EOW PROTOCOL

1. STUDY SITE SELECTION

The following are the criteria for the selection of a good study site for the project:

- Study site extension ideally between 2000 - 6000 ha.
- It is safe for camera trap deployment.
- It contains forest habitat (interspersed with other habitats)
- Intensive feeding is not provided to wild ungulates (occasional feeding when the cameras are not in field or baiting for hunting is not a problem)
- Hunting statistics are recorded by event (hunting*day) (in case collective hunting is practiced). When ungulates are hunted mainly by communal hunting (drive hunts), fine resolution hunting statistics per event (nº animals shot, sighted and surface beaten) must be recorded (see form attached).
- A temporal overlap of camera trapping and hunting activities must be avoided to the extent possible. The optimum situation is hunting activities to start immediately once the camera trap field trial ends, but partial overlapping is possible (e.g., camera trapping carried out in Sep-Oct and hunting is from Oct onwards).

2. STUDY DESIGN

Unmarked camera trap density estimation methods require representative sampling, placing cameras randomly with respect to animal movement. This is best achieved by preselecting camera deployment locations using computer-generated random points. Usually, these points should be in a systematic grid with fixed spacing between them across a defined study area (if you don't have the necessary GIS skills in your team, follow the instructions found at this link: GridMaker).

In cases where the study area covers more than one clearly distinct habitat, and especially when animals of interest are strongly attracted to a relatively rare habitat, it may be useful to stratify your grid, selecting a similar number of points in each habitat, rather than planning a single consistently spaced grid across the whole area.

Study designs that CANNOT be used to estimate the density of unmarked populations include preferentially placing cameras on animal or human trails, targeting spots preferred by the animals such as water sources, mineral licks, or high value foods, and using bait to attract animals. Using unmarked density estimation analysis on data gathered in these ways will give results that are biased to an unpredictable extent, and therefore of no value.

For a study area of 2000-6000 has the suggested minimum number of CT locations is 36 but we strongly recommend monitoring a higher number of locations whenever possible, ideally 60. In fact, a higher number of camera locations will ensure higher precision of the estimates, especially for highly aggregated species. The distance between locations in the study area can vary, however, in cases of larger study areas a higher number of CT points is recommended. Each CT should be active for at least 4 weeks, ideally 6 weeks. Unless you have enough CTs to simultaneously cover all the CT points you will be performing more rounds, in this case each round is going to uniformly cover the whole surface of the study area (see Figure 1). To obtain that you can simply select one point every other (or more depending on the number of rounds). The number of rounds should be the minimum possible to monitor all the camera locations. If possible, the grid should cover at least one patch beaten for hunting big game during the hunting season.

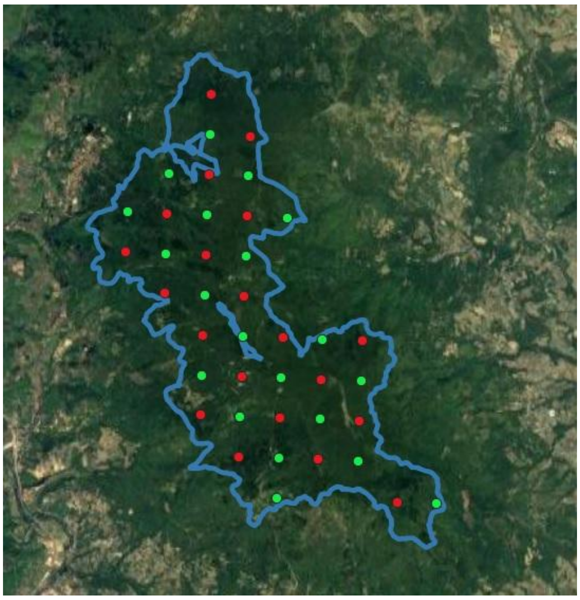


Figure 1. Example of study design. Red dots represent *round #1* while green ones represent *round #2.*

