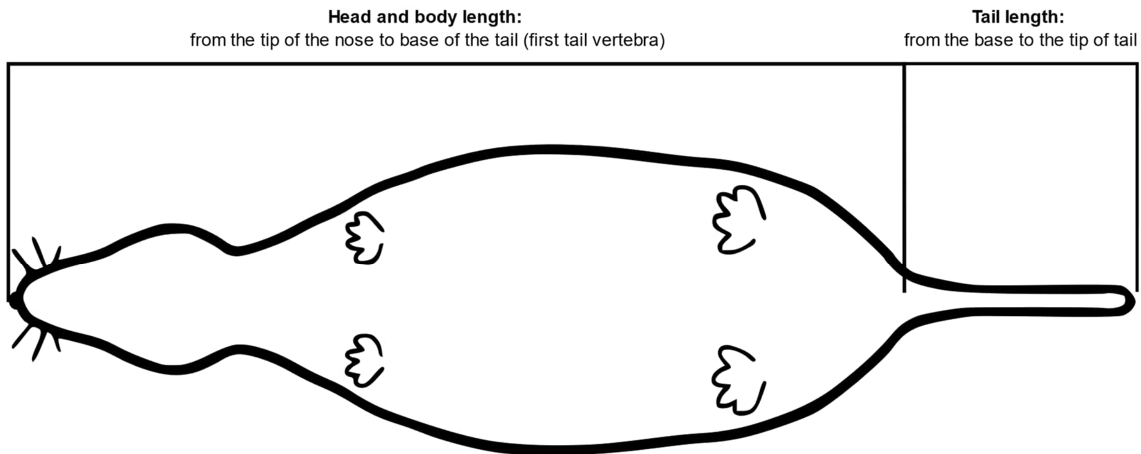

Figure 6. Measurement points for rodent body and tail length.

10. Determine the animal's sex and write down the reproductive traits.
11. Collect an oral swab

    Gently insert a sterile swab into the rodent's mouth. Put the sample in DRS or on dry ice.
12. Take a punch biopsy from the middle of the ear (diameter 2-3 mm). Put the sample in DRA or on dry ice.
13. Put animal in a dorsal recumbency and proceed with the dissection:
    - 13.1. Pin the animal to the dissection board using pins
    - 13.2. Clean the ventral side of the body using 70% ethanol, either using a soaked cotton ball or a wash bottle
    - 13.3. Open the animal using forceps and scissors starting from just above the anus and proceeding until the upper part of the thoracic cavity.
    - 13.4. Pin the skin to the sides to prevent contamination from the skin/fur.
    - 13.5. Rinse the tools in a beaker with water and place them into a beaker with Virkon 1% solution (or household bleach). **Proceed using a clean set of tools**.
    - 13.6. Collect, in order, the lungs, spleen, liver (optional) and kidneys.
    - 13.7. If possible, collect the head of the animal.
14. Cross-check that all measurements and samples have been taken.
15. Rinse the tools in a beaker with water and place them into a beaker Virkon 1% solution (or household bleach).
16. Dispose of the carcass and paper towels in the biohazardous waste bin. If the carcass is to be preserved as a museum specimen, put a field tag on the animal's hind foot and inject animal with formalin and store it in plastic jar with formalin.

17. Clean the dissection surface with Virkon, followed by water and 70% ethanol.
18. Rinse the dissection instruments in a beaker with water and place them in a beaker with 70% ethanol.
19. Put the trap in a large basin filled with disinfectant (Virkon or equivalent)
20. Change the gloves and proceed with the next animal, using a different set of tools.

8 References

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9 Appendices

9.1 Appendix 1: Trap data – first time data collection

When traps are set for the first time, the following information should be collected for all traps:

Information	Content
Year	Year of sampling
CSA	Number of case study area
BACI	Before / after / control / impact
Location	If used. If not, please use "0"
Trapping site	Trapping site number
Plot	Plot number
Plot type	line, square grid or cross grid
Bait	What bait is used in the trap
Time set	Approximate time when traps are set
Trap type	Ugglan Special, Sherman trap, Longworth trap or other
Trap number	In lines - use 1-10 For square grids - use a combination of A-H (rows) and 1-7 (columns) For cross grids - use letters to designate each quadrat (A-D) and numbers for traps within each quadrat.
Trap ID	Unique ID for trap used for data collection sheets and on traps if needed (e.g., in lines). Made from the combination of: location - trapping site - plot number -trap number, e.g., a trap in line: 0-1-1-1.
Trap latitude	For lines, coordinates of the first and the last trap. For square grids, take the coordinates of the corners, for cross grids, take the coordinates from the middle of each quadrat.
Trap longitude	For lines, coordinates of the first and the last trap. For square grids, take the coordinates of the corners, for cross grids, take the coordinates from the middle of each quadrat.

9.2 Appendix 2: Trap data sheet – each check data collection

When checking the traps, each time record the following information:

Information	Content
Trap ID	Unique ID for trap. Made from the combination of location - trapping site - plot number - trap number, e.g., a trap in line: 0-1-1-1.
Date	Date when trap is checked
Time	If multiple checks per day are done.
Trap state	0 – trap is closed and empty, 1 – trap is closed and has the target species/genus inside, 2 – trap is closed and has bycatch (not sampled in BEPREP or CSA) inside, 3 – trap is missing or has been damaged/destroyed.
Species	Species captured, Latin name or abbreviation if explained.
Count	The number of individuals trapped
Re-capture	Whether the individual is a re-capture
Notes	Add information on the co-trapped animal or anything else

9.3 Appendix 3: Rodent data collection field form – example

Animal sampling data form, CSA

Date:

Time sampled:

Trap ID:

Weather: sunny cloudy partially cloudy windy light rain heavy rain snow

Animal ID:

Species:

Co-trapped:

Sex				
	male	T: small	T: medium	T: large
		SV: developed	SV: undeveloped	
	female	V: perforate	V: imperforate	G: yes, scars:
		N: visible	N: not visible	G: no

Age group: juv sb ad

Weight, g:

Body length, cm:

Tail length, cm:

Sampled by:

Recorded by:

Ectoparasites

Ticks: yes no Nymphs: Larvae: Adults:

Fleas: yes no

Chiggers: yes no

Other:

Samples			
swab - rectal	☐yes	☐ no	☐ NA
swab – oral	☐yes	☐ no	☐ NA
faeces - µbiome	☐yes	☐ no	☐ NA
faeces - parasites	☐yes	☐ no	☐ NA
blood – whole	☐yes	☐ no	☐ NA
blood – serum	☐yes	☐ no	☐ NA
spleen	☐yes	☐ no	☐ NA
kidney	☐yes	☐ no	☐ NA
lung	☐yes	☐ no	☐ NA
ear-biopsy	☐yes	☐ no	☐ NA
nose	☐yes	☐ no	☐ NA
brain	☐yes	☐ no	☐ NA

Notes: