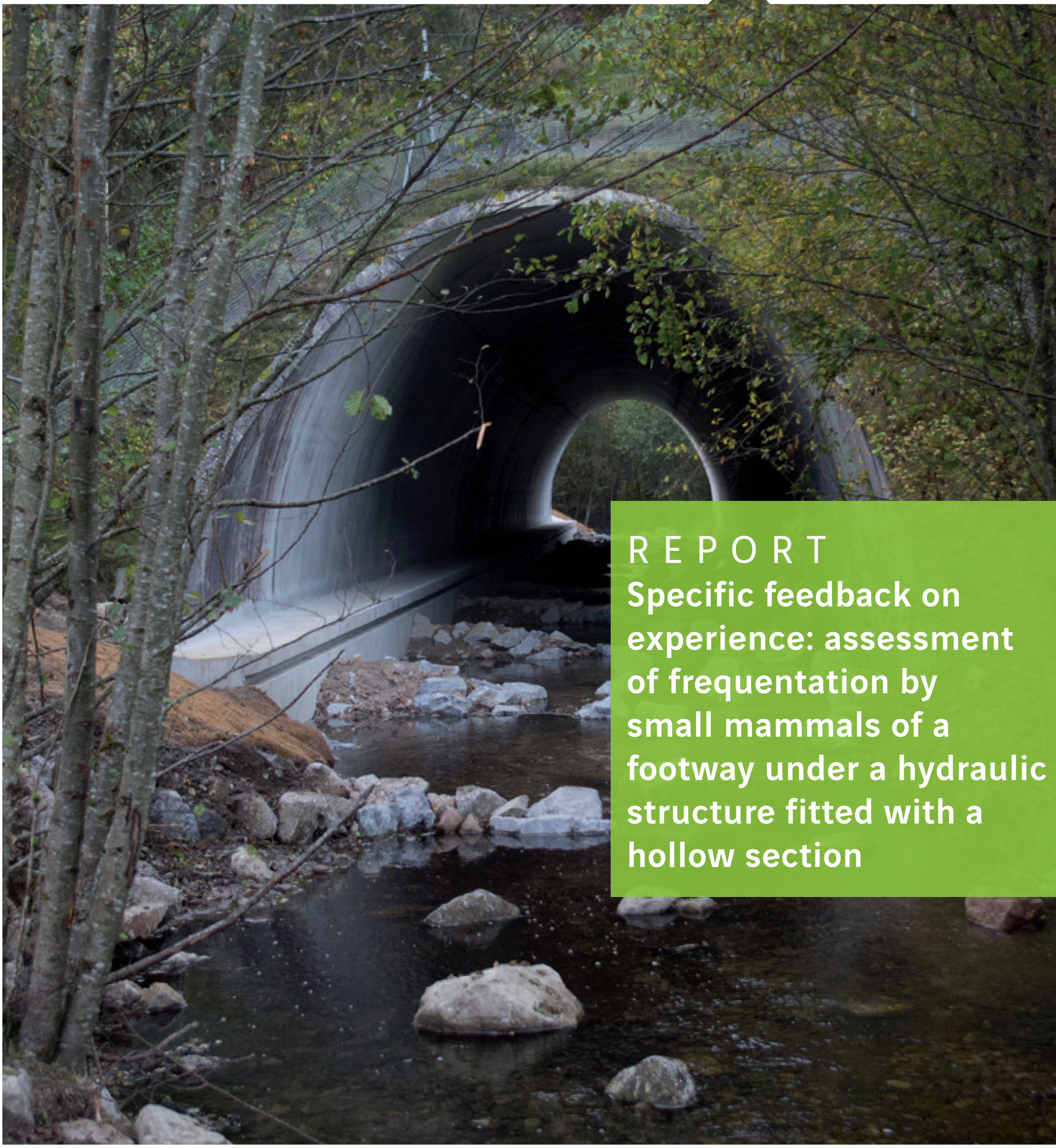
THE RESTORATION OF ECOLOGICAL CONTINUITY CORRIDORS ON MOTORWAYS

MARCH 2023



REPORT

Specific feedback on experience: assessment of frequentation by small mammals of a footway under a hydraulic structure fitted with a hollow section

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Groupe de Recherche et d'Etude pour la Gestion de l'Environnement

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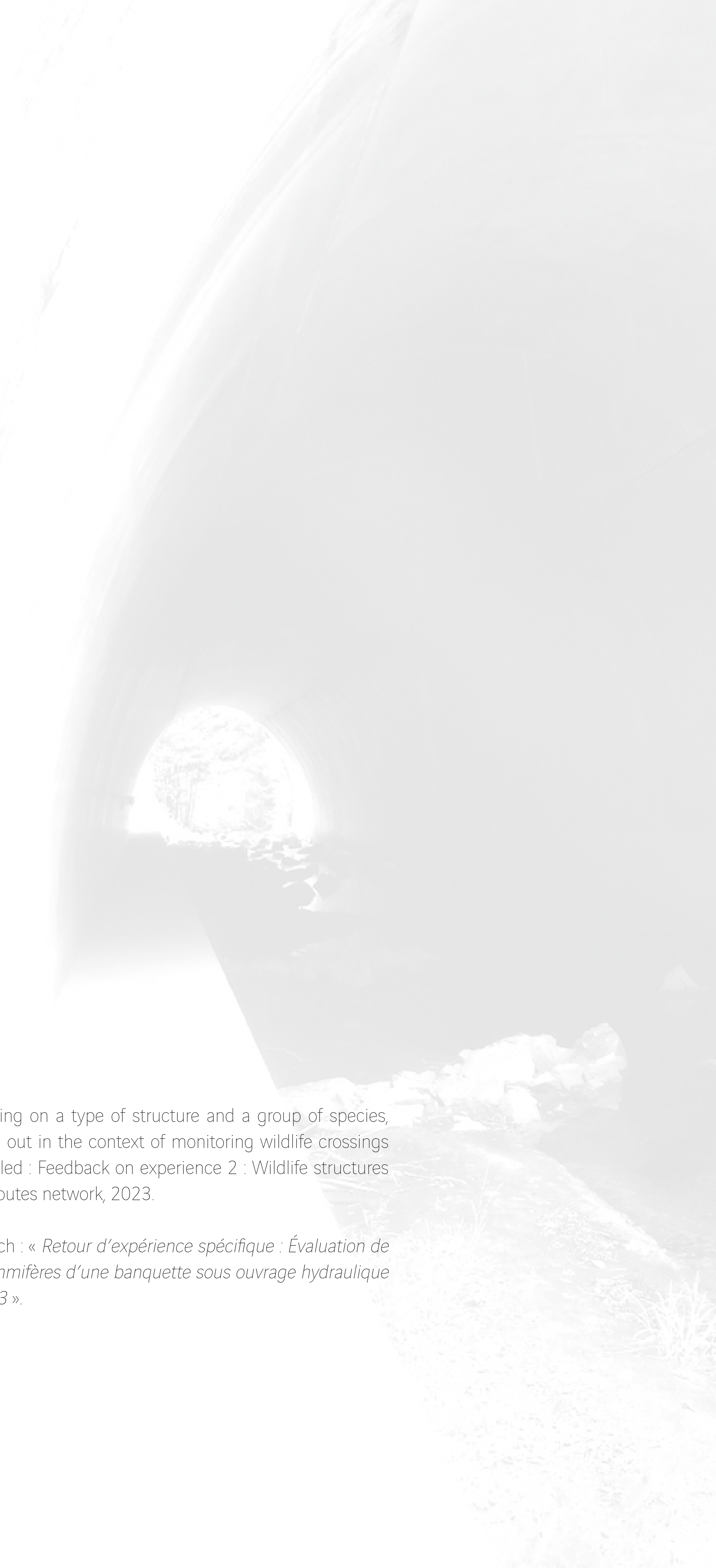

The results presented here, targeting on a type of structure and a group of species, are part of a set of studies carried out in the context of monitoring wildlife crossings presented in a general report entitled : Feedback on experience 2 : Wildlife structures and monitoring in the VINCI Autoroutes network, 2023.

This document is available in French : « *Retour d’expérience spécifique : Évaluation de la fréquentation par les petits mammifères d’une banquette sous ouvrage hydraulique équipée d’une encoche, mars 2023* ».

SUMMARY

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“ This monitoring demonstrates the effectiveness of the hollowed pathway for the passage of micromammals ”

I. SUMMARY

Within the framework of restructuring carried out by the VINCI Autoroutes network ASF in the Deiro hydraulic structure (A89 - SOUDEILLES) to re-establish ecological continuity for wildlife, a specific 120 centimetre-wide "small wildlife" footway was created in the structure and spurs were set up along the footway in the riverbed. On this occasion, ASF wished to test the setting up of a "micromammal" hollow section originally developed by GREGE for water shrews and water voles. This thirteen-centimetre-wide and ten-centimetre-high "notch" was hollowed in the wall of the "small wildlife" footway to create a covered pathway for micromammals.

In order to assess the effectiveness of this innovative adaptation, the GREGE joined forces with GMHL to evaluate the micromammals' frequentation of the hollowed section by deploying specific protocols adapted to the counting and identification of micromammals. Four techniques were combined to count the passages and identify the species: footprint trackers, camera traps, sample collection tubes (fur and faeces) and the sampling of environmental DNA in the hollowed pathway with genetic identification of the detected species.

Out of the 935 nights when the camera trap was operational, 1330 passages of five species or groups of species were recorded in the hollowed pathway: 923 passages of the group of two field mice of the genus *Apodemus sp.*; 361 passages of the group of two water shrews of the genus *Neomys sp.* eight passages of Red Squirrels (*Sciurus vulgaris*); five passages of the group of small voles and one passage of the group of large voles of the genus *Arvicola sp.* Over the two years of monitoring, the circulation of micromammals in the hollowed pathway was around 36 passages per month. These results are globally higher than in the very few bibliographical references, and confirm a high frequency of micromammals in the structures set up. This monitoring demonstrates the effectiveness of the hollowed pathway for the passage of micromammals.

The numerous passages attributed to the genus *Neomys sp.*, with a notable peak between July and October, are particularly remarkable (no other reference on the subject to our knowledge, either in terms of frequency or even monitoring) and show the interest of this structure for this group of protected species. In addition, the specifically developed protocol for collecting eDNA in the hollowed pathway or clues in the dedicated sample tubes, and the identification of the species through a genetic approach, confirmed the circulation of the European Water Shrew (*Neomys fodiens*) and the Wood Mouse (*Apodemus sylvaticus*).

II. CONTEXT OF THE STUDY

Within the framework of the restructuring carried out by VINCI Autoroutes ASF network in the Deiro structure to restore ecological continuity for wildlife, a specific footway was created in the structure and spurs were set up in the riverbed.

On this occasion, ASF wished to test the implementation of a "micromammal" hollowed pathway developed by the GREGE originally for the Aquatic Shrew (*Neomys fodiens*). In the present case, the hollowed section was aimed preferentially at the circulation of the Southwestern Water Vole (*Arvicola sapidus*) known to be present in the vicinity and was positioned in the vertical wall of the footway. To evaluate the effectiveness of this structure, ASF asked the GREGE to carry out monitoring of the device with appropriate protocols.

At the same time, the upper footway was to be equipped with a camera trap, and it was decided to seize the opportunity of this monitoring to compare the results of the "GREGE footprint trap" and "ASF camera trap" protocols on this site and to draw some lessons from them.

Monitoring by footprint trackers was therefore proposed: this technique, constantly used by the GREGE for monitoring the use of hydraulic structures, makes it possible to identify the species or groups using the facilities created for the wildlife and to evaluate the frequentation rate. To carry out this monitoring, the GREGE joined forces with the GMHL, which carried out standardised field surveys.

The proposed monitoring combines three complementary techniques:

- Assessment of the presence of the Southwestern Water Vole in the vicinity of the structure using transects to search for signs of its presence,
- Tracking species movement in the notch, using a dedicated footprint tracker and a dedicated camera trap,
- Monitoring of species movement on the wildlife footway using a footprint tracker set up across its entire width. It should be noted that this monitoring was backed up by ASF's standardised camera trap monitoring (ASF, 2017). The results thus obtained by the ASF camera trap were analysed by GMHL and can be compared to those recorded by the footprint trackers on the footway.

In addition, in 2020, additional monitoring using sample collection tubes was proposed to provide genetic insight to this inventory, in and around the structure.

Initial monitoring was conducted between May 24, 2018 and May 29, 2019. However, as the initial protocol was not fully adhered to, particularly due to a lack of footprint tracker surveys between December 2018 and April 2019, it was agreed to implement a new monitoring phase over an equivalent period the following year and to continue it until summer 2021.

This report presents the results of all monitoring implemented from May 2018 through August 2021

In addition, in 2020, complementary monitoring using collection tubes was proposed in order to provide a genetic input to this inventory, in the structure and the surrounding areas..

The following individuals within GREGE and GMHL were involved, along with proofreading by ASF:

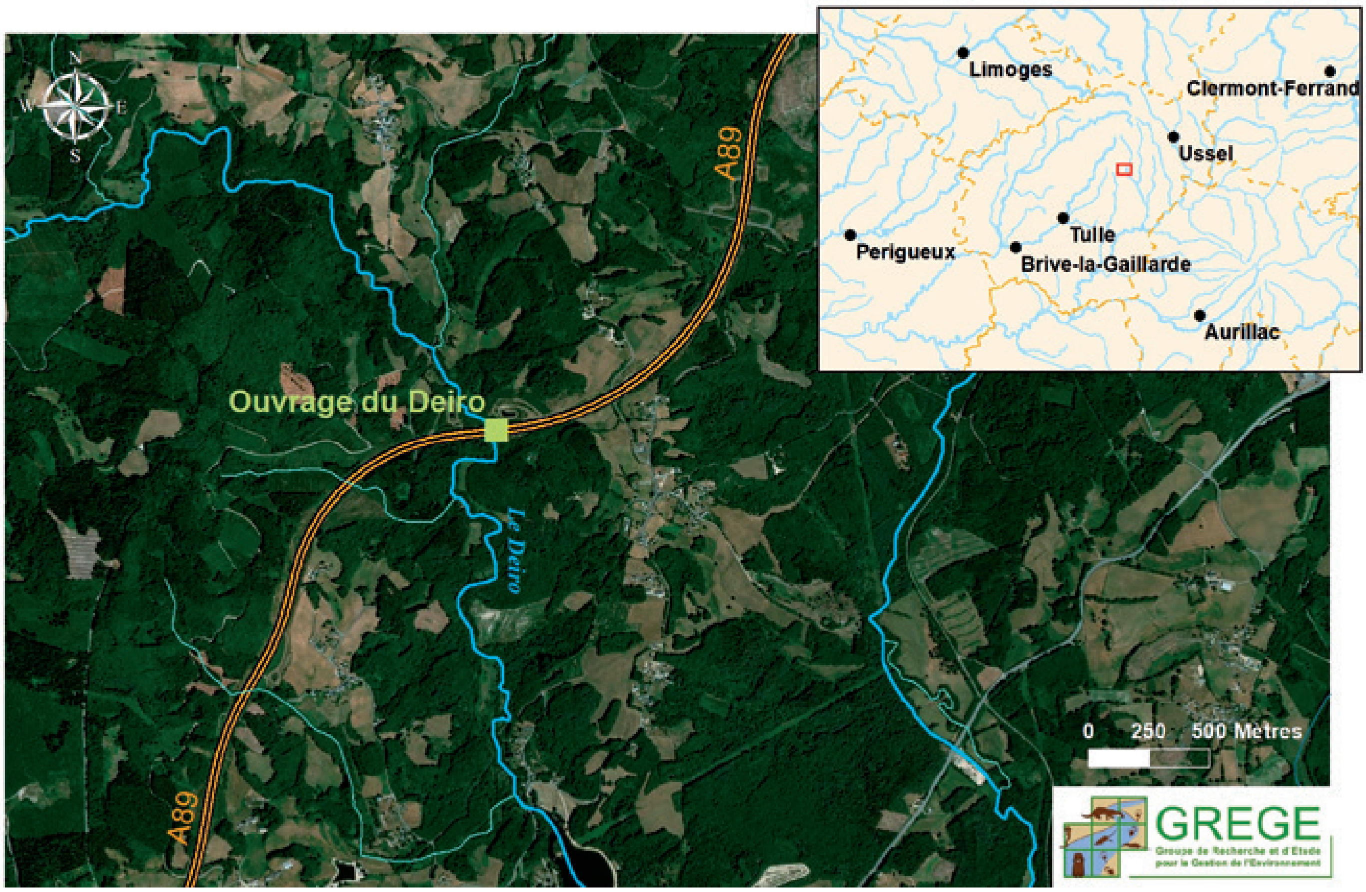
Marie ABEL	GMHL	Field sampling Analysis of camera-trap photos of the wildlife footway
Manon DEVAUD		
Cristian ESCULIER	GMHL	Field sampling
Vanessa MAURIE	GREGE	Identification of prints Data analysis Drafting of file and mapping
Chloé BADUEL	GREGE	Analysis of camera-trap photos of the hollowed out "small mammal" section Data analysis Preparation of genetic samples Drafting of file and mapping
Célia FOURNIER	GREGE	Analysis of camera-trap photos of the hollowed out "small mammal" section
Christine FOURNIER	GREGE	Collection of fur/stool samples in the hollowed out "small mammal" section Interpretation of genetic results General proofreading Quality control
Mathieu BOURBOULON	ASF	General proofreading
Gabriel METEGNIER	GMHL	
Pascal FOURNIER	GREGE	Training of GMHL Setting up sensors and collection of fur/stool samples in the hollowed out "small mammal" section File coordination
Adam CLARK		Translation

III. PRESENTATION OF THE MONITORED STRUCTURE

Located in the department of Corrèze, in the municipality of Soudeilles, the hydraulic structure enables the Deiro river to flow under the A89 motorway (Map 1).

In 2017, this 9-metre-wide structure was redesigned to re-establish the ecological continuity necessary for the crossing of the A89 by small wildlife (Photoset 1). Previously, the structure had already been fitted for wildlife by the creation of three tiered footways. However, the existing footways were considered unsuitable, given the permanent immersion of the lowest footway and the regular immersion of the second. Remodelling was therefore carried out.

A 1.20-metre-wide wildlife footway, 60 centimetres above the highest existing footway, was created on the left bank to allow species to move through the structure without getting wet throughout the year. In the side of this footway, 85 centimetres above the lowest existing footway, a "micromammal" pathway was specifically hollowed out for the Southwestern Water Vole. This arrangement, conceived and designed by the GREGE, has already been created previously in four structures on the Tours-Bordeaux high-speed trainline. The principle is based on obtaining better-covered and safer circulation of micromammals, by means of this 13-cm-wide and 10-cm-high hollowed section.



Map 1 : Location of the Deiro structure, monitored between 2018 and 2021.

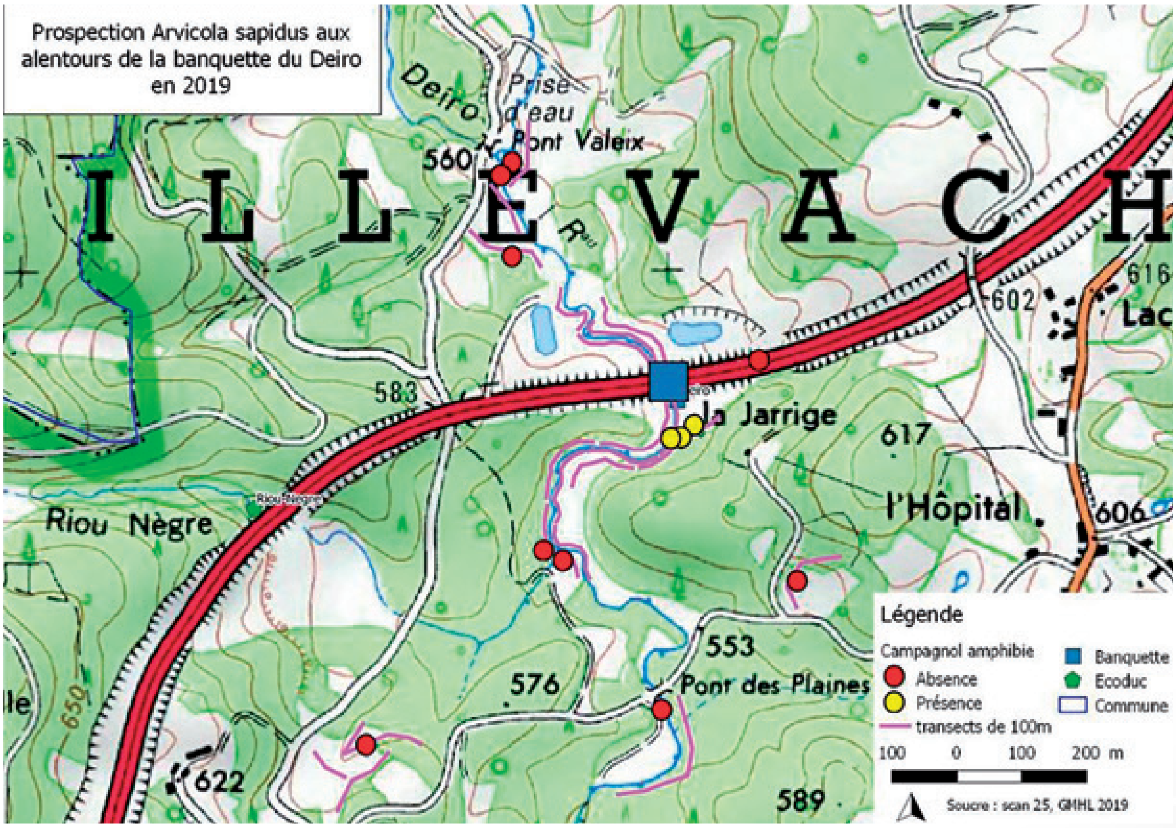
Photoset 1: Illustrations of the structure and its remodelling for small wildlife (photos taken during the construction phase). © GREGE
Up: View of the Deiro structure - Down: View of the wildlife footway and hollowed out "micromammals" section created and access to the downstream riverbank.

IV. INVENTORY OF THE SOUTHWESTERN WATER VOLE DOWNSTREAM AND UPSTREAM

The inventory of this rodent was based on systematic surveys to find specific indirect indicators of its presence (stools, trackways, feeding spots...).

The inventory was produced by creating a total of twenty 100-metre survey transects, spaced regularly downstream and upstream from the structure, surveyed twice a year in different seasons.

The 20 transects created downstream and upstream of the Deiro structure (Map 2) were surveyed on 15 and 17 April and on 30 July 2019. Stools and trackways were discovered in 3 locations during the second survey close to the wildlife footway, solely downstream from the structure (Map 2, Photoset 2).



Map 2: Location of survey of Southwestern Water Vole conducted by GMHL and results (detection or non-detection of indirect indicators). © GMHL

Photoset 2: Stools of Southwestern Water Vole and probable burrow found during surveying. © GMHL

V. MONITORING OF THE FREQUENTATION OF THE ADAPTED STRUCTURE BY SMALL FAUNA

V.1. EQUIPEMENT AND PROTOCOL

Since the primary objective was to evaluate the use of the pathway created for the Southwestern Water Vole, several techniques and experimental protocols were combined and adapted to the specific nature of the installation and the very small species to be counted.

The effectiveness indicator of the device is based on counting all passages (if necessary, evaluated by appropriate time period), with, if possible, identification of the species. When this is not possible, the count is done by group of species (see V.1.6), which still enables quantification of the use of the facility.

Given certain difficulties encountered during the monitoring, some operations were repeated, others were abandoned. Three techniques, described in the following paragraphs, were implemented in parallel in order to evaluate the frequentation of the Deiro structure and its facilities (Figure 1) and to identify the species. .

V.1.1. MONITORING BY FOOTPRINT TRACKERS

The proposed footprint trackers were specifically developed to ensure optimal impression of micromammal prints. They are made of felt soaked with a specific ink located in the centre, surrounded on both sides by sheets of paper previously soaked with revealing fluid and dried. Thus, the prints of any animal passing over the tracker, in one direction or the other, are instantly and indelibly shown on the revealing sheets.

In the framework of this monitoring, trackers were placed along the entire width of the various possible pathways, namely the wildlife footway, the "micromammal" pathway and the strip of sediment at the foot of the footway (Photoset 3; Figure 2).

The initial protocol provided for one-year monitoring with three one-month sessions (one per quarter) during which the trackers were checked every ten days, in order to guarantee an exhaustive count of the passages. Indeed, for the footprint trackers set up in the hydraulic structures, their effectiveness, which depends on the drying of the inked felt was evaluated by GREGE during the development phases of the protocol (Maurie, 2013; GREGE - unpublished data). Given the large surface area of the tracker (0.06 m²) which limits evaporation observed in the tunnels (Ferrand, 2019), its installation near water and the prevailing hygrometry in the structures, the effectiveness of this type of tracker is considered optimal for ten days, the survey interval for the monitoring sessions. The counts were considered to be exhaustive.

Between these standardised sessions monitored between 2018 and 2021, monthly checks take place for which the counts are not exhaustive, as the felt may become ineffective. However, they allow for the detection of species that frequent the structures more occasionally.

The first monitoring operation was conducted from May 24, 2018, but as the rhythm of monitoring was not completely respected during the winter of 2018-2019, complementary monitoring was initiated from December 2019 to April 2020, in order to have three sessions of exhaustive monitoring (Figure 1).

At each survey, all footprint tracker sheets were retrieved and replaced, then sent to GREGE for footprint analysis.

Analysis of the sheets collected in this way allowed us to determine the number of passages (i.e., the number of different tracks in each direction) and to assign each track and its corresponding footprints to a species or group of species.

The identifications are based on a specific key for inkprint trackers developed by GREGE (Maurie, 2013). This key distinguishes the prints of all small wild or domestic carnivores (except the weasel (*Mustela nivalis*) from the stoat (*Mustela erminea*), due to the lack of reference stoat prints allowing to find differentiation criteria with the weasel), and also other mammal species, such as the Red Squirrel, dormice, the Coypu (*Myocastor coypus*) or the European Hedgehog (*Erinaceus europaeus*). On the other hand, for micromammals other than dormice, in the absence of exhaustive reference prints of the species, the key has not yet been finalised and only two groups have been identified according to the size of the prints: "small micromammals" corresponding to small murids, small voles and shrews, and "large micromammals" corresponding to large murids *Rattus sp.* and large voles *Arvicola sp.* In addition, amphibian and reptile passages can also be identified. The identification error rate for the four GREGE employees, all categories combined, is 1.8%.

For medium-sized species (from weasels to dogs), the number of passages is counted precisely. For micromammals, the counting method depends on the abundance of footprints: if the tracks are quite distinct, the total number of passages is counted precisely; on the other hand, if the tracks overlap, the footprints are counted on the first ten centimetres bordering the felt, and then this number is divided by the average number of footprints left by a micromammal over ten centimetres. This parameter was evaluated from experimental boards collected in recent years from various structure-monitoring studies.

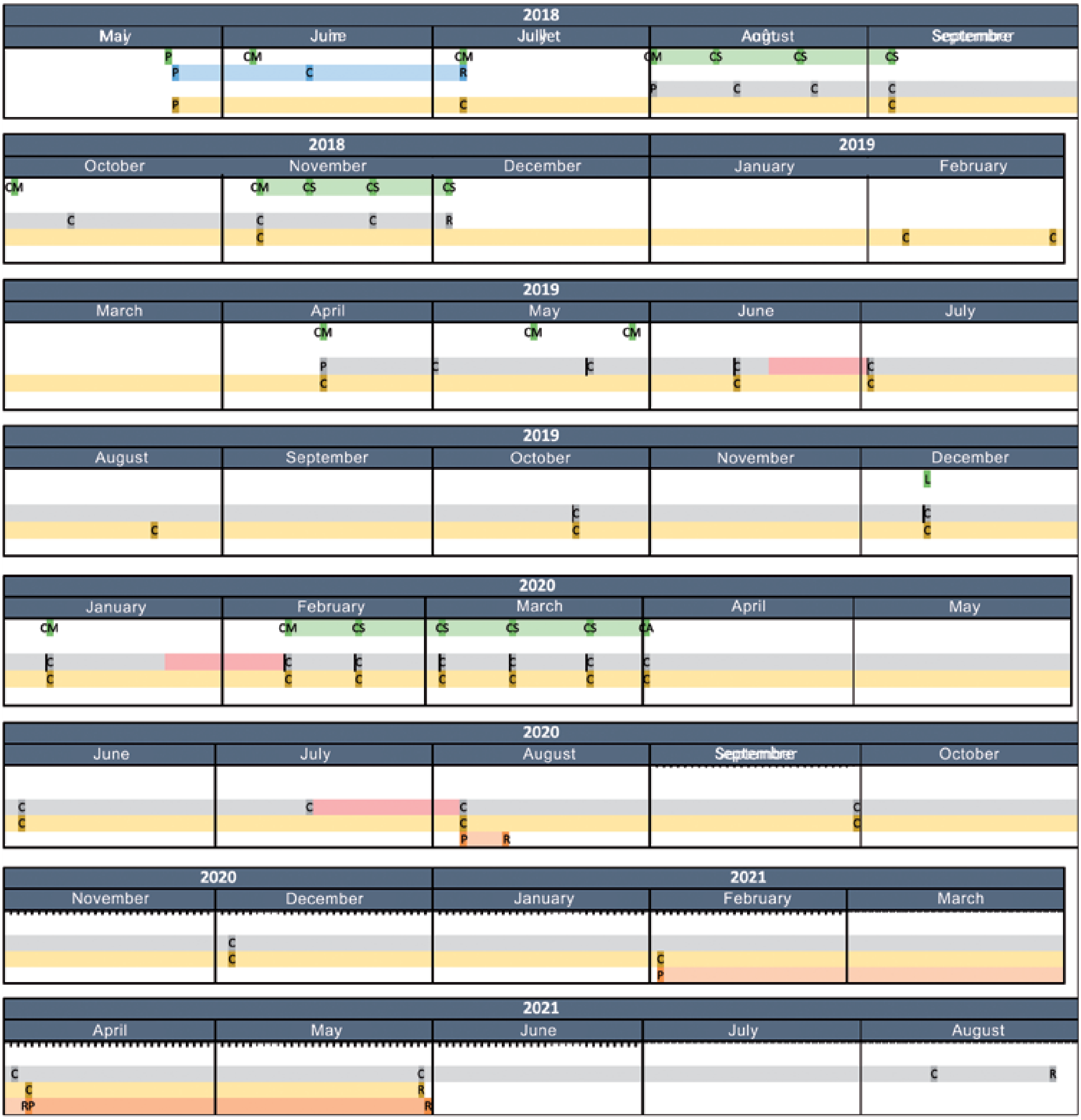


Figure 1: The various monitoring operations in the Deiro structure between May 2018 and August 2021.

Monitoring by footprint trackers: P = Set-up and bringing to operation; L = launch of monitoring phase 2; CM = monthly check; CS = session check; CA = session check and stop monitoring.

Monitoring by camera traps in the riverbed of frequentation of the hollowed pathway: P = Set-up and bringing to operation; C = verification; R = removal.

Camera trap monitoring of the wildlife footway and the hollowed "micromammals" pathway: P = setup; C = check.

Monitoring of frequentation by small micromammals using faeces and fur tubes: P = placing; R = removal.

Camera trap malfunction.

ILLUSTRATIONS OF THE THREE TRACKERS USED TO MONITOR THE FREQUENTATION OF THE DEIRO STRUCTURE

Photoset 3: Illustrations of three trackers used for monitoring frequentation of the Deiro structure. © GREGE
1 - Footprint trackers to monitor frequentation of wildlife footway © GREGE
2 - Footprint tracker set up in "small mammal" hollowed pathway © GREGE
3 - Footprint tracker set up in the riverbed to highlight potential movements (photo taken on 24/05/2018) © GREGE
4 - View of camera trap aimed at hollowed pathway and camera trap in the background aimed at the wildlife footway © GREGE
5 - View of the strip of sediment formed due to the presence of spurs created during remodelling (photo taken on 30/7/2019) © GMHL

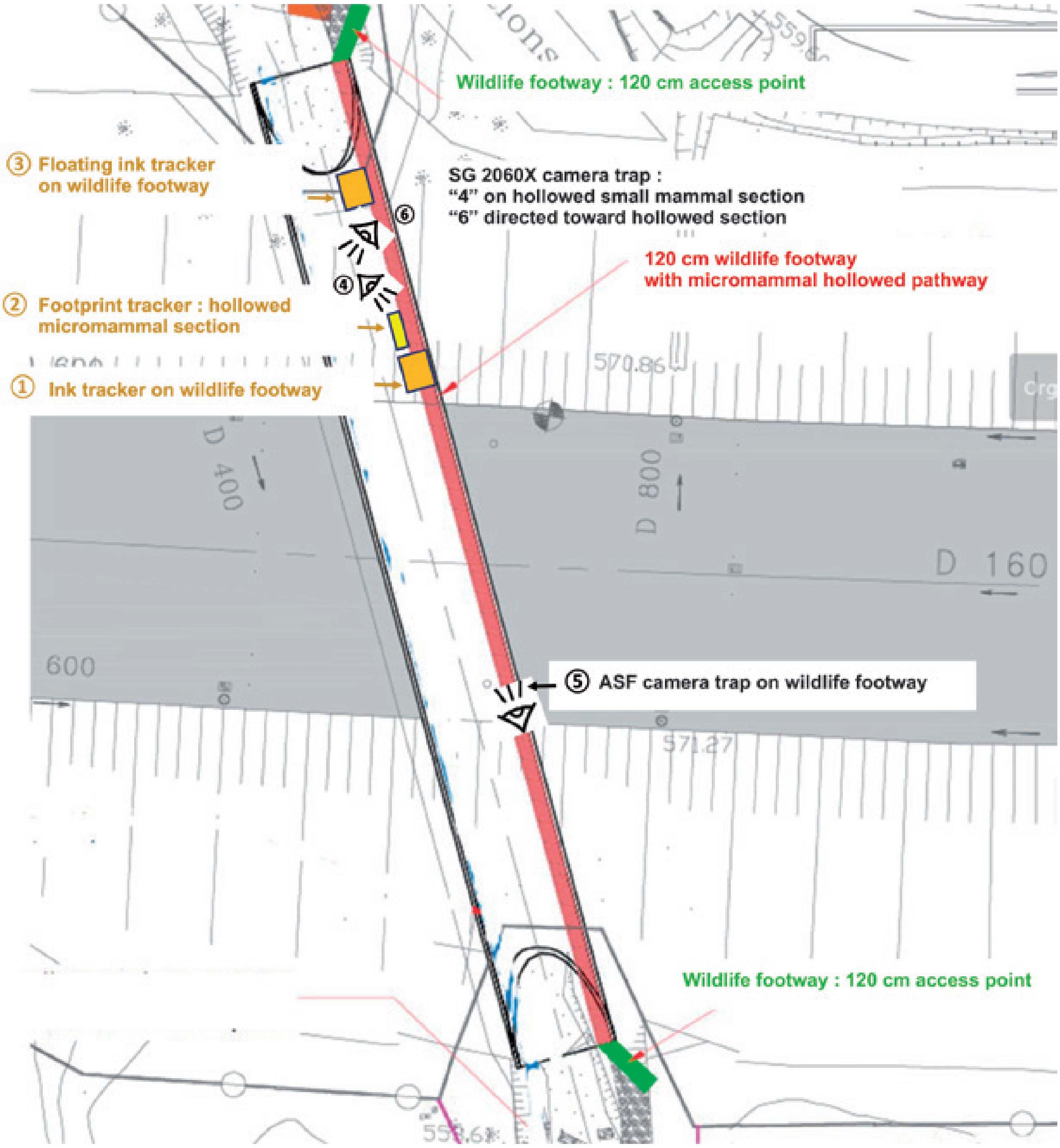


Figure 2: Diagram of monitoring systems on the plans of the Deiro structure

V.1.2. CAMERA TRAP MONITORING

Different types of camera traps were set up for the duration of the monitoring.

The first was carried out between 25 May and 5 July 2018 (41 nights), using a **ScoutGuard SG2060-X**, and monitored frequentation from one of the spurs in the riverbed (device installed 50 centimetres above the spur and directed at 45 ° in relation to the movement axis along the bottom of the footway - Figure 2).

The second was set up between 25 May 2018 and 30 May 2020, using a **Reconyx Hyperfire 2** attached high up, pointing towards the wildlife footway to evaluate its frequentation (Figure 2). The device was fixed by GMHL to the support installed during the construction phase based on ASF recommendations and adjustments provided by GMHL (2019, 2020, 2021), in accordance with the monitoring protocol requested by ASF (ASF, 2017). Beyond assessing the frequentation of the structure, the data collected were intended to compare the results obtained with those of the footprint tracker. Periods of premature interruption of the device between checks were deducted from the total number of nights monitored.

Finally, the "micromammal" hollowed pathway was also equipped with a dedicated camera trap (ScoutGuard SG2060-X) in addition to the footprint tracker in order to identify the micromammal species frequenting it (Figure 2). The camera was chosen because of its high sensitivity towards micromammals, its triggering speed for the first shot (0.8 seconds) combined with a black incandescent flash. This flash is less detectable than a normal white incandescent flash and freezes the shots of these species very quickly, allowing better identification of the species, in particular compared to Reconyx brand cameras which generate a movement blur (GREGE - unpublished data). The camera was fixed against the hollowed pathway on a swivel with a view at less than 45° with respect to the path.

Several periods were thus monitored: from 1 August to 3 December 2018 (124 nights), from 15 April to 17 June 2019 (63 nights), 2 July 2019 to 23 January 2020 (205 nights), 10 February to 14 July 2020 (155 nights), 05 August 2020 to 28 August 2021 (388 nights). The data presented are therefore based, in total, on 935 nights of monitoring. Moreover, the orientation of this camera trap revealed part of the spurs and strips of sediment at the foot of the footway, enabling non-exhaustive monitoring of their frequentation.

V.1.3. MONITORING BY FUR AND FAECES TUBES

On the basis of the data from the first phase of monitoring, tubes to collect indicators were set

Photoset 4: Illustration of two baited trackers used. © GREGE
Left : Faeces tube / Right : Fur tubes

up during the period of greatest frequentation of the "micromammal" hollowed pathway by the genus Neomys in order to shed genetic light on this inventory, inside the structure and in its surroundings. The indicators collected were firstly prepared by the GREGE and then the DNA of the host species was genetically identified, in close collaboration with the GeCoLAB conservation genetics laboratory of the University of Liege (Johan Michaux's team).

Identification is achieved using new generation sequencing methods and bioinformatics tools. These "metabarcoding" techniques are based on the high-throughput amplification and sequencing of short, highly variable fragments of the cytochrome oxidase 1, CO1, gene. The DNA sequences obtained after amplification are then compared to the public sequences of the BOLD database, as well as to the sequences of the private database of the GeCoLAB laboratory, which has developed the reference sequences of all the species of micromammals present in Metropolitan France, some of which are not present in the public databases. The final interpretation is carried by GREGE, jointly with GeCoLAB.

In 2020, two types of baited trackers were used (Photoset 4): faeces tubes, the bottom of which is covered with small pebbles to retain the droppings that the animal would deposit there, and fur tubes, which are equipped with adhesive plates that collect fur as the animal passes.

These tubes are set up inside the structure on each pathway level in the upstream and downstream thirds, and outside the structure, upstream and downstream about 15 meters from the structure, in the vegetation closest to the water way.

A total of four faeces tubes and eight fur tubes were placed outside the structure, and six faeces tubes and twelve fur tubes were placed inside the structure. These tubes set up installed on August 5, 2020, and retrieved on August 11, 2020, after six consecutive nights of monitoring.

In 2021, only faeces collection tubes were placed in the structure to try to identify Neomys, with as little time as possible to be spent on the operation by combining it with the camera-trap monitoring. Thus in 2021, between two and five tubes were placed in the structure from February 2 to April 4, 2021 and then from April 4, 2021 to May 31, 2021.

V.1.4. COLLECTION OF POTENTIAL INDICATORS ON THE FOOTWAYS

In addition to the direct gathering of micromammal indicators using tracker tubes, which required a frequentation of the structures by the individuals, a collection of environmental DNA (eDNA) was carried out on the areas where the individuals circulated, i.e. the micromammal hollowed section and the wildlife footway. The objective was to try to identify shrews of the genus *Neomys* and field mice of the genus *Apodemus*, identified by the camera traps, and, if necessary, voles of the genus *Arvicola*.

In this case, the collection of this eDNA was based mainly on the collection of faecal matter potentially left by individuals during their movements. Thus, each footway was meticulously inspected, and a representative sample of the stool or faecal matter was collected along the entire length of the footway, whether it was suspected to belong to mammals (micromammals or bats), reptiles or amphibians or invertebrates. The samples collected were grouped, for genetic analysis, into two independent pools according to their origin "wildlife footway" and "hollow section" in order to evaluate the specific difference between the two paths.

In addition, as an experiment, once this collection operation was completed, sand and dust present in the hollow section were collected by scanning in order to analyse the presence of detectable DNA without visual identification of faecal matter remains.

Once the samples were collected, they were preserved in 96° alcohol and then underwent the same analysis techniques as the faecal tube samples (see § V.1.3).

V.1.5. COMPARISON OF DATA FROM FOOTPRINT TRACKERS AND CAMERA TRAPS

This comparison, carried out for the two structures (wildlife footway and "micromammal" hollow section), was carried out on the basis of data from periods during which these two techniques were simultaneously functional.

Thus, the comparison of data between the two techniques was carried out on four independent data sets and three comparisons to take into account the optimal effectiveness of the footprint trackers, which is limited to between ten and twelve days.

- The first two datasets are based on the results obtained during standardised sessions. During these one-month long sessions in which the footprint trackers are checked every ten to twelve days, their effectiveness is evaluated and with few exceptions, it is ensured over the period allowing a comparison of the results over the 30 days of joint tracker and camera trap monitoring. The datasets from each footprint tracker monitoring were compared to the compilation of the camera trap results over the same periods.
- A second dataset was developed from the results obtained outside of the standardised sessions, from the monthly monitoring. For these monthly surveys, as the effectiveness of the tracker can only be guaranteed over ten to twelve days due to its drying out, the results collected on the footprint trackers were in this data set, compared to the results of the camera traps from the first ten days of the same period.
- Next, a third dataset was constructed with the camera trap results recorded between the eleventh and thirtieth day of the monthly footprint tracker survey period. These data were then analysed one by one and compared with the data from the tracker to shed light on and verify discrepancies in the monthly results between the trackers and the camera traps (for example, a species detected by the camera trap around the twenty-fifth day has very little chance of being detected on the footprint tracker).

In the case of the camera traps, the observations dated one day at the limit of the periods are divided between the two periods concerned: those between midnight and 11:59 am are attributed to the first period, and those between 12:00 and 11:59 pm, to the second. Five of the passages were concerned by this procedure.

The data used for this comparison thus concern 341 nights of monitoring.

Period	Type of comparison	
	“ 10 days ” period	“ 20 days ”
24 May to 5 June 2018	X	
5 to 15 June 2018	X	
15 June to 5 July 2018		X
5 to 15 July 2018	X	
15 July to 1st August 2018		X
1st to 10 August 2018	X	
10 to 22 August 2018	X	
22 August to 4 September 2018	X	
4 to 14 September 2018	X	
14 September to 2 October 2018		X
2 to 12 October 2018	X	
12 October to 6 November 2018		X
6 to 13 November 2018	X	
13 to 22 November 2018	X	
22 November to 3 December 2018	X	
15 to 25 April 2019	X	
25 April to 15 May 2019		X
15 to 25 May 2019	X	
25 to 29 May 2019		X
10 to 20 December 2019	X	
20 December 2019 to 7 January 2020		X
7 to 17 January 2020	X	
17 January to 10 February 2020		X
10 February to 20 February 2020	X	
20 February to 3rd March 2020	X	
3rd to 13 March 2020	X	
13 to 24 March 2020	X	
24 March to 1st April 2020	X	
Total number of periods	**20**	**8**

Allocation of periods and types of comparisons made according to the dates of footprint tracker checking.
The “10-day” comparison data gathered 10 to 12 days following the checking date (=period of optimum effectiveness of footprint trackers), while the “20-day” comparison includes just the data gathered after this time frame for the camera traps.

V.1.6. NAMES USED FOR SPECIES AND GROUPS

In function of the techniques used, some identifications go as far as the species while others stop at the genus, species-group or order. All the results are presented in this report with the denomination correspond objectively to the reality of the identification.

In the case of species with the same genus name which cannot be distinguished from the species, the name is presented as "genus name + sp." (e.g., Wood Mouse (*Apodemus sylvaticus*) and Collared Field Mouse (*Apodemus flavicollis*): the identification was noted as *Apodemus sp*).

In the case of groups of species not belonging to the same genus, a group name has been created either on the basis of the identifiable characteristics of the group (e.g. the group of "small voles" including *Microtus sp*, *Myodes* ...), or on the basis of the family (e.g. "*Soricidae*" grouping all the shrews that cannot be distinguished separately), or on the basis of the order (e.g. "*Anura*" grouping all frogs and toads).

GREGE name	Common name	Scientific name
Rattus sp.	Black Rat	*Rattus rattus*
	Brown Rat	*Rattus norvegicus*
Martes sp.	Beech Marten	*Martes foina*
	Pine Marten	*Martes martes*
Polecat/Mink	European Polecat	*Mustela putorius*
	American Mink	*Mustela vison*
Arvicola sp.	Southwestern Water Vole	*Arvicola sapidus*
	European Water Vole	*Arvicola terrestris*
Apodemus sp.	Wood Mouse	*Apodemus sylvaticus*
	Collared Field Mouse	*Apodemus flavicollis*
Small voles	Short-tailed Field Vole	*Microtus agrestis*
	Common Vole	*Microtus arvalis*
	Bank Vole	*Myodes glareolus = Clethrionomys glareolus*
Large micromammal	*Arvicola sp.* grouping of voles in the *Arvicola* genus	
	Black Rat	*Rattus rattus*
	Brown Rat	*Rattus norvegicus*
Small micromammal	Grouping all the Soricidae (shrews) and small rodents. Excluding *Arvicola sp*, *Rattus sp.* and dormice (European Edible, Garden and Hazel Dormouse)	
Neomys sp.	Eurasian Water Shrew	*Neomys fodiens*
	Iberian Water Shrew	*Neomys anomalus*
Soricidés	Grouping all the shrew family	
Anoura	(Frogs, toads)	
Urodela	(Salamanders, newts)	

Table II: Names used for species-groups and species concerned

V.2. RESULTS

V.2.1. EFFECTIVENESS OF THE "SMALL MAMMAL" HOLLOW PATHWAY

V.2.1.1. General data

Out of a total of 350 nights monitored between 24 May 2018 and 1 April 2020, 188 passages were counted using a footprint tracker: 185 passages were attributed to small mammals and three to Urodela species.

Over the entire camera trap monitoring period of 935 nights, 1330 passages were observed in this hollowed pathway, and five species or groups of species were detected (Figure 3). These passages include 69.3% (923 passages) of *Apodemus sp.* and 27.12% (361 passages) of *Neomys sp.* In addition, red squirrels passed on eight occasions, small voles were detected on five occasions, and **an individual of the genus Arvicola sp. was observed in the hollowed pathway on November 29, 2019. (Photoset 5)**.

Monthly analysis by species group shows strong monthly variations with peaks in spring and summer and an increase in use until 2020, particularly for the field mouse group (Figure 4).

On the other hand, for the group of aquatic shrews of the genus *Neomys sp.*, the frequentation was very early, even almost exclusively, shortly after the end of the construction work. As for field mice, the peak of passages occurred in August 2018 and 2019, and the number of recorded visits decreased over the years (Figure 5), with only sporadic visits in 2020 and 2021.

