

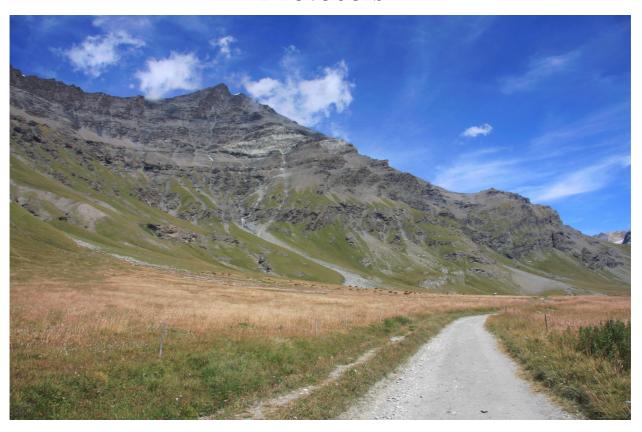






# MARMOT ALPINE PROJECT

# **Protocols**



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# **CAPTURE PROTOCOL**

# Activity 1 - CAPTURE PROTOCOL - Recognition of burrows and territories (map of marmot territories).

Upon your arrival, you will be given a map of the different marmot territories (families).

- Locate the main burrows on the map, then on the ground. The principal burrows are distinguished from the secondary burrows by multiple entrances and the absence of vegetation in the immediate surrounding area.
- Locate the different territories (families) on the map, then on the ground. *Use the following to help you locate their presence on the ground: large stones with unusual form and color, water feature, path, building, main burrows...*

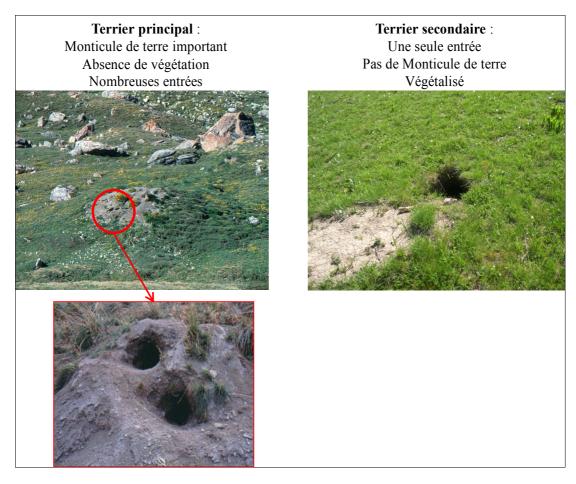
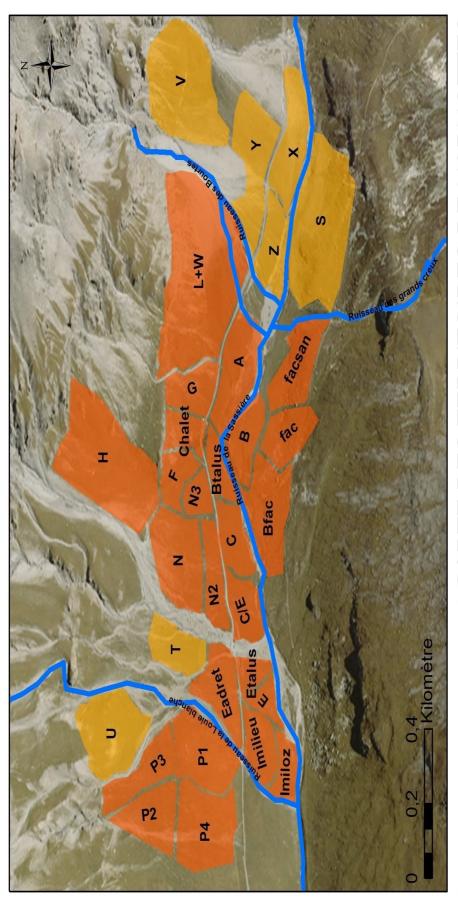
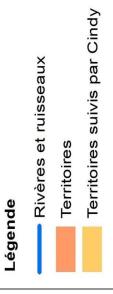


Figure 1: Comparison of main burrow and secondary burrow.







#### Activity 2 - CAPTURE PROTOCOL - Collect dandelions

Dandelion collection should be done daily upon return from the marmot area.

• Pull, as far as possible, the dandelion flowers with both their leaves and roots. Pulling up the entire plant allows it to stay fresh longer.

#### Critères de reconnaissance du pissenlit :

- Feuilles implantées à la base de la tige, allongées et profondément dentées
- Racine verticale
- Inflorescence jaune vif
- Tige creuse, verdâtre





Figure 2: Appearance of the dandelion

- Fill two trash sacks with dandelions.
- Store the filled sacks in a cool, shady place until the next morning.
- The next morning, carry the full sacks to the marmot terrain.



Photo 1: Collecting dandelions

#### Activity 3 – CAPTURE PROTOCOL – Bait the traps with dandelions

Each morning, your job is to put the dandelions that were gathered the previous day in each of the functioning traps.

- Cut the dandelion plants into pieces about 3 cm long.
- Using the dandelion pieces, cover the tool bar that serves as a tripping device to close the trap. The tool bar should be covered sufficiently with dandelions to be entirely hidden.
- Put down four or five dandelion pieces at each of the entries, creating a path to the tool bar.

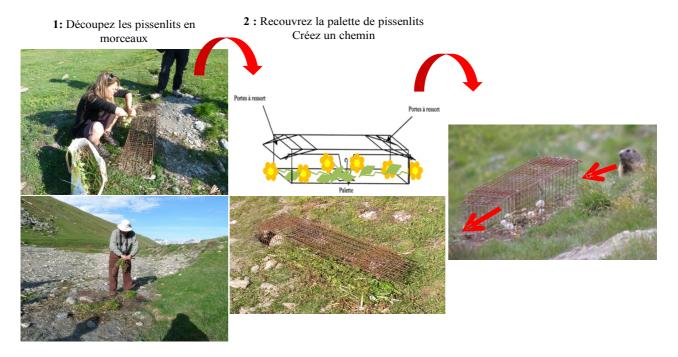


Figure 3: Placement of bait in the traps

#### Activity 4 – CAPTURE PROTOCOL – Monitoring traps

Once all the traps are baited, they should be watched, using binoculars, every half hour, from the path.

Verify the presence or absence of a marmot in each of the functioning traps. If you have the least doubt as to the presence or absence of a marmot in a trap or whether you have checked all the traps, quickly warn a member of the research team.



Figure 4: Observation of the traps from the path

- When a marmot is captured, a member of the research team should be notified immediately. He or she will take charge of putting the marmot in a sack and transporting it to the handling spot.
- Once the marmot is out of the cage, the cage should be cleaned of any excrement (carry the latex gloves for use in this task. Once used, return the gloves), then re-bait and reset the trap.

#### Activity 5 - CAPTURE PROTOCOL - Report all individuals with a colored mark (Data sheet 1)

All individuals with a dyed fur marking or a colored ear token, or both should be recorded in the data sheet below. For each observation, indicate the type of marking:

- Dye: color and symbol
- Ear mark: color and which ear is marked.

Note: left ear mark = colored mark on the left side of the animal; right ear mark = colored mark on the right side of the animal. *This plastic ring is put on the right ear of dominant males and on the left ear of dominant females*.

Couleurs des marquages auriculaires plastiques	Formes des marquages à la bombe	Couleurs des marquages à la bombe
Jaune	XX	Vert
Blanc		Bleu
Bleu clair		Noir
Bleu foncé	<del>\times</del>	Rouge
Vert clair		Orange
Vert foncé		Jaune
Rouge		Rose
Orange		

Figure 4 : Examples of different possible markings

- The territory where the marmot was found
- Date of observation
- Note: certain marmots have only an ear ring, others only a dye mark and others have both an ear ring and a dye mark.



Photo 2: Marmot marked with colored dye: horizontal yellow line between the back paws.



Photo 3 : Dominant male with white mark on right ear.

### Data sheet 1 – CAPTURE PROTOCOL

Observer	Date (DD/MM/YY)	Territory	Color of ear ring	Ear marked (right or left)	Dye color	Dye symbol

#### Activity 6 - CAPTURE PROTOCOL - Release

After handling, each marmot should be released alert, exactly where it was captured:

- Verify the label attached to the capture bag. This carries the letter corresponding to the territory where the marmot was captured and where it should be released.
- Refer to the map of territories in order to release the animal in the right territory

Attention: NEVER release an animal unless you are accompanied by a member of the research team. Releasing an animal in the wrong territory could be fatal to it.

Wait until the animal has returned completely to the interior of the tunnel and you can no longer see or hear it. Never force an animal to enter a tunnel. If it is resistant to entering the burrow that you propose, offer it another burrow.



Photo 4: Release of a marmot after handling.

### **COUNTING MARMOTS PROTOCOL**

#### Activity 7 – COUNTING MARMOTS PROTOCOL (Data sheet 2)

Each morning, in groups of two or three people:

- Spread out on the walking path along the length of the territory
- Observe the number of marmots leaving the main burrow and other burrows



Figure 5: Counting

With the help of the Data sheet 1, fill in the boxes on Data sheet 2:

- Date (DD/MM/YY)
- Total number of marmots
- Ear ring color of the dominant female
- Ear ring color of the dominant male
- Number of mature males
- Number of mature females
- Number of yearling males (one year old)
- Number of yearling females (one year old)
- Number of pups (less than one year old)
- Number of marmots without any markings (without either a metallic ear ring or a colored ear ring)

### Data sheet 2 – COUNTING MARMOTS PROTOCOL

Observers (names):

Date : DD/MM/YY	Territory	Color of		ma	ber of ture iduals	yearlin	mber of gs (1 year old)	Numbe	er of pups	Number of unmarked individuals
		Male	Female	Male	Female	Male	Female	Male	Female	

# REPRODUCTION PROTOCOL

#### Activity 8 – REPRODUCTION PROTOCOL – Date of emergence (Data sheet 3)

From mid-June to mid-July, all of the burrows should be scrupulously observed every day to determine the presence, the absence and the number of marmot pups in each of the territories followed.

#### Not seeing marmot pups is as important a piece of information as seeing marmot pups.

- In groups of two or three people, stay at the place shown to you by a member of the research team.
- Observe the burrows shown to you by a member of the research team. When you observe, be as discreet as possible (no abrupt movements, speak in a low voice) and under no circumstances should you leave the main path to go toward the burrows. That could frighten the marmot pups emerging for the first time.
- Observe all the burrows by scanning them on a regular basis. Focus particularly on the main burrow.
- As soon as a marmot pup is observed in a territory, someone should immediately inform a member of the research team.
- The other observers remain in their observation position and fill out Data sheet 3.



Figure 6: Observation of a marmot pup's emergence

### Data sheet 3 – REPRODUCTION PROTOCOL – Date of emergence

Observer	Date DD/MM/YY	Territory	Number of pups observed*	Comments

<sup>\*</sup>If no marmot pups are observed, write "0"

#### Activity 9 – REPRODUCTION PROTOCOL - Counting pups (Data sheet 4)

When all the marmot pups in a territory have been captured:

- In groups of two or three people, remain in position at the place shown to you by a member of the research team.
- Observe the burrows of the territory that you will be shown by a member of the research team. When you are observing, be as discreet as possible. (No abrupt movements, speak softly) and under no circumstances should you leave the main path to go towards the burrows. Doing this could frighten the marmot pups going outside for the first time.
- Observe all the burrows by regularly scanning every burrow.
- When the marmot pups leave a burrow, count them carefully.
- Note their number on the corresponding data sheet.
- Then, carefully check if all the marmot pups have a metal ear ring.
- Fill out Data sheet 4.

### Data sheet 4 – REPRODUCTION PROTOCOL – Counting pups

Observer	Date DD/MM/YY	Territory	Number of pups with ear marking	Number of pups without ear marking

<sup>\*</sup>If you do not observe any pups, write "0"

#### Activity 10 – REPRODUCTION PROTOCOL – Video of the pups

In a territory, while all the marmot pups are being captured, you will supplement Activity 2 by filming the marmot pups leaving the burrows.

For that, when you go to carry out Activity 2, "Counting pups"

- Carry a video camera with you.
- Position yourself at the location indicated by a member of the research team.
- Direct the camera toward the main burrow.
- When the first marmot pup comes out of the burrow, start the video and say loudly:
  - the date, including the year
  - the name of the territory observed, so that the information can be heard when the video recording is viewed.

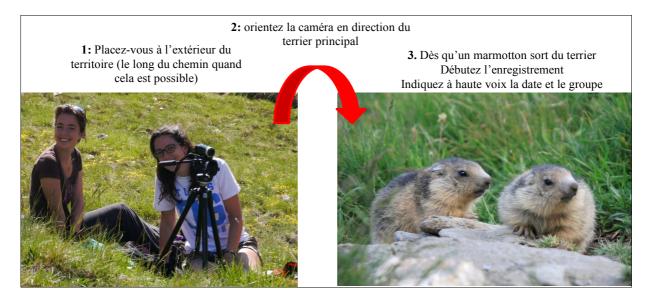


Figure 7: Development Protocol - "Video of the pups"

### **DEAR ENEMY PROTOCOL**

#### Activity 11 – DEAR ENEMY PROTOCOL – Behavioral experiments

In this protocol, you are responsible for assisting a member of the research team with setting up and carrying out the experiment. Scrupulously follow the instructions given to you by the member of the research team that you accompany.

*Be as discreet as possible – no abrupt movements, no loud conversation.* 

- Once the experimental device is in place, be sure you are outside the territory to be studied. *This avoids disrupting the marmots and affecting the results of the experiment.* A member of the research team will tell you where you should stay.
- Install the camera in such a way that it obtains a good picture of the experimental device, start the recording and announce loudly:
  - the date, including year and time
  - the observers' names
  - the territory observed
  - the contents of the tube to the left of the image
  - the contents of the tube to the right of the image
- Stop the recording
- With the aid of a telescope and/or binoculars, watch the dominant marmots. As soon as a dominant marmot approaches a tube, begin the video recording.
  - Be watchful and always ready: the behaviors can be very short. In order for the data to be useful, it is essential to film the whole behavior sequence.
- When a marmot interacts with the tubes, loudly specify its identity: social status and sex (i.e. dominant male, dominant female, subordinate male, subordinate female) and the time.
- As soon as the dominant marmot leaves the field of vision, stop the video recording and indicate the time.
- If, at the time of the experiment, a first dominant marmot or a subordinate marmot marks one of the tubes and moves away from the field of vision, change that tube in order to be able to take into account a subsequent observation of a dominant marmot.

Ideally, the video recording of behavior should be carried out on the same day for the dominant female and the dominant male. However, if the member of the research team feels that the experiment is not feasible because of behaviors observed, the experiment will be interrupted.



Figure 8: Development of the "Dear Enemy" Protocol

#### Activity 12 - DEAR ENEMY PROTOCOL - Video Analysis - Step 1

Following the experiment in the field, two of you are in charge of analyzing the videos obtained.

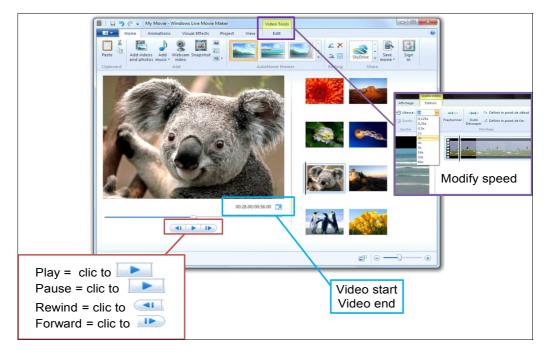


Figure 9: Use of Windows "Movie Maker"

- Go into the "Earthwatch" folder, in the subfolder "Dear-enemy".
- Open the subfolder "dear enemy"
- Start the Movie Maker software by clicking on the following icon:



- Open a video contained in the file "video to analyzed"
- First, view the whole video

• Enter the information in the file "dearenemy2014" by referring to the example below:

In other words, the film produced by "Mike" with, at the left of the image, a tube bearing the scent of male 1405 from family B and, at the right of the image, a tube bearing the scent of female 1608 from family P2.

Video id	Video observers	Left sex	Left marmot family	Left marmot id	Right sex	Right marmot family	Right marmot id
Video name	Mike	M	В	1405	F	P2	1608

- Watch the video a second time
- Indicate the following information in the file "dearenemy2014" by referring to the example below:

In other words, the experiment conducted July 7, 2014 [13/07/2014] between 8:19 and 8:50 on Territory A was an example of the Neighbor-Stranger; female 1134 reacted.

Date	Family group	Experiment	Sex	Id	Experiment- Start	Experiment End
13072014	A	Neighbor_ stranger	F	1134	08:19:00	08:50:00

#### Activity 12 – DEAR ENEMY PROTOCOL – Video Analysis - Step 2

This second step consists of listing all of the behaviors present in the video:

- View the whole video
- Fill out the column "behavior" of the folder "dearenemy2014" according to the list of behaviors below and from those that you observe on the video.

The behaviors observed are classified in three categories:

- *The territorial behaviors directed towards the tubes*
- The territorial behaviors which are not directed towards the tubes
- Non-territorial behaviors

The list of behaviors to note and their corresponding codes are below:

list of behaviors english	Code for the excel file
Smell right tube	SD
Smell left tube	SG
Mark right tube	MD
Mark left tube	MG
Smell near right tube	SVD
Smell near right tube	SVG
Smell another place	SA
Mark another place	MA
Move the tail	TQ
Interaction with another marmot	INTER
Scratches or make a hole	GC
Walk or run	MC
Sitting or lying	AA
Standing	ST
Toilettage	TOIL
Eat	MANG
Run away	FUIT
Return to the burrow	RENT
Go out from the burrow	SORT

Behavior
SVG

#### Activity 12 – DEAR ENEMY PROTOCOL – Video Analysis - Step 3

This third step consists of evaluating the length of each behavior.

Reduce the video running speed by x0.5 so that the measurement of time is more precise. To do this, go to the top of the computer screen, click on the tab "edition", then on the tool "speed", bring the cursor down to "0.5x".

• Fill out the Excel file "dear\_enemy2014.xls", noting for each action the beginning time of the action and the ending time (this information is visible at the lower right of the video).

Behavior_start	Behavior_end
08:46:00	08:48:00

#### Activity 13 - DEAR ENEMY PROTOCOL - Saving videos

Once the video is analyzed:

• Move the file containing the video into the folder "video done"

# WEATHER MONITORING PROTOCOL

#### Activity 14 – WEATHER MONITORING PROTOCOL - Step 1 – Device Recovery (Data sheet 5)

In this protocol, you are responsible for assisting a member of the research team in setting up and recovering the device used for recording temperature and luminosity; the device is present in the main burrows of all the territories followed.

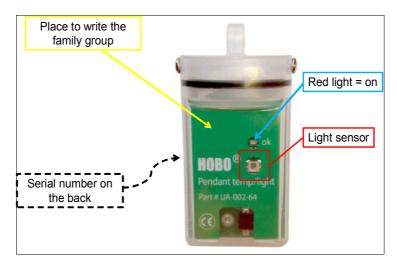


Figure 9: Description of temperature recording

- Be efficient and be careful to remain the least possible time on territories in order to disrupt the marmots as little as possible.
- Carefully follow the instructions given to you by the person in charge.

#### At the beginning:

- In the territory, place yourself at the level of the main burrow.
- Using a GPS and GPS coordinates, search for the exact place where the temperature recorder was buried.
- Remove it from the hole in which it was placed.
- Unscrew the recorder from its support.
- Use a marker to note down the name of the territory on the recording.
- On data sheet 5:
  - Note "recovery" in the 'action' box.
  - The date
  - The time
  - The territory
  - The GPS point
  - The number of the recording

#### Activity 14 – WEATHER MONITORING PROTOCOL - Step 2 - Setting up device (Data sheet 5)

- Using the same support, screw on a new recorder
- Check that it works; the red indicator light of the recorder should be blinking.
- Be sure that the part of the recorder that picks up luminosity is directed toward the bottom, as illustrated below:

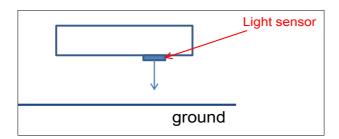


Figure 10: Orientation of the temperature recorder to the interior of the hole.

- Using a marker, note the name of the territory on the recorder.
- Put the temperature recorder in the hole. If the territory where you are situated doesn't have a recorder, then dig a hole using a hammer and chisel. Otherwise, use the same hole from which the previous recorder was removed.
- Note on data sheet 5:
  - 'Set up' in the 'action' box
  - The date
  - o The time
  - The territory
  - The GPS point of the hole in which you placed the recorder. This will allow it to be easily found the following year. Be equally careful to work efficiently so as to stay the least amount of time on the territory.
  - Note the number of the recorder



1: Munissez-vous d'un burin et d'un

marteau

2: Placez -vous à l'entrée du terrier principal et creusez un trou assez gros pour y déposer l'enregistreur



**3:** Déposez l'enregistreur au fond du trou et prenez les coordonnées GPS de ce trou.



Figure 11: Sequence of setting up the recorder.

When the hole isn't already dug, refer to steps 1, 2 & 3 of the illustration.

If it is already dug, follow steps 2 & 3 of the illustration.

### Data sheet 5 - WEATHER MONITORING PROTOCOL

Action	Date		GPS Point		Device	
(recovery or set up)	Date (DD/MM/YYYY)	Time	Territory	X Point	Y Point	number

# PHENOCLIM PROTOCOL

#### Activity 15 - PHENOCLIM PROTOCOL (Data sheets 6 and 7)

The monitoring begins in April/May and continues until July. The observations should be made once a week and always on the same day. (For example, if the first observation is made on a Wednesday, then the subsequent observations take place on Wednesday.)

- For each species illustrated on your data sheets (Data sheets 6 and 7):
  - Count the number of stalks in flower
  - o Count the number of fruits
  - Report these on the card corresponding to the species observed.
- Repeat the same operations for each of the species followed.
- At the end of the day, use the computer to go to "Earthwatch" in the "Earthwatch" folder, then in the subfolder "Monitoring of Alpine ecosystem", open the "Phénoclim" file on the sheet corresponding to the species, enter into the computer the observations noted on your field sheet.

#### Data Sheet 6 - PHENOCLIM PROTOCOL

### PASQUE FLOWER - Pulsatilla vernalis

The different stages to note down:

Flower	Fruit
og viole	

#### Observer:

OUSCIVEI.				
	Date (DD/MM/YY)	Nb. of blossoming stalks	Nb. of stalks with fruit	
Square 1				
Square 2				
Square 3				

# **EDELWEISS - Leontopodium alpinum** The different stages to note:



#### Observer:

	Date (DD/MM/YY)	Nb. of blossoming stalks	Nb. of stalks with fruit
Square 1			
Square 2			
Square 3			

#### Data sheet 7- PHENOCLIM PROTOCOL

### COBWEB HOUSELEEK - Sempervivum arachnoideum

The different stages to note down:

The different stages to note down.				
Flower	Fruit			

Observer:

	Date (DD/MM/YY)	Nb. of blossoming stalks	Nb. of stalks with fruit
Square 1			
Square 2			
Square 3			

# **COLTSFOOT** – **Tussilago farfara**The different stages to note down:

The different stages to note down.			
Flower	Fruit		

#### Observateur:

	Date (DD/MM/YY)	Nb. of blossoming stalks	Nb. of stalks with fruit
Square 1			
Square 2			
Square 3			

# PHENOPIAF PROTOCOL

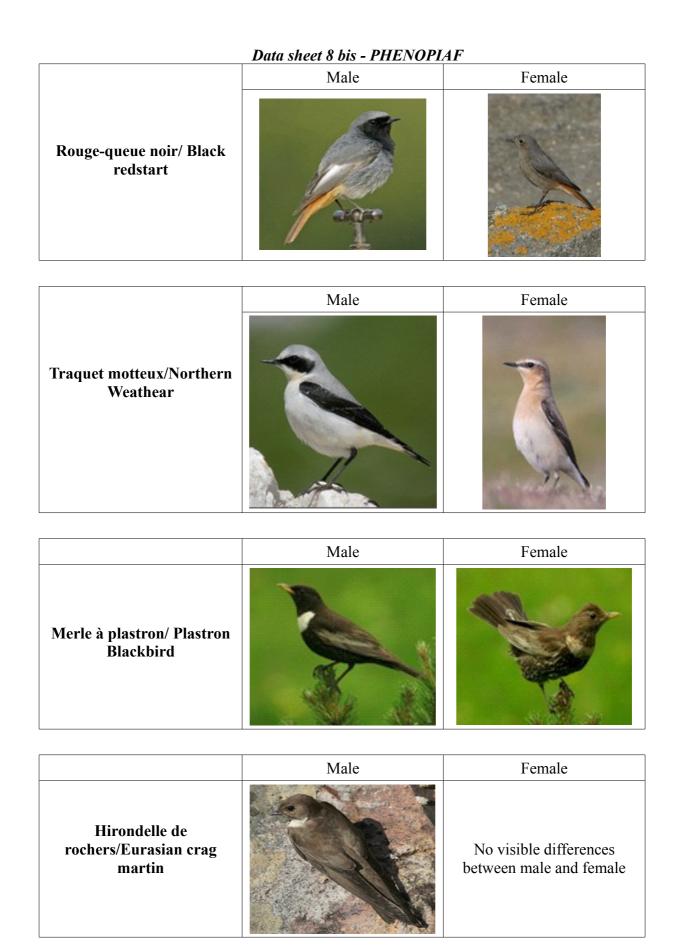
#### Activity 16 – PHENOPIAF PROTOCOL (Data sheet 8)

Upon your arrival, you will be given a list of bird species to follow (Data sheet 8). Learn to recognize them on the sheet, then in the field.

- As soon as you observe one of the four species listed, fill out the Data sheet 8 with the information shown below:
  - o Date
  - Species
  - Number of birds observed
- At the end of the day, go to "Earthwatch" on the computer, in the folder "Earthwatch", then in the subfolder "Monitoring the alpine ecosystem", open the file "Phénopiaf", noting down:
  - The observer's name
  - Location
  - o Date
  - Species observed
  - Number of birds observed. The first returning migratory birds arrive individually or in small groups. Then the main part of the group follows in a few weeks at intervals. For this reason, it is important to specify the number of birds observed.

#### Data sheet 8 - PHENOPIAF

Observer	Location (site/village)	Date DD/MM/YY	Species observed	Nb. of individuals



# PHENOZOO PROTOCOL

#### Activity 17- PHENOZOO PROTOCOL - Monitoring grass frog (Data sheet 9)

The monitoring begins in April/May and continues until July. Observations should be made once a week and always on the same day (i.e., if the first observation is made on Wednesday, the subsequent observations should always take place on Wednesday).

For eachwater point that you are assigned by a person from the research team, note down:

- Number of clutches (groups of eggs)
- Presence of tadpoles
- Put this information on Data sheet 9 in the chart corresponding to the frog.
- At the end of the day, go to the "Earthwatch" computer. Then, go in the folder "Earthwatch". In the subfolder 'Monitoring the alpine ecosystem", open the file "Phénozoo" and on the page "grass frog" enter the observations noted in the field.

#### Activity 18 - PHENOZOO PROTOCOL – Monitoring green dock beetle (Data sheet 9)

The monitoring begins in April/May and continues until July. Observations should be made once a week and always on the same day (i.e., if the first observation is made on Wednesday, the subsequent observations should always take place on Wednesday).

In the marked squares, note the following on Data sheet 9 in the chart corresponding to the green dock beetle.

- Number of green dock beetle
- Number of matings
- Number of clutches (groups of eggs)
- Number of larva
- Transfer this information to Data sheet 9 in the chart corresponding to the green dock beetle.
- At the end of the day go to the "Earthwatch" computer, then in the "Earthwatch" folder. In the subfile "Monitoring the alpine ecosystem", open the file "Phénozoo", on the sheet "green dock beetle", enter the notes from your data sheet.

### Data sheet 9 - Field sheet for the grass frog

Different stages to record:

Clutch	Number of clutches	1st larva observed			

#### Observer:

	Date (DD/MM/YY)	Nb. of clutches	Larva (yes/no)
Pool 1			
Pool 2			
Pool 3			

### Field sheet for the green dock beetle

The different stages to note:

Adulte number	Couple number	Clutch number	Larva number

### Observer:

	Date (DD/MM/YY)	Individuals nb.	Matings nb.	Clutches number	Larva number
Square 1					
Square 2					
Square 3					

# **COMMUNICATION PROTOCOL**

#### Activity 19 - COMMUNICATION PROTOCOL - Photo reporting

In this protocol, you participate in communicating information about the Alpine Marmot Project by sharing photos made during your stay.

#### In order to do this:

- On the computer made available to you
- A folder "Photo" is set up in the "Earthwatch" folder
- In this folder, create a file with your first and last name, as well as the dates of your stay. (i.e. Pierre Dupont week1 2014).
- Post your best photos (and only your best photos) taken during the week.

#### Activity 20 - COMMUNICATION PROTOCOL - Updating the facebook page

This activity calls on your talents as a reporter.

#### For this:

- A schedule is available in the common room (Data sheet 10).
- If you are interested in this activity, put your name down on the schedule (You can also carry out this activity in groups of two or three people designs).
- At the end of the week, on Friday evening, write the weekly field report. This report will be posted on the project's Facebook page and accompanied by photos taken by volunteers during the week.

#### Activity 21 – COMMUNICATION PROTOCOL – Popularization of scientific articles

This activity consists of summarizing the scientific articles about research carried out by the marmot team.

#### For this:

- Several articles are available to you in the "Earthwatch" box
- If interested, sign up on the schedule (Data sheet 10)
- Summarize these articles in one page or less, using non-technical language, so they are understandable to the largest number of people.
- Then, put your summaries in the folder "Popularization of scientific articles" and use the title of the article you have summarized as the file name.

#### Data sheet 10 - COMMUNICATION PROTOCOL

	Updating facebook page	Popularization of scientific articles
Week 1		
Week 2		
Week 3		
Week 4		
Week 5		
Week 6		
Week 7		
Week 8		
Week 9		
Week 10		

### **DATA ENTRY PROTOCOL**

Attention: Carry out these two activities with two people: one person enter the data told by a second person, who read them on the data sheets

#### Activity 22 – DATA ENTRY PROTOCOL – Capture file (Data sheet 11)

Each afternoon, the data collected in the field is recorded in computer files. This data contains the basic information needed to answer all the biological questions regarding the alpine marmots. The capture data is listed in the file "capture".

#### Don't hesitate to ask for help if necessary

- In the "earthwatch" computer, go to the "Earthwatch" folder. In the subfolder "CMR data", open the file "capture2014". All the data relating to captures is in this file. Each line of the chart corresponds to a marmot captured on a given day.
- Enter all the information written on the capture sheet into the file "capture 2014".

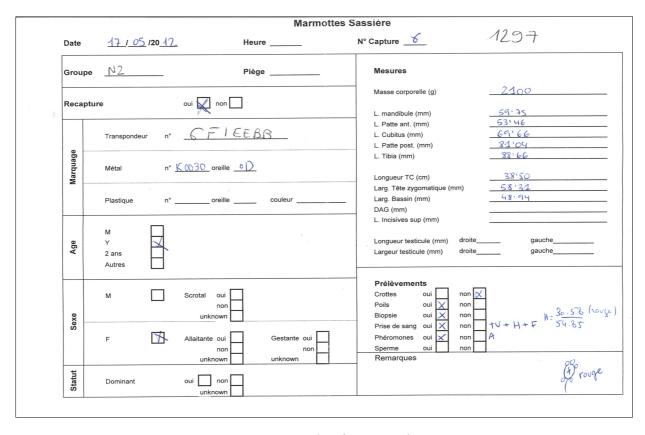


Figure 12: Example of capture sheet

#### Data sheet 11- DATA ENTRY PROTOCOL - Capture file

Scrupulously follow the formatting (in particular, the use of capital letters and small letters):

1	1 11																
Day	Month	Year	Time	N tcap	ture	Handlii	ng ]	Identity numb		Group	Fa	amily		Recapt		tran	sponder
11	06	1990	NA	1		NA		1		b		b90		1			NA
12										<b>→</b>			•		•		22
Left ringc	Color	Righ ring	_	gger A	Age	Sex Scrotal Lactating Pregnant Social statu		ng Pregnant		al status		Mass g					
z6326	NA	NA	N	A	1	f		NA		NA		NA			NA	(	3600,00
23			•							<b>→</b>						·	33
Jaw_ mm	Forefoo length_n	_	na_length _mm	Hind _leng _mi	gth_	Tibia_le th_mn		Body_le		omatic_ th_mm	Bass widt mr	th_		er_inc :_mm	Testes_ ht_m		Testes_ left_m m
50	65		NA	8:	5	NA		45	]	NA	N/	A	]	10	NA		NA
34										<b>→</b>							44
Fece	es Ha	air	Biopsy	Green_ tube	R	ed_tube	Sme	ear	Eurytic	Leuc	otic	Hen	natoc	cryte	Jugal		Bucal
NA	. (	0	0	0		0	N/	A		N/	A		NA		NA		NA
	45 50																
	ana	al	TV_ex	tract		TR_extract H_to		H_total H_red_blo		H_red_blood_cell Commen		Comments	S				
	N.A	A	0			0	NA		NA		NA NA		NA				

Figure 13: Extract from the data table "capture"

Fill out the file "capture2014" in the order indicated below:

When information is lacking on the capture sheet, fill out the corresponding field with "NA"

- 1. Day = find in Date (DD/MM/YYYY)
- 2. Month = find in Date (DD/MM/YYYY)
- 3. Year = find in Date (DD/MM/YYYY)
- 4. Time = Time
- 5. N Capture = Capture number
- 6. Handling = name of the mesurer
- 7. Identity = marmot identity number (written in red at the top right corner of the capture sheet)
- 8. Group = name of the group
- 9. Family group = name of the group following by year (eg: for n3 in 2014, put n314)
- 10. Recapture count = Recapture (if  $1^{st}$  capture of the year put 1, if  $2^{nd}$  capture of the year put 2....)
- 11. Transponder = Transponder number

- 12. Left ring = either "metal" or "plastic" code of the marking section if the "ear" next to it is "OG", else put "NA"
- 13. Color = color of the "plastic" mark followed by the side of the ear (e.g. "white1eft")
- 14. Right ring = either "metal" or "plastic" code of the marking section if the "ear" next to it is "OD", else put "NA"
- 15. Logger = Data Logger presence (yes or no)
- 16. Age = 0 if pup, 1 if yearling, 2 if 2 years old, put the number written in the box if other
- 17. Sex = "m" if male, "f" is female
- 18. Scrotal = scrotal presence if male (yes or no)
- 19. Lactating = Lactating female (yes, no or unknown)
- 20. Pregnant = Pregnant female (yes, no or unknown)
- 21. Social status = in status section, if "dominant" is yes put "d", if "no" and the age class (in age section) is pup: put "m", if "no" and the age class is not pup put "s", if "unknown" put "NA".
- 22.  $Mass_g = Body mass(g)$
- 23.  $Jaw_mm = Jaw(mm)$
- 24. forefoot\_length\_mm
- 25. ulna\_length\_mm
- 26. hindfoot\_length\_mm
- 27. tibia length mm
- 28. body length cm
- 29. zygomatic width mm
- 30. pelvis width mm
- 31. upper incisor mm
- 32. testes right mm
- 33. testes left mm
- 34. feces = put 1 if yes, put 0 if no
- 35. hair = put 1 if yes, put 0 if no
- 36. biopsy = put 1 if yes, put 0 if no
- 37. green tube = put 1 if yes, put 0 if no
- 38. red tube = put 1 if yes, put 0 if no
- 39. smear = put 1 if yes, put 0 if no
- 40. Eurytic = put 1 if yes, put 0 if no
- 41. Leucotic = put 1 if yes, put 0 if no
- 42. Hematocrit = put 1 if yes, put 0 if no
- 43. jugal = put 1 if yes, put 0 if no

- 44. bucal = put 1 if yes, put 0 if no
- 45. anal = put 1 if yes, put 0 if no
- 46. TV\_extract = put 1 if yes, put 0 if no
- 47. TR extract = put 1 if yes, put 0 if no
- 48. H\_total
- 49. H\_red\_blood\_cell
- 50. Comments = copy what's written in comments section. (Ask for help if needed)

When information is lacking on the capture sheet, fill out the corresponding field with "NA"

#### Activity 23 – DATA ENTRY PROTOCOL – Family structure (Data sheet 12)

Each afternoon, the data on family structure at the time of the captures is entered into a computer. The family data is listed in the file named "structure famille" (family structure).

Attention: This activity requires two people who work together: the first person reads loudly the data written on field data sheets, and the second person enters the data on the computer.

- In the "Earthwatch computer, open the "Earthwatch" folder, then the subfolder "CMR data", open the file "structurefamille2014". This file represents all the data relative to the annual structure of each family group. Each sheet represents a family, and on each page the lines correspond to the year of observation. For this year, only the line corresponding to the year 2014 should be filled out.
- Enter into the computer all of the information contained in the field notebook.

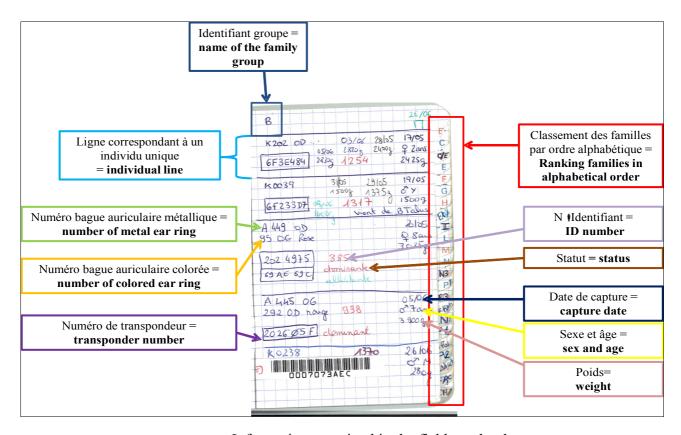


Figure 14: Information contained in the field notebook

#### Data sheet 12 - DATA ENTRY PROTOCOL - Family structure file

The information file named <<structure\_familleYYYY>> consists of several sheets whose names correspond to the identified family groups. For example, sheet B is going to correspond to the family structure located on territory B.

- Take the field notebook where the family compositions are recorded. *In this notebook, each marmot is classified according to the territory where it was captured.*
- Using the information in the field notebook, fill in the columns corresponding to the marmots captured in each family group.

The sheets of the file <<structure\_familleYYYY>> are presented as follows:

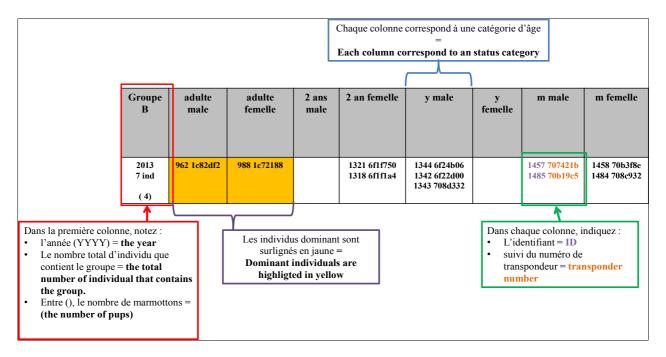


Figure 15: Information contained on Excel sheets

- Scrupulously respect the formatting (in particular the use of capital letters and small letters)
- Dominant marmots should be underlined in yellow
- All the marmots present in the field notebook should be in **bold type**.\*

Fill out the file "structure famille YYYY" in the order indicated below:

- Adult Male: ID and transponder number of all adult males present in a family group
- Adult female: ID and transponder number of all adult females present in a family group.
- 2 year male: ID and transponder number of all 2-year old males present in a family group
- 2 year female: ID and transponder number of all 2-year old females present in a family
- y male: ID and transponder number of all yearling males present in a family
- *y female: ID and transponder number of all yearling females present in a family*
- m male: ID and transponder number of all male pups present in a family
- m female: ID and transponder number of all female pups present in a family

<sup>\*</sup>If, in a family, there is not a marmot of an age group or a sex indicated, leave the box empty.

# HAIR PROTOCOL

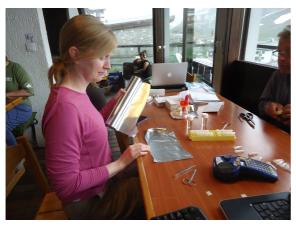
### Activity 24 – HAIR PROTOCOL – Preparing samples for genetic analysis (Data sheet 13)

In this protocol, you prepare samples of hair for later DNA analyses: To separate the hair root bulbs:

• Follow the method explained and illustrated on Data sheet 13 below.

#### Data sheet 13 - HAIR PROTOCOL

**1: Prepare a piece of aluminium foil** *like a table place mat to create uncontaminated work space* 



3: Sterilize scissors and tweezers by quick dip into alcohol and run through lighted candle, and place tools on the place mat

Close the alcohol bottle



5: Take a micro-centrifuge tube



2: Open small alcohol bottle



4 :Take an envelope, check the number ,  $\underline{G}1,\underline{G}2,...etc.$ 



6: Check that the number of the tube is the same as that of the envelope.



7: Carefully open the envelope so as not to destroy the seal.

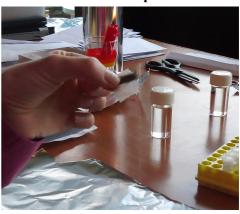


- 9: Line up the bulbs of the hairs, for ease of cutting. 10: Remove excess/ uneven hairs, have tweezers very
- close to hair bulbs
  11: Once hairs are lined up, use small piece of tape to



- 15: Check that bulbs are in tube
- 16: Place matched tube in holder to keep it upright.
- 17: Close microcentrifuge tube and place in box with other finished samples. Be sure that it is placed in sequence (G1, G2, G3, etc.)

8: Using clean tweezers, select a small amount of hair, between 30 & 60 hairs, from envelope\*.



- 12: Confirm envelope number with micro centrifuge tube.
- 13: Place matched tube in holder to keep it upright.
- 14: Use scissors to clip bulbs off the hair into the tube. Avoid excess hair pieces.



- 18: Close the envelope and place it in the finished stack of envelopes in the correct sequential order (G1, G2, G3).
- 19: Fold up al. foil, clean surface of working area.

Start each hair sample with clean aluminium foil work area

\* If small amount of hair or it is mixed up and not most of hairs going in same direction, get professional help to sort out where the bulbs are.

# **COUNTING BLOOD CELLS PROTOCOL**

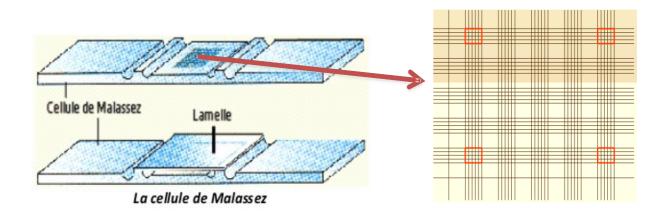
# Activity 25 – COUNTING BLOOD CELLS PROTOCOL – Establish CBC from captured marmots – Step 1 (Data sheet 14)

In this activity, you are in charge of counting red blood cells (erythrocytes) and white blood cells (leukocytes). To do this, follow the method explained on Data sheet 14 below:

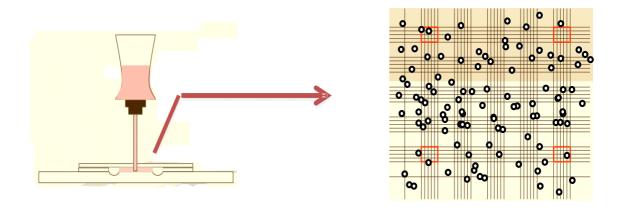
#### Data sheet 14 – COUNTING BLOOD CELLS PROTOCOL

# **Microscope step**: Already done

A special slip, a hemocytometer, with a grid pattern has been used.

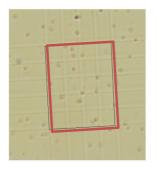


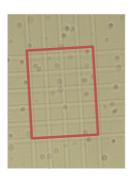
After blood deposit, a picture has been taken to count blood cells.



# Find the rectangles and the stripes: Work to be done

**ERYTHROCYTES**: Selected the four of the more distant rectangles (in red on the picture above)

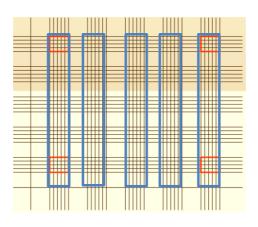


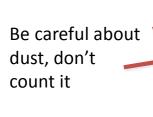


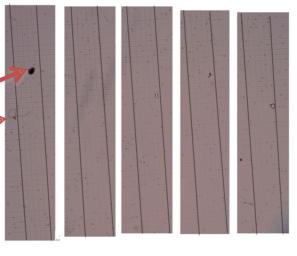




**LEUCOCYTES**: We have selected five strips (in blue on the picture below)







Blood cells are round and clearer at the center most often

## **Counting step**: Work to be done

**ERYTHROCYTES**: Count cells in the four rectangles. Each rectangle includes 25 squares.

# Example of calculation

Rectangle	Number of cells
Rectangle 1	13
Rectangle 2	20
Rectangle 3	15
Rectangle 4	2

Make the sum of each rectangle in order to get the total number of erythrocytes : 13 + 20 + 15 + 2 = 50. Retain this result to enter it in the data folder.

### **LEUCOCYTES**: Count cells in the five stripes

Example of calculation

Strip	Number of counted cells
Strip 1	98
Strip 2	53
Strip 3	67
Strip 4	48
Strip 5	77

Make the sum of each strip in order to get the total number of leucocytes : 98 + 53 + 67 + 48 + 77 = 343. Retain this result to enter it in the data folder.

### **Data step**: Work to be done

Enter data in the document 'datafile\_bloodcellcount'

File_name	cell_number
170513_XXX	XX

Name of the picture

The retained result

# Activity 25 – COUNTING BLOOD CELLS PROTOCOL – Establish CBC from captured marmots – Step 2

Once the erythrocytes and leukocytes are counted:

- In the folder "Earthwatch" open the file "Data File Bloodcellcount.ods"
- On the "erythrocyte" sheet, enter the data regarding the erythrocyte counts.
- Next, on the "leukocyte" sheet, enter the data regarding the leukocyte count.