3. CAMERA SET-UP

- If the exact location is not suitable for the deployment of the CT (extremely steep, too dense vegetation…etc) aim for the nearest suitable point but always aiming for the same environment. If the there is no suitable spot with the same environment of the original one, then aim for the nearest suitable point within 100 m even if the environment is different. If it is not possible to find a suitable spot to set up the camera within 100 m from the original point skip the point.
- The CT will be placed on poles or vegetation 50cm above the ground.
- The CT is configured with the operation of 24 hours per day and to take up to eight consecutive images (the maximum number possible), with the minimum waiting time (0 sec. if possible) between activations and, when possible, chose the rapid-fire setting (less time between pictures of the same sequence). Use medium sensitivity. Make sure the time lapse between consecutive pictures is not > 2-3 sec. as this might influence the protocol application.
- The flash intensity should be set at medium (if possible) to avoid “overexposed photos”.
- Check that the date and time are correctly set, and that they are printed automatically on each image.
- If CTs are active for about 4 weeks, then no check should be necessary, but if the monitoring period of each camera location is much longer (i.e. 8 weeks or more), then a check of batteries and SD card might be necessary. Please note that at every check a new calibration would be required. Choose a field of vision of the CT that is cleared of vegetation (it is not necessary to be totally clean, but that allows the detection of any wild boar that passes within the first 5 m), being better a north orientation.

- A form (see Table 1) must be filled in, collecting the information of each CT during its placement (see below). All the information that is subsequently extracted must keep the traceability of the CT (mark the source camera of each memory card extracted and keep this nomenclature in the folders that are created on the computer to archive the images). Shortly, Enetwild will provide an app based on Smart which will be useful to collect this information in the field.

See “Field protocol“ for the recording of the course about chapter 3.

4. CALIBRATION POLE

Thoroughly follow the detailed explanation found at this link to make your calibration pole: Calibration_pole_instructions

5. DEPLOYMENT CALIBRATION

Once the CT has been firmly set up and all the settings have been checked you are ready to switch it on (please note that it won’t have to be touched again until it is removed or checked) you will take the deployment calibration pictures which are fundamental to allow Agouti to perform the automatic estimation of camera parameters (radius and angle of detection) and animal day range.

- Starting about 1m directly in front of the camera, hold the pole with its base on the ground so that it is clearly visible to the camera. Take care to ensure that the pole is held perpendicular to the camera’s line of sight. On level ground with camera line of sight roughly parallel to the ground surface, the pole should be roughly vertical, but if the camera is angled to observe a slope the pole may need to be tilted accordingly (see Fig. 2).

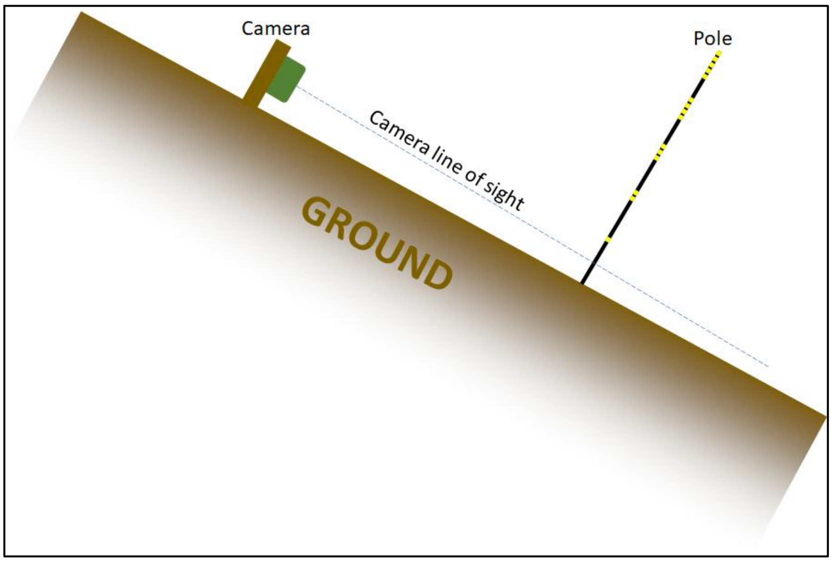


Figure 2. Diagram illustrating a camera set up to observe sloping ground, and the orientation of the calibration pole required to keep it perpendicular to the camera line of sight. Orientation can be judged by eye and need not be measured precisely in the in the field.

- Hold the pole still long enough to ensure a clear image (5-10 seconds). In order to indicate when the pole is resting on the ground, give a distinctive hand gesture when this is the case. For example, thumbs up! **This is fundamental, as often it is not going to be visible whether the pole is actually touching the ground or not and, if it is not, the picture is not going to be useful**.
- Repeat this for further pole placements (at least 25-30) across the field of view and away from the camera, with placements spaced about 0.5 m apart. Continue away from the camera to the maximum extent that any animals are likely to be captured, or if possible, a bit beyond. As you reach greater distances, it may help to have a second person next to the camera to keep it triggering. See figure 3 for an example of a good coverage of deployment calibration pictures. **Before going to the field, it is important to run trials of the deployment calibration process.** Complete the deployment calibration process described above in a convenient location and inspect the images making sure that the results is good.

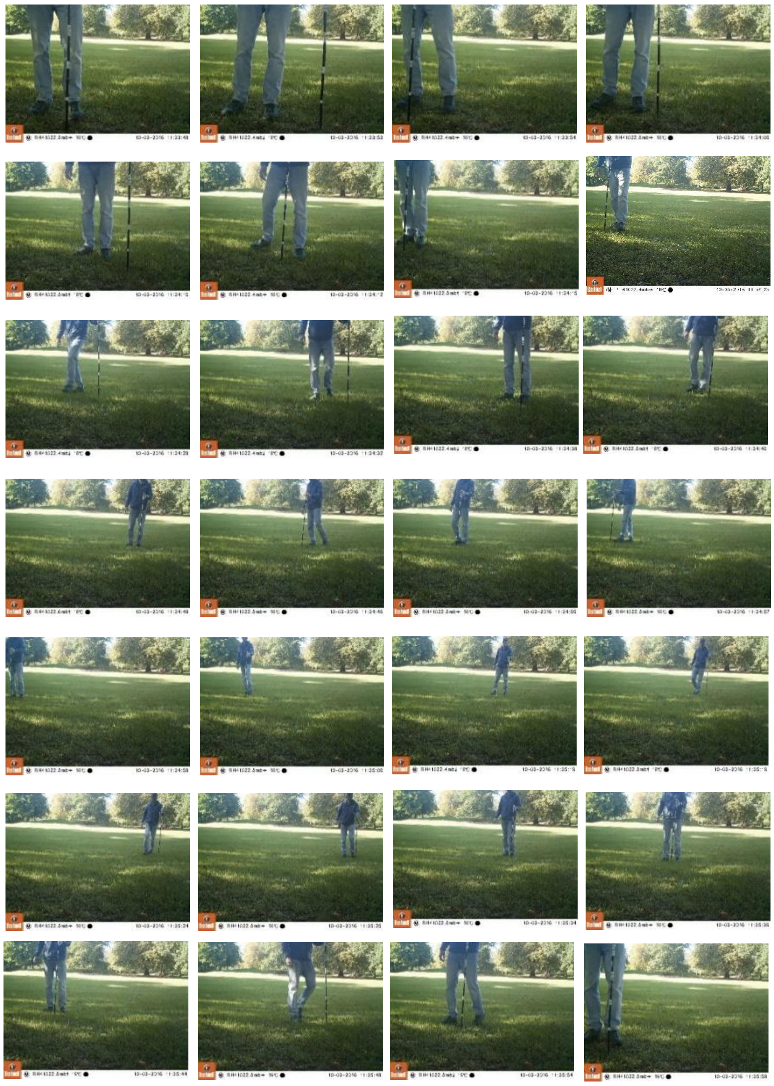


Figure 3. A set of deployment calibration images showing 28 pole positions with good coverage of the detection zone.

- This is another crucial stage of the calibration as you are not going to be able to see how many pictures have been taken and if the pictures are not enough the precision of the estimates for that deployment is going to be low. Make sure you spent enough time on this process and, **if in doubt, take some more pictures as the precision of the estimates strongly depend on this process**. See figure 4.
- Every CT deployment needs its own calibration. If you change the batteries and/or card, which indeed typically changes the camera view in most circumstances, you effectively start a new deployment on the same location. **Therefore, the calibration should be repeated when removing the camera, as well as when setting and checking it**.
- In figure 4 you can see an example of a good distribution of pictures for a deployment calibration.

See "Deployment calibration" for the recording of the course about chapter 5.

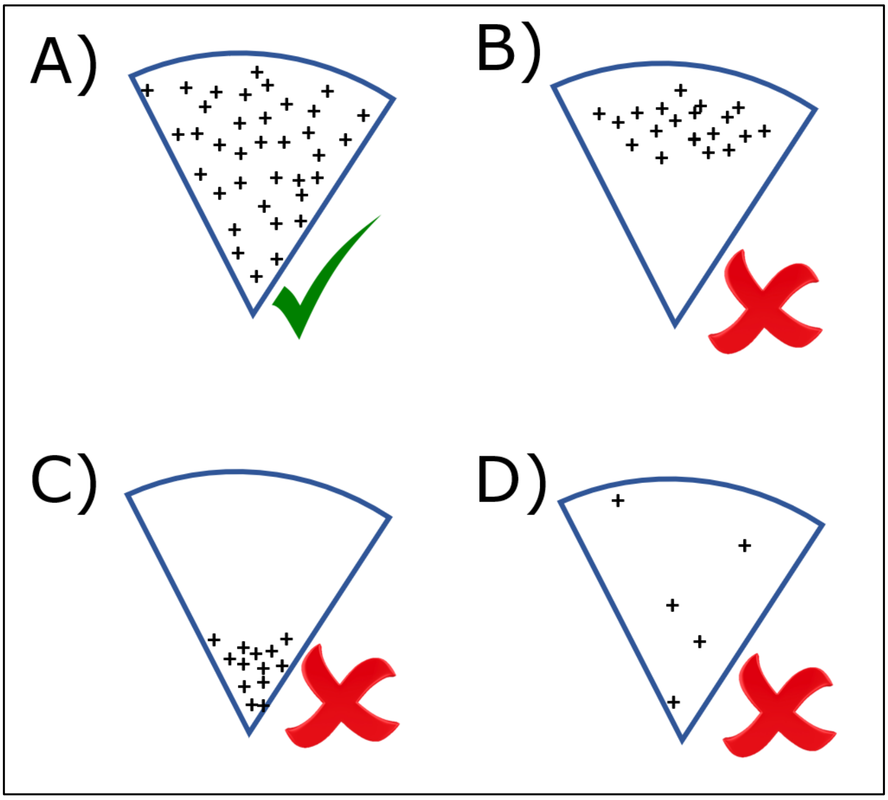


Figure 4. Example of four schemes of calibration of a single camera trap. Crosses represent all the locations of the calibration pole. Panel A represents an adequate calibration (more than 20 points covering homogenously all the detection zone). Panels B, C and D represent wrong calibrations; in panels B and C the points are not homogenously distributed; in panel D, few points were recorded.

6. TAKING CAMERA CALIBRATION IMAGES

The goal is to take pictures of objects of known size at a range of known distances from the camera to calculate the camera model's intrinsic properties, which then allow us to calculate the distance of calibration poles in deployment calibration. This needs to be done for each combination of camera model and image resolution setting used in the field. It's best to keep image resolution consistent throughout deployments; if you do this, and use a consistent camera model, you only need to calibrate one camera, once (no need to repeat it if you already did it last year for the same combination of camera model and resolution). The steps are as follows:

1. Set up the camera in a convenient location in front of a level surface, either indoors or outside.

2. Mark out nine positions at a range of radial and angular distances from the camera, measuring the distances from camera accurately. Fig. 4 gives an example of placement positions, with poles at three distances (1, 2 and 4 m), and a range of angles. It's not necessary to measure angle, but it should be variable, and within the camera's field of view (usually about 20 degrees either side of the midline), but you may need to check the field of view for your camera.

3. With a camera positioned in front of the arena and switched on, take images of a calibration pole (making instructions: [Calibration pole instruction]) at each position on the array, holding up some visible marker of the distance. For example, in Fig. 5, the pole is placed at 2 m from the camera, with distance indicated in metres by the number of fingers displayed. As in the deployment calibration process, care should be taken to hold the pole perpendicular to the camera's line of sight.

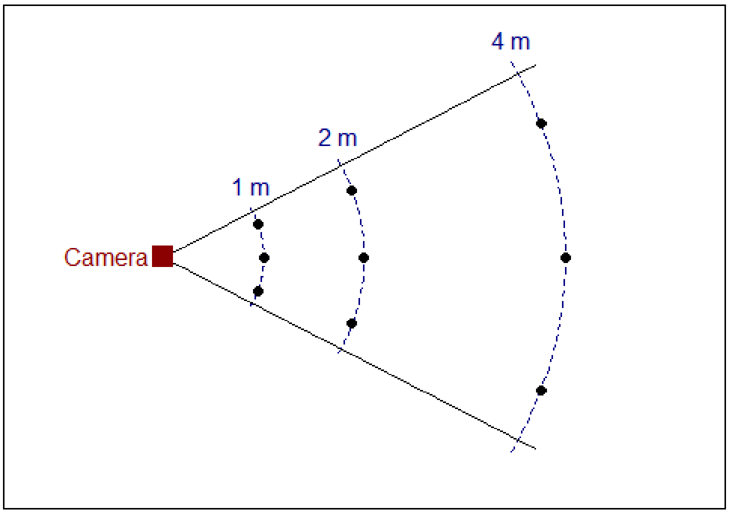


Figure 5. Plan view of an example layout for a camera calibration pole grid.

Figure 6. A camera calibration image with pole in position 2 m from the camera.

7. AGOUTI PLATFORM

7.1. Project creation

Navigate to https://www.agouti.eu/ and create your own account. Then follow the following steps for the creation of a new project:

- From the agouti homepage or directly from your email write a message to agouti@wur.nl. please include whether you are working in a commercial or non-profit setting.
- You will receive a response with an invitation link to your newly created project. Please use the link and notify agouti@wur.nl when that's done.
- The admins will make you PI on the project. You can now enter the project from your personal dashboard and get started.

7.2. Project settings

- In the main project menu go to "Project settings". In the "General" page choose the "Default UTC offset" keeping in mind to also consider the eventual daylight-saving time (DST). So, if the CTs are deployed in a country using the DST and during a period of DST application remember to also consider that when choosing the UTC offset. So, for example, in a project implemented in Italy (UTC +1:00) during in summer is going to have the Default UTC offset of +2:00 (Kaliningrad, South Africa), in this way all the projects are going to have the Greenwich standard time.
- Below, if you like you can add project description and picture. Further down specify the project owner, PI and organisation.
- In the "Sampling design" section select "No bait", 0 seconds quiet period and "Systematic random".
- In the "Annotation" section the sequence cut-off will be set on 120 seconds by default, and it won't be possible to modify this setting as it has to be the same for all the projects.
- In the automatic annotation box, you can select an AI model to automatically process your deployments. You can either select a "species model" (e.g., "Western Europe species model", if more versions are available make sure you selected the latest) or the "Generic

blank/human model”, that is only going to annotate for you the blank pictures and those with humans, leaving to you the sequences with animals. After selecting a model, a button ‘Annotate by AI’ will show up for each deployment listed on the Annotate page.

- In the “Species” page press “Add species list” and select the list for your study area. Once you saved the species will be available for the manual annotation. If you were to record any species that is not included in the list, you can then manually add it using the “add species” button and then browsing the species.
- In the “Behaviour” section you can decide which behavioural classes to add to the annotation page.

Se “Project creation and settings” for the recording of the course about chapters 7.1 and 7.2.

7.3. Add new deployments

In your project, in the “Locations” section you can add the locations of your study site by uploading a CSV file (see “Example CSV” in the top right corner of the page).
Within the “Deployments” section, select “Add Deployment” and then select the location from the drop-down menu. If you didn’t already add this location you can add it now by pressing “add sampling point”. You can now press “Select Files” in the window that pops up (or drag all the pictures of the deployment onto the window), then browse to the folder of the deployment and select all the pictures that you want to upload for that camera deployment and press “ok”. Note that files with the same name will be considered duplicates and will not be uploaded, it will be therefore necessary to rename the files that have the same name. If possible, make sure that you have a good internet connection. Especially if the deployment counts many pictures, it might take some time to complete the import.

7.4. Image processing and animal tracking

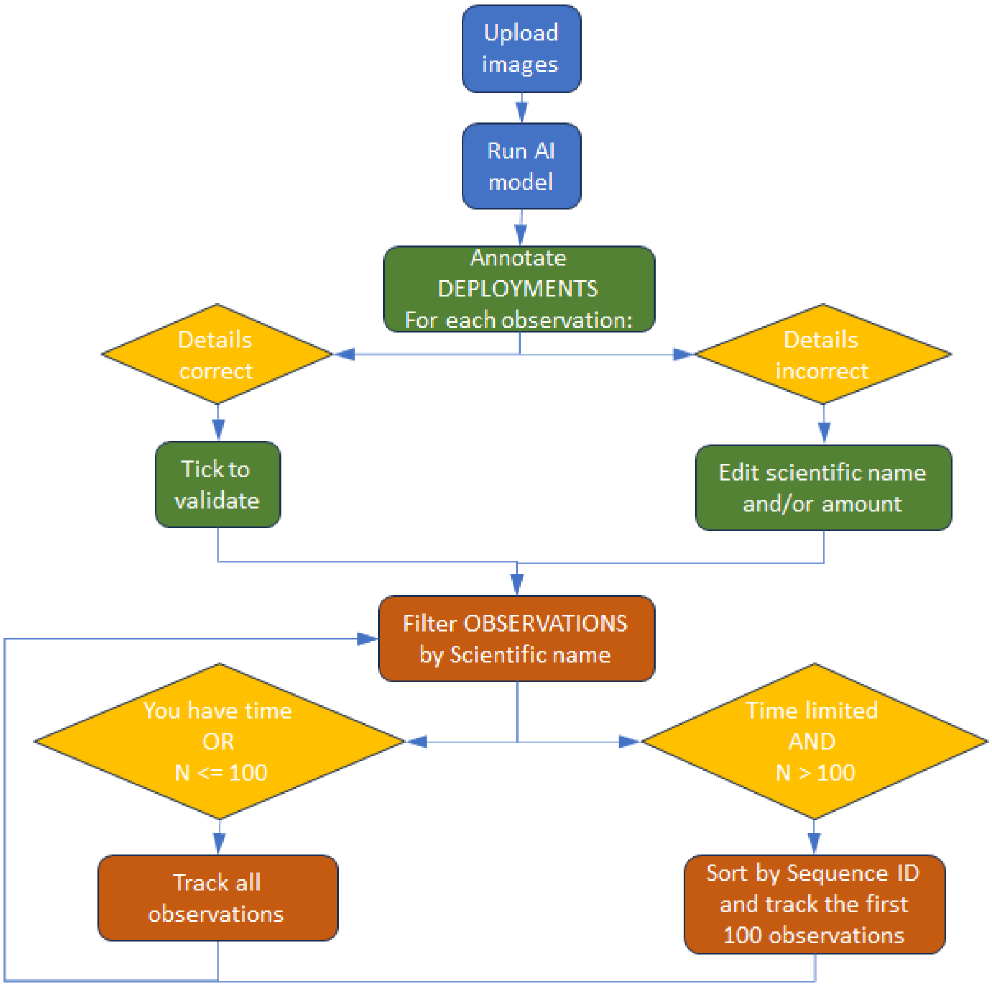
The animal tracking procedure, together with the deployment calibrations is going to allow the calculation of both, camera parameters and animal movements.

1. Upload images and run the appropriate AI detection and classification model.
2. From the “Deployments” section, annotate each deployment. For each AI-defined observation, EITHER:
   a. Tick the Validate box to confirm the AI-defined species and amount; OR
   b. Edit the observation to correct species and/or amount. NB amount should be a count of the number times an animal passes through the camera detection zone, which AI will often get wrong. Be careful to pick up cases where animals leave and enter multiple times within a sequence; keeping an eye on the timestamp can help to identify such cases.
3. In the “Observations” section, filter by species (type a species scientific name into the “Scientific” box), then edit observations to digitise animal tracks. In the sequences where a group of animals is recorded you only have to digitize one of them.

You can EITHER:

   a. If the total number of observations after filtering is less than or equal to 100, or you plan to track all observations regardless, edit all the available sequences for this species; OR:
   b. If you are sampling sequences for tracking to reduce time requirements, and the total number of results after filtering is greater than 100, randomise the sequences by sorting the Observations table by “Sequence ID”, then track just the first 100 observations. Please note that, despite 100 digitised observations are enough, whenever possible, digitizing about 200 observations would be preferable.

4. Repeat step 3 for each species.



The performing of the tracking is going to be allowed only when you are editing a sequence, to do so you just have to click on the point on the picture to create a point. For each picture of a sequence (and within a specific observation) you can only add one point. The animal tracking must be performed on the point of contact of the **frontmost foot with the ground** (often further down the image than you think). The first point is the point of entry, and you then keep tracking **the same foot** (when on the ground) throughout the sequence. If the foot is not visible (e.g., covered by a stone or behind a tree) you can still mark it as the point on the thing covering it where you believe the foot is. The most steps tracked the more accurate is going to be the estimate, however if the path is straight the most important are the first and the last points.

7.5. Manual Annotation

In “Annotate” section select “Annotate” next to the deployment. The pictures of the deployment will be shown as divided in sequences; each sequence is made of the group of pictures the camera took until it stopped being activated for 120 seconds. Browsing through the pictures of each sequence you will then press “Add Observation” when you recognize an animal activating the camera. In the “Identification” menu select the species, quantity, sex, age and behavior, then press “Save Observation”. It is fundamental that you create different observations in the same sequence for animals belonging to different classes (e.g., sow with piglets will need an observation for the sow, adult female, and one for the piglets, juvenile with unknown sex). Remember that **every time an individual goes out from the field of view of the camera and then comes back in it must be counted as a new individual**.

In case there is no observation to add you will be able to select one the following options:

- Blank: empty sequence (e.g., activated by the wind)
- Setup/Pickup: people deploying or collecting camera.
- Deployment calibration: pictures of the "taped stick" for the photogrammetry method.
- Unknown: unknown species in the sequence.
- Vehicle: camera activated by some kind of vehicle.

See "Sequence annotation and animal tracking" for the recording of the course about chapters 7.3, 7.4 and 7.5.

8. REM ANALYSIS

1. If you don't already have them, install:
   - R: https://cran.r-project.org/
   - RStudio: https://posit.co/download/rstudio-desktop/

2. Extract your data from Agouti
   - in your Agouti project, go to the "Export data" section, create a new export file and download the zip file.
   - Unzip the Agouti export file to a specific destination folder.

3. Download example files:
   - Go to: https://github.com/MarcusRowcliffe/camtrapDensity - here you will find a description of steps for the REM analysis (scroll down to the ReadMe section, click the table icon in the top left of that section for a table of contents).
   - Download the camtrapDensity_example.R file (linked from the page or using this link), this contains R codes for running REM density analysis. Move the file from the downloads to the folder where you unzipped the Agouti export.

4. Open RStudio and set up a new project
   - File > New Project > Existing Directory > Browse to the folder you just extracted from the Agouti export > Create Project.
   - In the lower right RStudio pane you should see a tab called Files – click on this (if not already highlighted) and you will see your project files. Click on camtrapDensity_example.R.
   - Before you can start analysing, you will need to install some additional packages as a one-off (code chunk 1 in the camtrapDensity_example file). These will then be available in future analysis sessions without having to reinstall. Running the first chunk of code

(INITIAL SETUP) does this installation. To run a line of code, place the cursor on it and press Ctrl+Enter (Win) or Cmd+Enter (Mac), or click run at the top of the code pane.

5. Start analysing.
   - At the start of each session, load the necessary libraries (run code chunk 2, LOAD PACKAGES).
   - Load the data using the function `read_camtrapDP` (code chunk 3). If you set up the project in the directory created by unzipping the Agouti export (as directed above), you only have to run the code

     ```
     pkg <- read_camtrapDP("./datapackage.json")
     ```

     (without the "." On Mac). See here for details on loading data from other folders if you have stored it elsewhere.

6. Create a subpackage (code chunk 4). **Note: you only have to do this in case you have deployments from previous years in the same Agouti project.** In the example line with the function `subset_deployments` should return only the deployments made in the 2023 campaign, but will need to be edited if any of your deployments cover dates outside that year. **Note: in this case you must then change the object of the following lines from `pkg` to `subpkg` (or whatever name you choose to give to it). Take care to always use the correct data object.**

7. Check deployment schedule (code chunk 4). This allows you to check visually whether all the expected deployments are present in the data, and whether all observation timestamps fall within their given deployment periods (see here).

8. Check deployment calibration models (code chunk 5). Running the lines in this section, R asks you to check all the deployment diagnostic plots and say which species you want to analyse before running the analysis. Diagnostic plots should show reasonably good fits between trend line and data points. Very poorly fitting models should be marked for exclusion so that the unreliable animal position data from these deployments can be excluded from detection zone and speed models. If you don't remember the explanation of how to evaluate diagnostic plots, see this recording of the online courses. Also refer to the document "*Interpreting deployment model diagnostic plots*" for guidance on how to decide whether a calibration model is adequate, available here.

9. One step REM analysis (code chunk 6). Run the line in this section and enter the row number of the species you want to run the analysis for. Here you can also see the number of sequences available for the analysis of each species. Clearly the analysis can only be run for the species for which sufficient sequences have been digitized in Agouti.

10. Evaluate how well the activity, detection radius and detection angle models fit (plots produced by code chunk 7). This can reveal potential problems with limited data that might suggest a less reliable result.

    Run the analysis (steps 9-10) for other species.

11. Inspect model estimates and export the results csv table (code chunk 8). Run the lines to inspect the estimate values and save results with additional information about the species and project, including the option to save a single data table of results for multiple species. Having fitted one or more rem_estimate models (e.g. resulting in objects red_deerREM, roe_deerREM, wild_boarREM), pass the outputs to this function: write_rem_csv(red_deerREM, roe_deerREM, wild_boarREM).

12. When leaving RStudio, save your workspace so your work is preserved (code chunk 10).

See "REM analysis" for the recording of the course about chapter 8.

9. Results reporting

Once all the analysis processes are complete, you can proceed with reporting the results for your study site/s. From 2024 results must be reported using the EOW Results Form where all the necessary information is going to be collected. You should fill in a separate form for each of your study sites. In the "Results" section of the EOW Results Form you will be asked to attach the results' csv table resulting from the analysis process.

10. TRAINING RECORDINGS

At the following links you can find the full recordings of the 2024 training sessions:

- First Training Session – Field protocol
- Second Training Session – Agouti and data analysis

EOW Vocabulary

- **Agouti**: An online platform for the processing and storage of camera-trap images and data. Also used for processing calibration images and digitizing animal movement paths. The output of Agouti is a zip-file in camtrapdp-format.
- **Annotation:** process, performed in Agouti, during which a sequence of images is labelled with one or more observations. Animal observations typically include species, n. of individuals, sex and age classes and, when possible, behaviour.
- **Calibration pole:** 1-m long pole marked at known intervals, used to perform calibrations of the field of view of the camera deployments.
- **Camera model calibration:** process of taking pictures with the calibration pole laterally and centrally with respect to the camera field of view at known distances. It only has to be performed once per combination of camera model and image resolution (picture quality) and is performed at a convenient location (not in the field).
- **Camera locations**: the actual points in the field where the cameras are deployed.
- **Day range:** average distance travelled by an individual of a certain species in a day.
- **Deployments**: use of a camera trap to record wildlife at a specific location during a specific period. Each deployment has its own deployment calibration. A single "camera location" typically has multiple deployments: one per year.
- **Deployment calibration:** process of having an installed camera trap take pictures with the calibration pole standing at many different positions within the camera trap's field of view, with the calibration pole held resting on the ground and perpendicular with respect to the line of sight of the camera trap.
- **Detection zone:** sector-shaped area in which cameras detect animals, defined by the interaction between sensor and environment. Usually defined by radius and angle.
- **Digitizing:** process of marking key points in images. It is performed using the Agouti tools to digitize deployment calibration images and the tracking of the target species.
- **Encounter:** each time that an individual enters detection zone.
- **Encounter rate:** number of encounters per unit of sampling effort.
- **Field of view:** space in front of the camera where animals can be photographed, defined by camera optical properties. It may be larger than the detection zone.
- **Observation:** event of detection of a given animal or group.
- **Photogrammetry:** mathematical technique to extract information on the physical three-dimensional scene captured by the camera from two-dimensional images.
- **Rounds**: the number of subsets the overall number of "camera locations" has been divided into. If, for instance, you have 20 cameras to sample 60 "camera locations" you will deploy the cameras in 3 different "rounds/sessions".
- **Sequence:** group of photos taken by the camera trap that are separated by less than 120 sec, usually representing a single event. A single image sequence can contain one to hundreds of photos, but more typically contains around 10 images.
- **Session:** synonym for round.
- **Study design:** distribution of the camera locations over the study site. Typically, a grid with fixed interspacing.
- **Survey:** field activity aimed at collecting data on wildlife populations.
- **User**: Person involved in a camera-trap survey. Users can have various roles that come with different data access rights, which are assigned in Agouti.

Nº of the study point	Nº CT and SD card	Coordinate X	Coordinate Y	Date setting-up CT in the field	Time setting-up CT in the field	Deployment calibration performed? (Y/N)	Date CT removal	Time CT removal	Deployment calibration performed? (Y/N)	Observations: any eventuality, aspects of functioning of the CT, if it dropped down, if still correctly attached, any failure etc.
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	CT - SD -									
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Table 1. Field sheet, print as many as you need to report the data from all the CT locations.