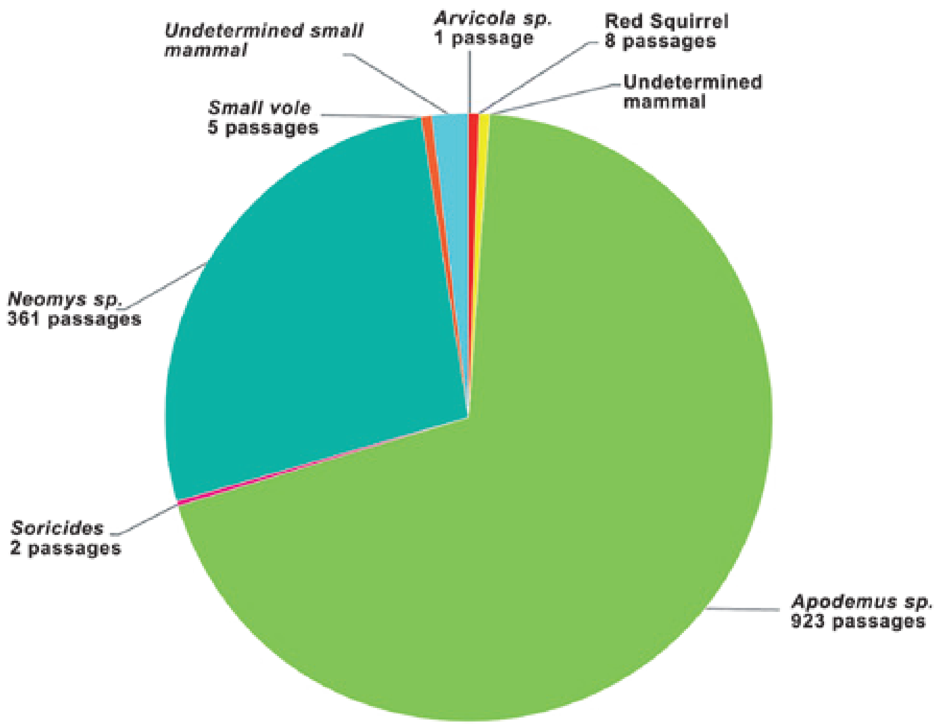
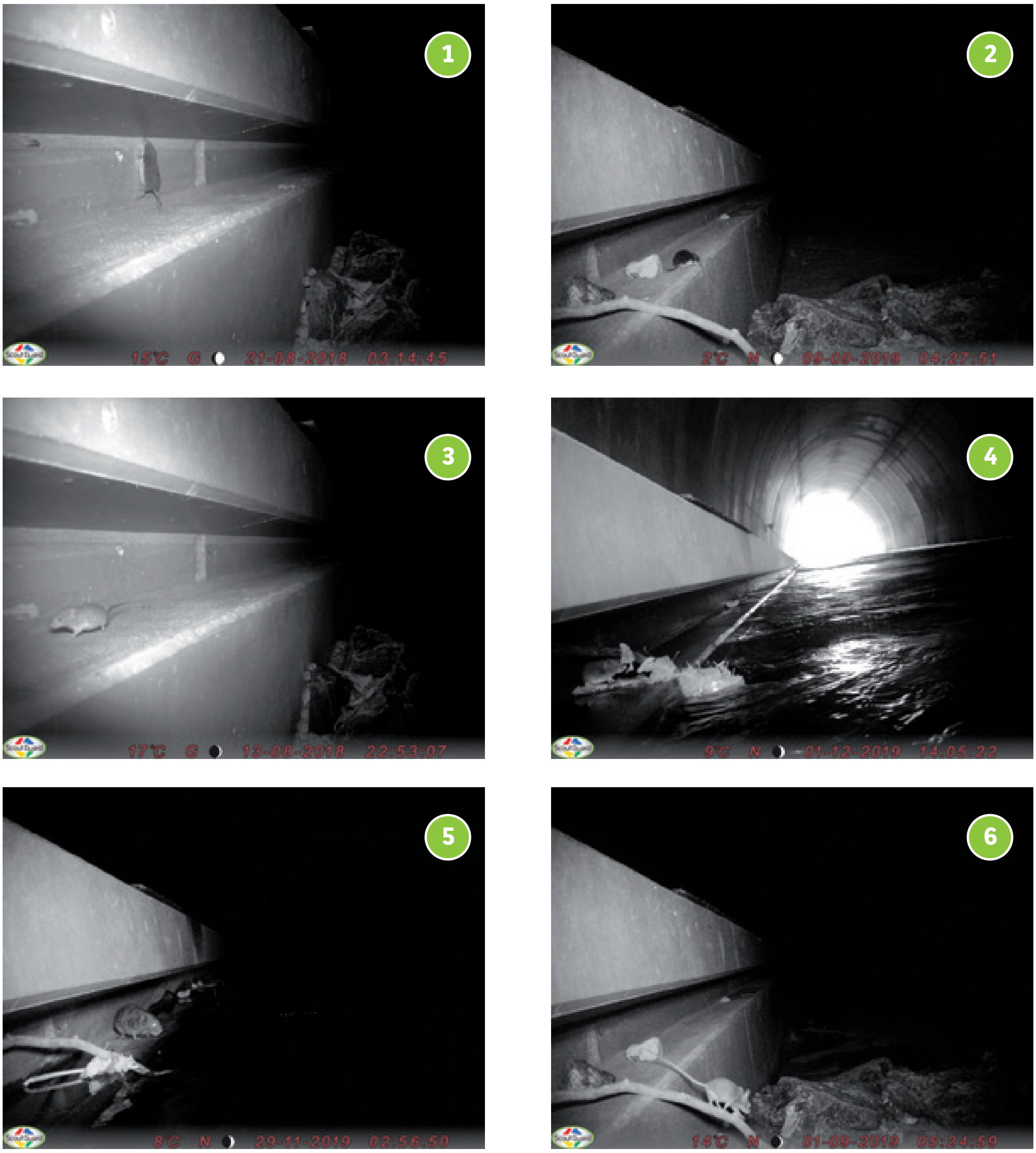


Figure 3: Distribution of the 1331 passages detected in the "micromammal" hollow pathway by camera trap over the 935 nights of monitoring.

ILLUSTRATIONS OF THE FOUR SPECIES GROUPS DETECTED BY CAMERA TRAP IN THE "MICROMAMMAL" HOLLOWED PATHWAY.



Photoset 5 :
1 - Neomys sp. *hunting © GREGE*
2 - Neomys sp. *© GREGE*
3 - Small vole in the "small mammal" hollowed pathway © GREGE
4 - Small vole in the "small mammal" hollowed pathway a flood of the Deiro © GREGE
5 - Arvicola sp. *in the "small mammal" hollowed pathway a flood of the Deiro © GREGE*
6 - Apodemus sp. *using a piece of wood as a footbridge to pass from the hollowed pathway to the strip of sediment at the foot of the footway © GREGE*

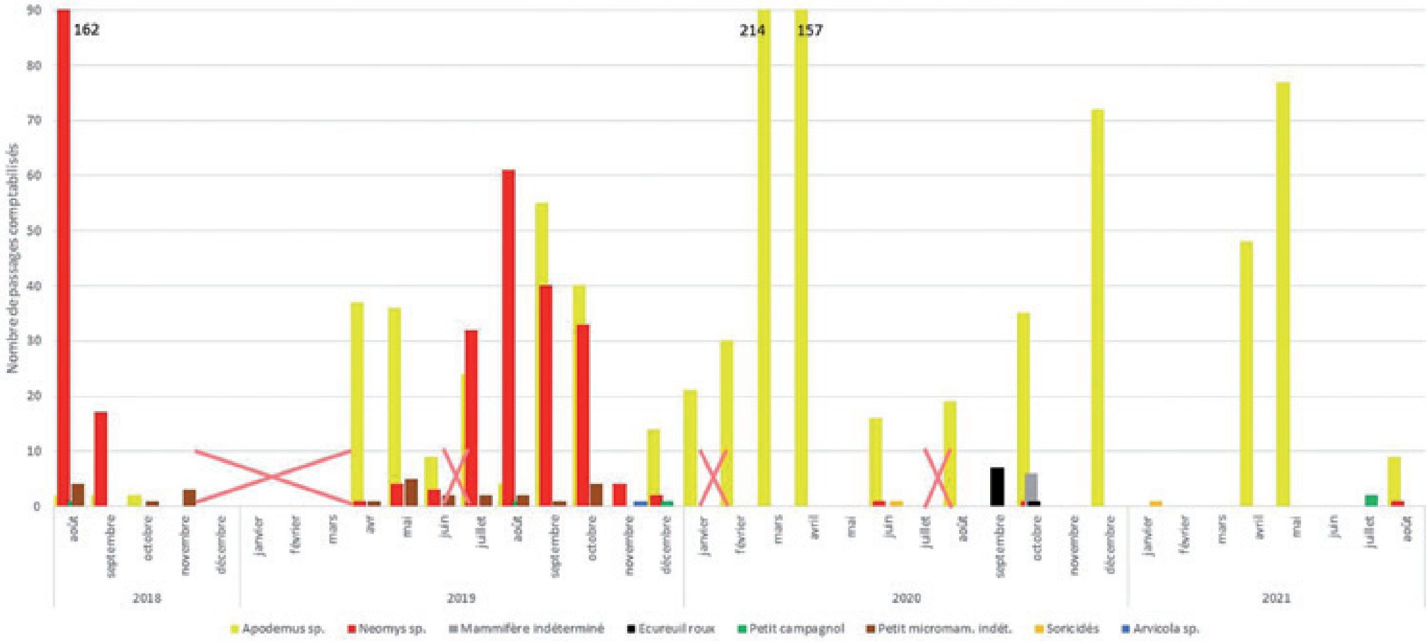


Figure 4 : Evolution of the frequentation of the "small mammal" hollowed pathway from passages detected by camera trap.
The camera trap (ScoutGuard SG2060-X) was not operating between 18 June and 1 July 2019, between 24 January and 9 February 2020 and between 15 July 2020 and 4 August 2020, i.e., a total of 862 nights of monitoring. Moreover, monitoring was interrupted between 3 December 2018 and 14 April 2019.

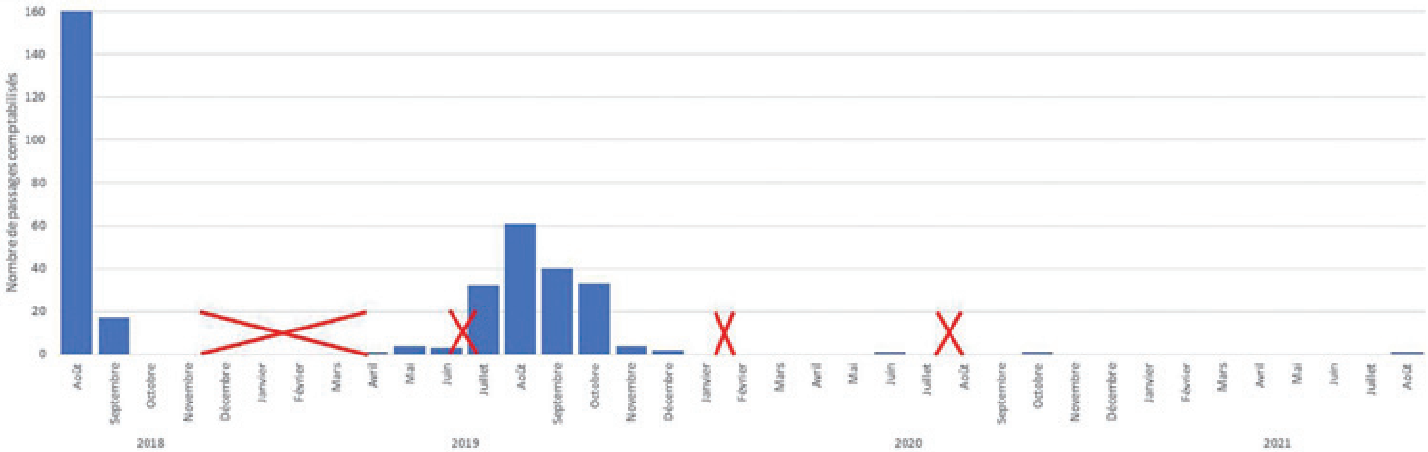


Figure 5 : Evolution of the frequentation of the "small mammal" hollowed pathway by water shrews Neomys sp. from passages detected by camera trap.
The camera trap (ScoutGuard SG2060-X) was not operating between 18 June and 1 July 2019, between 24 January and 9 February 2020 and between 15 July 2020 and 4 August 2020, i.e., a total of 862 nights of monitoring. Moreover, monitoring was interrupted between 3 December 2018 and 14 April 2019.

V.2.1.2. Comparison of methods

Over the 341 nights of simultaneous monitoring, 172 passages were recorded using footprint trackers compared to 540 by camera trap (Table III).

While the identification key of the footprints does not currently enable precise distinction of species of small mammals, the camera trap allows the identification of the species at least up to the genus, and has revealed the presence of three species over the period considered (Table III). On the other hand, the *Urodela* species detected by the tracker during the monthly monitoring was not observed on the pictures viewed.

The data show that small mammals were detected overall three times more by camera traps than by footprint trackers, showing good effectiveness of the devices.

The difference noted with footprint trackers can be explained based on two main factors:

1. From April 15, 2019, a branch located between the apparatus and the footprint tracker, is visible on the photographs (Photoset 5). It can be seen that, in most cases, this branch was used as a footbridge by the micromammals to move from the hollowed pathway to the continuous strip of sediment until the natural bank, and vice versa. Thus, the footprint tracker was avoided, distorting the detection.
1. The effectiveness of this tracker with a very small surface area is reduced to a few days (around 48 hours in dry atmosphere (Ferrand, 2019)). Finer analysis of the differences during the summer season (August and September 2018) confirms the same trend with 2.2 times more passages on the camera trap than on the footprint tracker. In wetter periods, the differences certainly diminish due to natural rewetting by river spray.

In the case of the "small mammal" hollowed pathway of the Deiro structure, the selected camera trap (ScoutGuard 2060X) with its specificities already tested by the GREGE on other monitoring operations and its being set up as close as possible to the hollowed pathway, is appropriate for monitoring this adapted structure, since it makes it possible to determine more precisely the species (at least the genus) because of the absence of motion blur.

Any other equipment, including the Reconyx HP2X, rightly considered as particularly high-performance, does not seem to be suitable because of either detection bands that hinder the illumination in the hollowed pathway, or because of a lack of sensitivity and excessively slow triggering speed, and above all because of a motion blur that would not allow the identification to be made.

Therefore, in the event that the monitoring is renewed, the same device should be used, and if necessary, the HP2X by Reconyx should be simultaneously tested.

Species	Data monitoring "session"		"Monthly" monitoring data		
	Footprint tracker	Camera trap	Footprint tracker	Camera trap " 10 first days "	Camera trap " 20 last days "
Urodela sp.	-	-	1	-	-
Field mice	-	246	-	58	40
Neomys sp.	-	177	-	2	4
Small voles	-	1	-	-	-
Undetermined small micromammals	73	6	98	3	3
TOTAL	73	430	99	63	47

Table III : Number of passages detected by the "small mammal" hollowed pathway, by species and depending on monitoring protocol. For monthly monitoring, comparable data between footprint trackers and camera traps are obtained exclusively over the first ten days.

V.2.1.3. Genetic identification

In terms of attempts to genetically identify species moving through the structure, the faeces tubes placed in the hollowed area of the pathway yielded about ten droppings. Three pools were formed by grouping the faeces according to the date of the survey and the collection route. These pools only revealed the presence of the Wood Mouse (*Apodemus sylvaticus*) in the hollowed area. In addition, the complementary search for indicators directly on the footways to try to distinguish between Neomys species enabled the constitution of four samples of faeces and sand/dust. The analyses revealed the presence of the Eurasian Water Shrew (*Neomys fodiens*) in the hollowed area.

V.2.1.4. Effectiveness of the hollowed area of the pathway

To date, very little data exists in France on the levels of frequentation of small wildlife tunnels by small mammals. In order to assess the overall effectiveness of the hollowed section, the results of this study were compared to the orders of magnitude reported on various small fauna structures by the two 2016 publications of the VINCI Autoroutes group (Fagart et al. 2016; Vinci Autoroutes et al. 2016) and by our own data from the monitoring of nearly 150 structures on behalf of LISEA on the Bordeaux - Tours high-speed trainline (GREGE et al. 2020).

The order of frequentation of the paths, even of the structures is most often lower than about twenty passages of small mammals per month:

- 34 to 206 passages per year over 43 lower structures monitored by Vinci Autoroutes *et al.* 2016
- 85% of the structures with have than 20 passages per month and a maximum of 116 monthly passages recorded on one structure out of 153 structures monitored on behalf of LISEA (GREGE 2016, 2017, 2018, 2019, 2020)..

In the case of the Deiro, the total number of small mammal passages recorded in 2019 and 2020 was 419 and 581 respectively, for a monthly average of 36 passages per month. These results are significantly higher than the references consulted, confirming a high frequency of micromammals visiting the adapted structure. This monitoring demonstrates the effectiveness of the hollow section for this species. Moreover, the numerous passages attributed to the genus *Neomys sp.* are particularly remarkable (no other reference on the subject to our knowledge, either in terms of frequentation or even monitoring) and show the interest of this adaptation for this group of protected species, even if the genetic identifications have only revealed the presence of the Eurasian Water Shrew (*Neomys fodiens*).

For the Southwestern Water Vole, the passage attributed to the genus Arvicola sp. detected in the hollowed section at the time of a flood of the Deiro confirms, by analogy, that the adaptation also works for this, initially targeted species, and above all, that it fully plays its role in maintaining ecological continuities in high water periods (Photoset 5).

V.2.2. MONITORING OF SEDIMENT STRIPS

The camera trap set up to monitor frequentation at the level of one of the spurs in the riverbed at the beginning of the monitoring, then the width of field of the camera targeting the "micromammal" hollowed pathway, as well as the footprint tracker installed at the foot of the fauna pathway, made it possible to detect a minimal number of passages having taken place in the strips of sediment along the footway (Table IV).

It should be specified that the strips of sediment were strengthened during the three years of monitoring through the action of the specifically installed spurs. Thus, a path at the foot of the footway was progressively created and reinforced with increasing drying over the months by widening the spaces located between the spurs.

As such, 74 passages, attributed to seven species or groups of species were detected by the footprint tracker, against 298 passages and twelve species for the camera traps (Photosets 6 & 8).

A total of thirteen species were recorded roaming on this strip of sediment (Table IV): the two amphibian groups, two domestic carnivores, a Coypu, four small carnivores, including the Stoat and the European Otter (*Lutra lutra*), the Red Squirrel, two small micromammals (including the genus Neomys sp.) and two large micromammals (including the genus *Arvicola sp.*)

Planche-photos 6 : Otter detected by the camera trap targeting one of the spurs in the riverbed. © GREGE

These data show that the proportion of passages on this strip of sediment is significant compared to the use of the two pathways, particularly for amphibians or terrestrial voles, since six passages of individuals of the genus Arvicola sp. were observed there.
Thus, the spurs played a full role in creating a “natural” pathway at the foot of the footway that could be used for a good part of the year, although this effectiveness was not monitored during this study. Then, in case of higher water level or flooding, the hollowed section played its major role in maintaining the continuity of the pathways in the structure.

Species	“Spur” camera trap	“Pathway” camera trap	Footprint tracker
Toad	-	1	-
Undetermined Anura	-	-	4
Undetermined Urodela	-	-	1
Domestic cat	-	5	2
Genet/Cat	-	-	1
Dog	-	2	-
Canid	-	1	-
Coypu	1	14	-
European Otter	9	18	-
Otter / Coypu	3	-	-
Stoat	-	2	-
Pine Marten	-	3	-
Martes sp.	-	1	-
Polecat/Mink	-	-	2
Red Squirrel	-	2	2
Arvicola sp.	-	6	-
Rattus sp.	-	7	-
Undetermined large micromammals	-	-	2
Apodemus sp.	-	190	-
Neomys sp.	-	18	-
Soricidé	-	5	-
Undetermined small micromammals	-	6	56
Undetermined mammals	-	4	-
Undetermined species	-	-	4
TOTAL	**13**	**285**	**74**

Tableau IV: Total number of passages detected, by species, during the overall monitoring period for all material providing data on frequentation of strips of sediment at the foot of the wildlife pathway.

V.2.3. MONITORING OF THE WILDLIFE FOOTWAY

V.2.3.1. General data

Out of a total of 350 nights monitored between 24 May 2018 and 1 April 2020, 137 passages were recorded on the wildlife footway using a footprint tracker, divided between 9 species or groups of species (Figure 6). 49% of the passages correspond to domestic carnivores and 6% to mustelids. Surprisingly, no "large micromammal" footprints were detected on the wildlife footway.

Over the entire camera trap monitoring, i.e., 443 nights, 70 passages were observed on the wildlife footway, attributed to six carnivore species (Figure 7), among which domestic carnivores represent 91.4% of the passages. An exceptional datum is to be noted: a Raccoon was detected at the beginning of the night on June 20, 2019. .

Finally, of the two faecal tubes and four fur tubes placed on the wildlife footway, no faeces or fur were captured during the single set-up carried out by GMHL. This disappointing result is not consistent with the capture rates usually obtained by GREGE on the structures. It can be explained by the small number of devices set up (adaptation of the protocols to share the operational input with the camera traps) and the low number of repetitions.

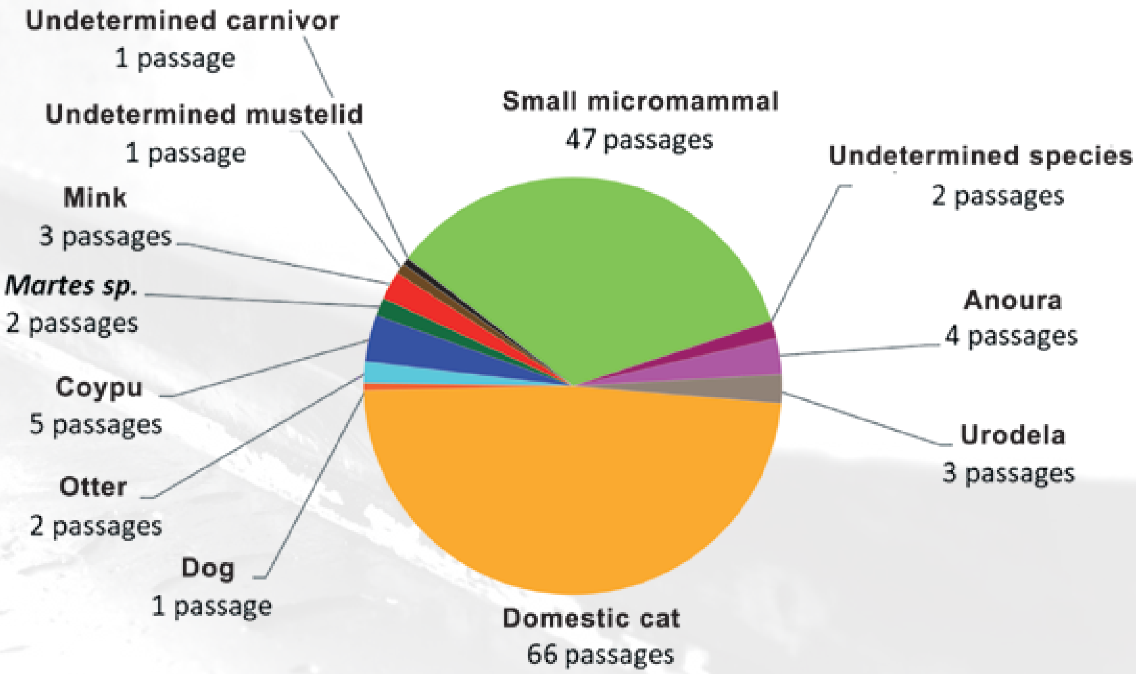


Figure 6 : Distribution of the 137 passages recorded on the wildlife footway using footprint trackers over 350 nights of monitoring.

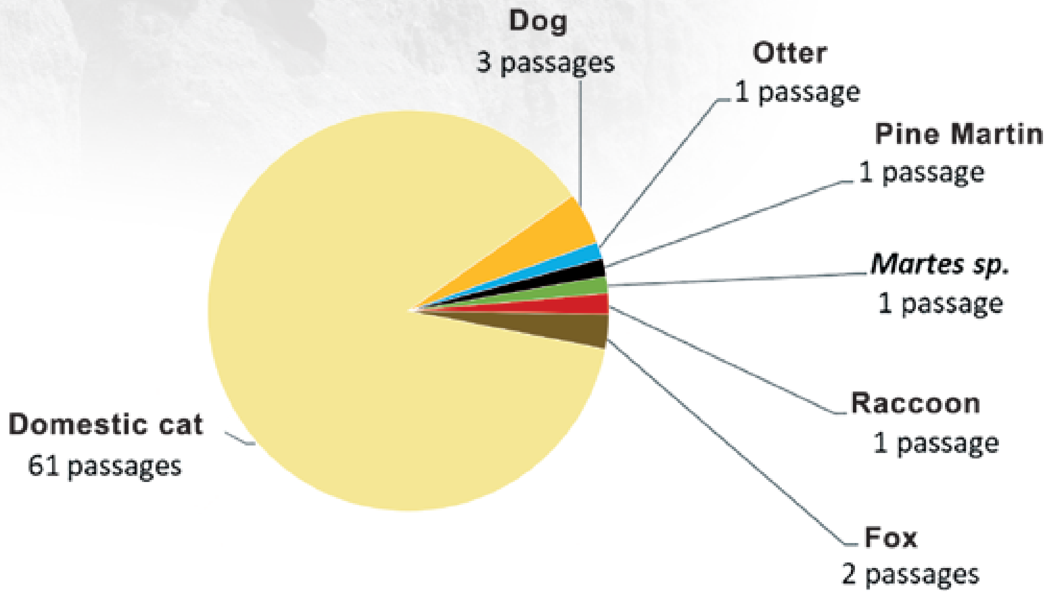


Figure 7 : Distribution of the 70 passages recorded on the wildlife footway using a camera trap over 443 monitoring nights.

V.2.3.2. Comparison of methods

Over the 341 nights simultaneously monitored, 119 passages were recorded using footprint trackers, against 27 by camera traps. (Table V). In addition, the number of species detected is 3 times higher with a footprint tracker than with a camera trap.

The Reconyx Hyperfire 2 is considered a good camera trap compared to other existing models on the market but, in the case of the monitoring the wildlife footway, it was placed at a height of about two meters and in the axis of the wildlife footway, which poses a number of detection problems, as shown in the comparative data above.

At this distance, it is not possible to detect either micromammals or amphibians, whereas the footprint tracker revealed the passage of these two groups on this structure.

For small carnivores, the experiments conducted by GREGE on many structures and with various models of camera traps show the difficulty of achieving exhaustive detection. Even with Reconyx models, non-detections vary between 25 and 50% and are not constant over time for a given device and pathway monitored, making assessments complicated (GREGE - unpublished data to date). This is a result of the size of the species, the insufficient sensitivity of the devices and their detection zones which, as for the Reconyx, do not cover the entire visual field of the device.

Thus, the installation height of the camera trap in the Deiro structure is too high compared to that used by GREGE or usually recommended by many authors. In addition, axial photography with Reconyx is also known to generate significant failures due to the detection mode.

Finally, anecdotally, some of the different results stem from some individuals leaving or accessing the footway while crossing, and thus not passing within the field of view of the camera trap, such as a fox detected in 2018 coming down from the footway (Photoset 7).

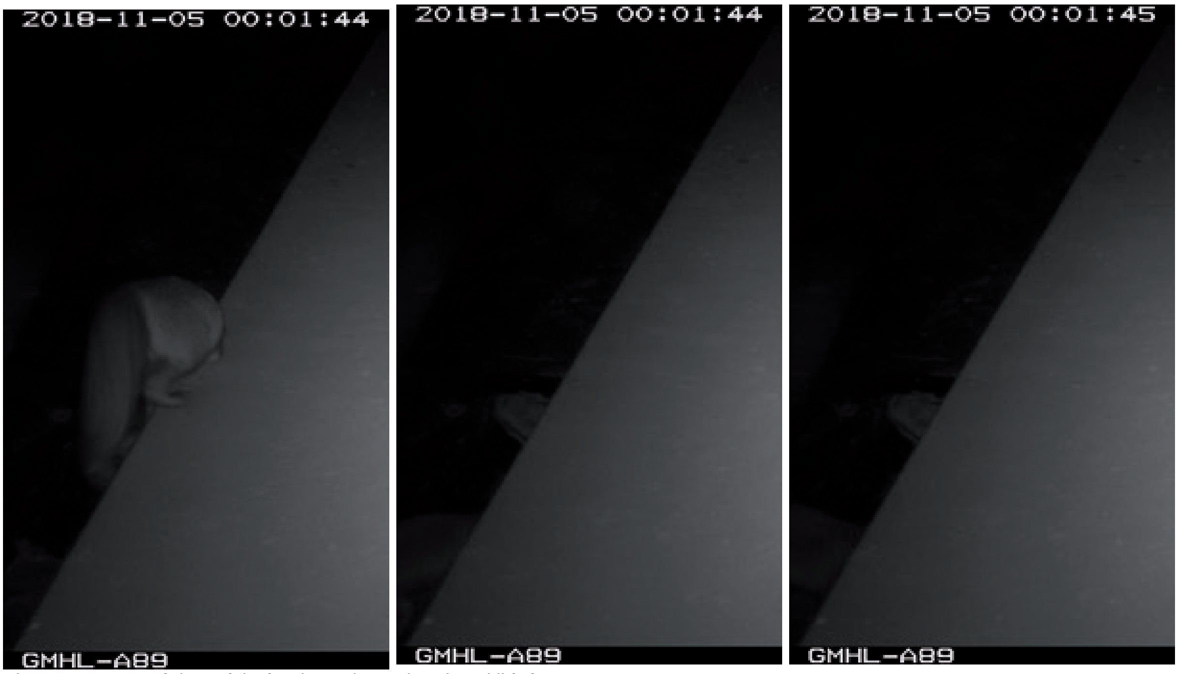
In the case of the wildlife footway of the Deiro structure, the camera trap as it was placed, quite high and in the axis, significantly underestimates the level of frequentation of the pathway and the diversity of species.

Several corrective solutions appear necessary:

- Lower the camera trap to about 50 centimetres off the ground and shift its orientation, then retest the correlation between the techniques.
- Test the Reconyx HP2X equipped with an external battery-mounted cell, which has shown its effectiveness in joint tests conducted by the GREGE and the LPO. Note that the number of checks will have to be increased to change the battery..

Species	**"Session" monitoring data**		**"Monthly" monitoring data**		
	Footprint tracker	Camera trap	Footprint tracker	Camera trap "first 10 days"	Camera trap "last 20 days"
Anura sp.	3	-	1	-	-
Urodela sp.	-15	-	3	-	-
Cat	16	8	40	9	7
Dog	-	-	1	1	2
Otter	1	1	1	-	-
Coypu	-	-	5	-	-
Martes sp.	1	-	1	-	-
Polecat	-	-	3	-	-
Undetermined mustelid	1	-	-	-	-
Undetermined carnivore	-	-	1	-	-
Undetermined small micromammal	5	-	34	-	-
Undetermined species	2	-	-	-	-
TOTAL	**29**	**9**	**90**	**10**	**8**

Tableau V: Number of passages detected per technique used over the wildlife footway, by species and by monitoring protocol. For monthly monitoring, comparable data between footprint trackers and camera traps were obtained over the first 10 days.



Photoset 7 : Series of shots of the fox shown descending the wildlife footway. © GMHL

V.2.3.3. Effectiveness of the wildlife footway

In addition to the problem of positioning the camera trap, which limited the detection of species, the frequentation highlighted throughout the monitoring demonstrates the effectiveness of the wildlife footway for semi-aquatic mammals (otter and polecat) and other mustelids, for other carnivores, such as the raccoon or fox, and also to a lesser extent for smaller species (amphibians and small micromammals).

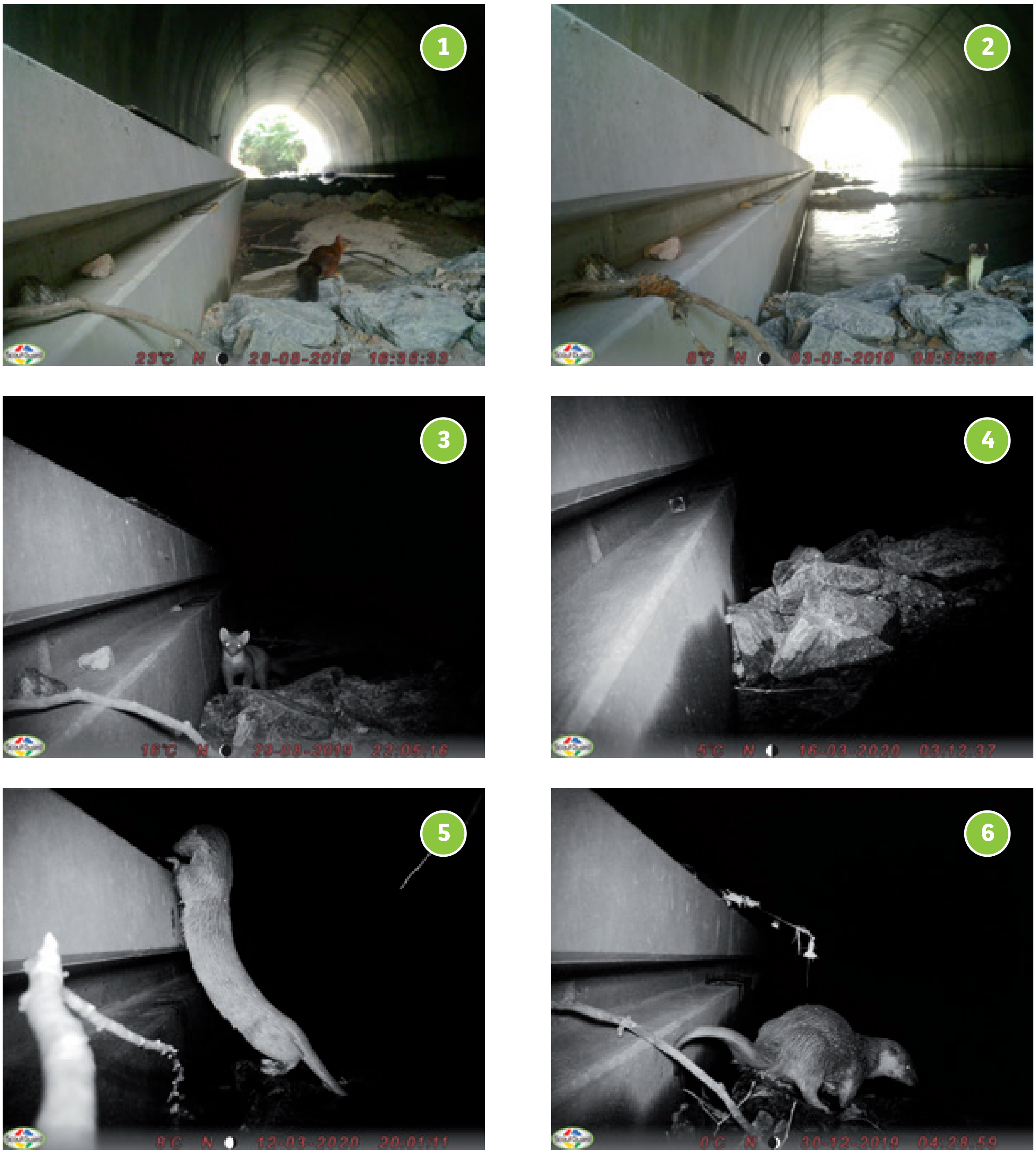
It should nonetheless be specified that the frequentation of this footway is entirely dependent on the possibilities of moving in the riverbed and that, for micromammals, the presence of the hollowed section effectively reduces the utilisation of the upper footway.

Simultaneous monitoring of the three pathways with appropriate camera traps and devices, set up together with a time lapse of eight hours to monitor the water levels would shed light of the ways the different species move around in function of the pathways present..

V.2.4. OTHER INFORMATION ON THE EFFECTIVENESS OF THE ADAPTATIONS

The camera trap targeting the hollowed section was used to observe flooding events and to estimate, without being exhaustive, the duration of certain periods of submersion of the hollowed section (Photoset 8). Several flooding episodes were thus observed from November 2019 to August 2021, during which the photos show water arriving just under the hollowed section, then partially or totally flooding it. In total, the "micromammals" hollowed section seems to have been flooded, or even totally underwater, for at least 33 days spread between November/December 2019 and August 2021, with between one and nine consecutive days per flooding event. The duration of non-effectiveness therefore remains very low.

ILLUSTRATIONS OF VARIOUS OBSERVATIONS MADE BY A CAMERA TRAP SET UP IN THE FRAMEWORK OF MONITORING THE “MICROMAMMALS” HOLLOWED SECTION. © GREGE/GMHL



Photoset 8 :

1 - Red Squirrel

2 - Stoat

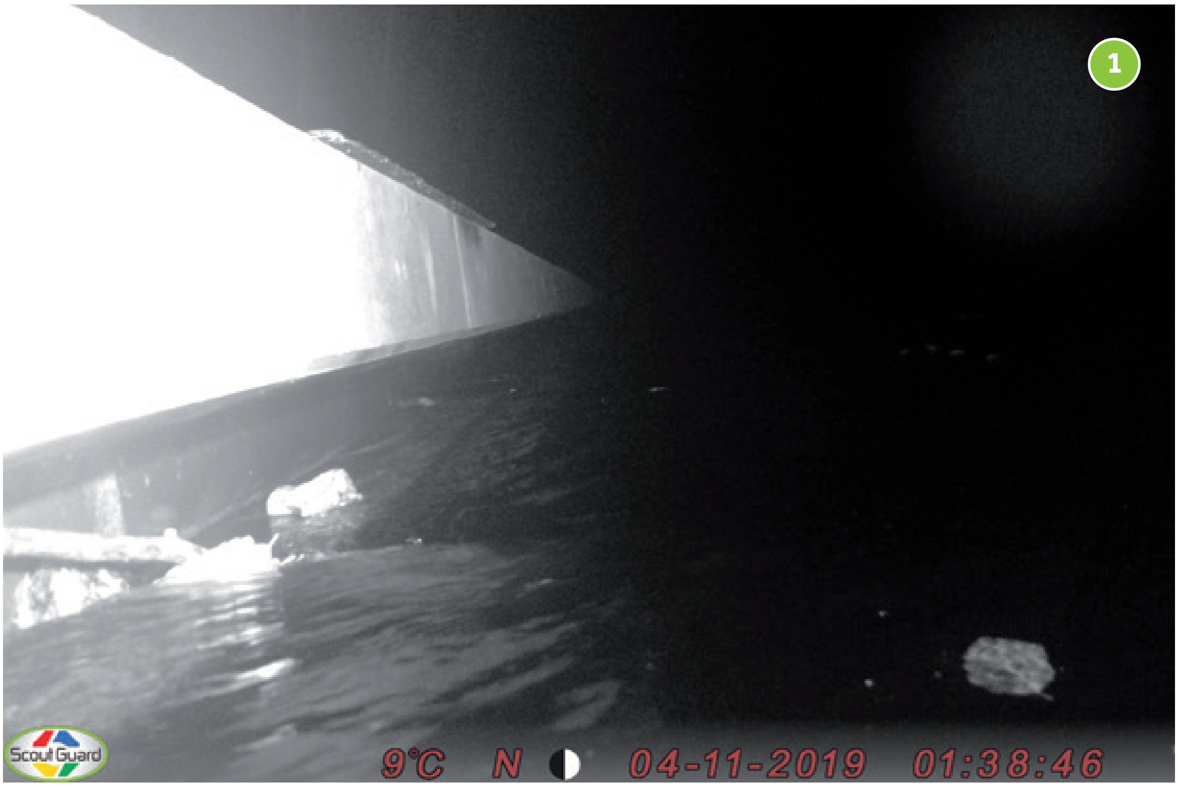
3 - Pine Marten

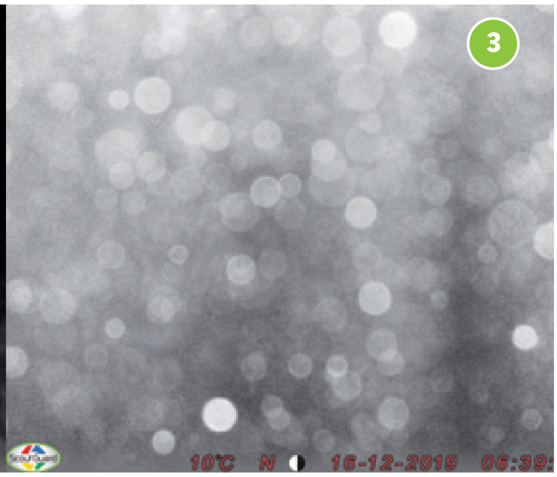
4 - Individual identified as Arvicola sp.*, that could be a Southwestern Water Vole.*

5 - Marking behaviour of the European Otter

6 - European Otter having a look at the wildlife footway

ILLUSTRATIONS OF VARIOUS OBSERVATIONS MADE BY A CAMERA TRAP SET UP IN THE FRAMEWORK OF MONITORING THE "MICROMAMMALS" HOLLOWED SECTION. © GREGE/GMHL.





1 - "Micromammals" hollowed section flooded © GREGE/GMHL
2 - "Micromammals" hollowed section totally underwater © GREGE/GMHL
3 - Camera trap underwater © GREGE/GMHL

VI. ASSESSMENT AND PERSPECTIVES

This study assessed the frequentation of the modified Deiro structure, and validated experimental protocols and monitoring techniques adapted to the particular features of the target species and the devices set up in the structure (footprint trackers, camera traps, genetic sampling).

The results exceeded expectation by being able to measure the levels of movement of these difficult-to-census species, and to genetically identify some of the high-stake species moving through the structure.

The level of frequentation of the structure thanks to the specific hollowed section, rarely equalled compared to other projects, and the range of species detected including heritage species, definitively demonstrates its effectiveness and interest for this group of species.

An exceptional number of passages of *Neomys sp.*, the group including the two aquatic shrews, both protected at national scale, was recorded, showing individuals hunting in the adapted structure. Manual collection of indicators in the hollowed section identified the Eurasian Water Shrew (*Neomys fodiens*), even if this approach requires considerable experience for spotting and selecting the matter to be sought and collected. Indeed, the searches carried out by non-specialists gave the impression than no there were no indicators present in the structure.

In the second monitoring phase in 2020, an individual of the genus *Arvicola sp.* was detected in the hollowed section during a period of flooding, at a time when the sediment deposits, apparently preferentially used by this genus (six passages out of seven), was not available. This datum confirms that the adapted structure works well for large voles, and above all that it fully plays its role of maintaining ecological continuity for these species, especially in high-water periods.

Given the range of species passing through it, we propose to rename this hollowed section, which initially targeted the Southwestern Water Vole, "micromammals hollowed section".

In addition, more globally, this monitoring confirms the frequentation of the three levels of pathway available, i.e., the wildlife footway, the "micromammals" hollowed section and above all the sediment deposits at the foot of the footway. From a technical point of view, this requires systematic simultaneous monitoring of all the pathways through a structure because their interdependence significantly influences the results.

The high frequentation of the sediment deposits confirms that many species prefer to travel as close to the water as possible and confirms the interest of making sure that pathways are not too high when there are no sediment deposits above the water level in the riverbed. The setting up of spurs to recreate progressive meandering and a better flow in the river is a very interesting solution for small fauna by bolstering the deposits at the foot of the wildlife footway, which will be their main pathway when it is dry.

Finally, concerning all the monitoring of the Deiro structure, fifteen wild species or species-groups were detected: the two groups of amphibians, four small carnivores with two semi-aquatic mustelids including the European Otter, plus the exceptional datum of the Raccoon in June 2019, the Red Fox, the Coypu, the Red Squirrel and five micromammals, including the genus *Arvicola sp.* and *Neomys fodiens*, together with the genus *Rattus*.

To gauge the level of effectiveness of the Deiro structure, by comparing the results with the figures obtained for the 200 structures monitored in the framework of other projects we were able to create a reference base in terms of species diversity and frequentation rate by species-group or by species (GREGE et al., 2020 – see Annexe)..

In the Deiro structure, the global species diversity (mammals and amphibians) is judged as being quite good. However, the passage rates of small carnivores and large micromammals are judged as "very low", unlike those of small micromammals which are judged as "high" with 54 passages in 30 days on average during the first phase, and 97 passages during the second phase. Part of this good indicator certainly results from the exceptional level of frequentation by the genus *Neomys sp.* Highlighted in the hollowed section of this structure.

Given the results, it would seem to be interesting to repeat the simultaneous monitoring of the three potential pathways to better assess their relative effectiveness for micromammals, using camera traps that make best use of all known recommendations in the field. The revised protocol could be based on the setting up of:

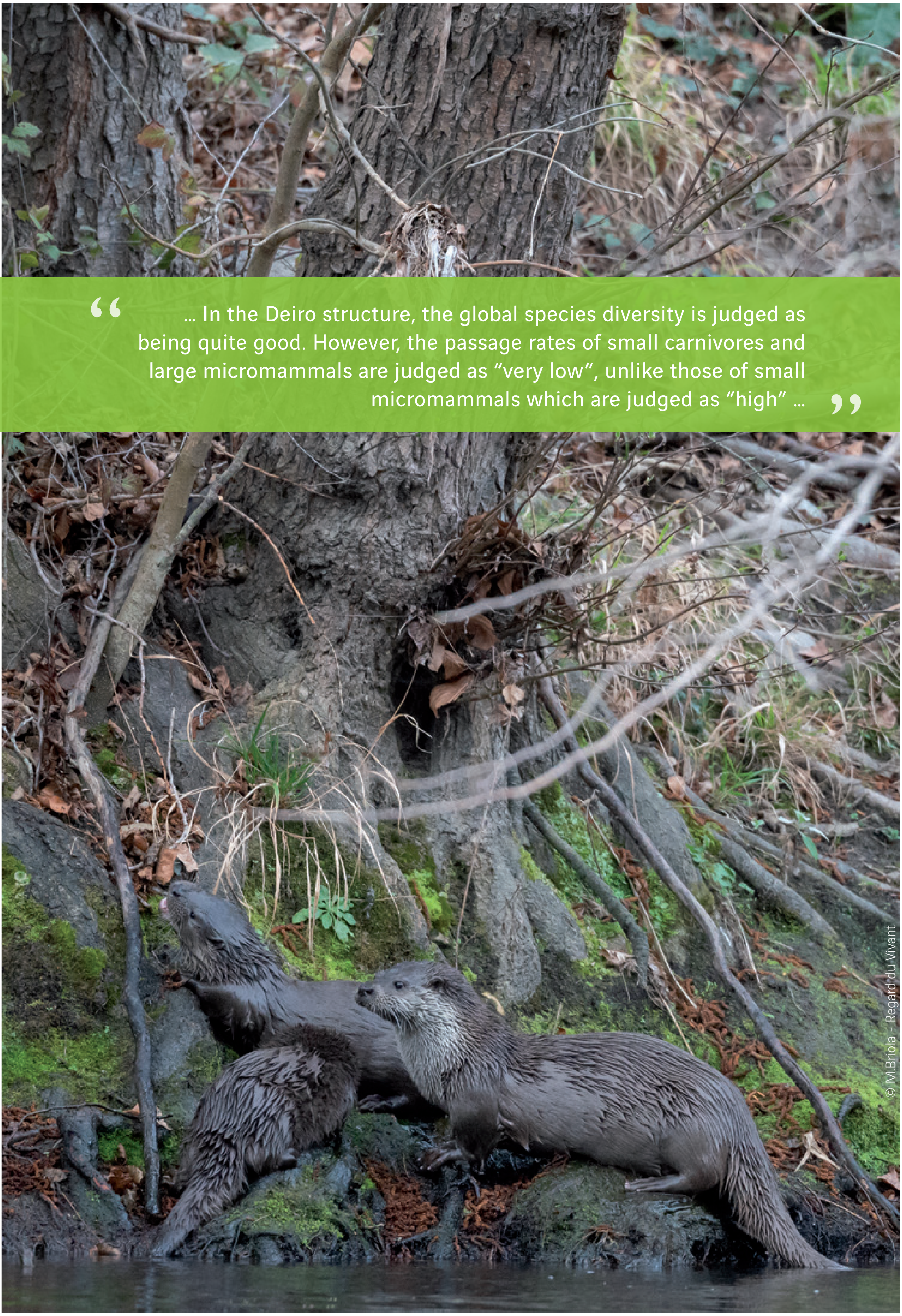
- A ScoutGuard 2060X-type camera trap to monitor the micromammals hollowed section with a time lapse of four to eight hours to monitor water levels.
- A Reconyx HP2X-type camera trap fixed 50 centimetres from the ground and oriented between 30 and 45° with respect to the axis of the footway; this trap will need a back-up battery if it is intended to ensure the exhaustivity of the micromammal count, in order to really compare this species-groups preferences in terms of pathway.
- A flood-resistant Reconyx HP2X or ScoutGuard 2060X-type camera trap fixed to a spur at 50-60 centimetres from the ground and oriented between 30 and 45° with respect to the axis of the footway.
- An equipment check every two months, reduced to one month if the detection cell is present.
- A search for and collection of all indicators of small mammals present on the three pathways for genetic identification of the species.

VII. ANNEXE

Indicator	Value or interval	Category attributed
Frequentation rate by small micromammals	0 passage	0
	]0 - 10[passages in 30 d	1
	[10 - 20[passages in 30 d	2
	[20 - 40[passages in 30 d	3
	≥40 passages in 30 d	4
Frequentation rate by large micromammals	0 passages	0
	]0 - 5[passages in 30 d	1
	[5 - 10[passages in 30 d	2
	[10 - 20[passages in 30 d	3
	≥20 passages in 30 d	4
Frequentation rate by small carnivores	0 passage	0
	]0 - 5[passages in 30 d	1
	[5 - 10[passages in 30 d	2
	[10 - 20[passages in 30 d	3
	≥20 passages in 30 d	4
Species diversity for "small carnivores"	0 species	0
	1 species	1
	2 species	2
	3 à 4 species	3
	5 à 7 species	4
Global species diversity (Mammals and amphibians)	0 species	0
	1 à 2 species	1
	3 à 4 species	2
	5 à 9 species	3
	10 à 20 species	4

List of the categories formed for each of the five indicators used to determine the effectiveness of structures (GREGE et al., 2020).

“… In the Deiro structure, the global species diversity is judged as being quite good. However, the passage rates of small carnivores and large micromammals are judged as “very low”, unlike those of small micromammals which are judged as “high” …”



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