Translocation strategy for *Prionotropis rhodanica*

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Adult male of *Prionotropis rhodanica* © Lisbeth Zechner

Action LIFE A.5 Analysis and translocation study for *Prionotropis rhodanica*

Saint-Martin-de-Crau

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Introduction

Declining farmland biodiversity and ecosystem degradation is observed throughout Europe, primarily caused by agricultural intensification (cf. European Court of Auditors, Special Report Biodiversity on farmland 13/2020). Arthropod communities have been shown to be greatly affected, with sharp and widespread declines in biomass, abundance, and diversity (Hallmann et al. 2017). Grasslands are particularly declining in Europe, while remaining patches are under pressure (van den Pol-van Dasselaar et al., 2019). In southern France, the "Coussoul" ecosystem (Natura 2000 habitat 6220*) is a unique dry grassland of the Crau plain (Figure 1) renowned for its biodiversity. Transhumant, extensive sheep grazing remains the dominant land use practice in the Crau, and greatly contributes to preserve the steppe character and its biodiversity. The Coussouls de Crau National Nature Reserve NNR (7,411 ha) is a strictly protected area. It is also part of the Natura 2000 network (SPA FR9310064 and SCI FR9301595). This nature reserve is known to maintain important populations of threatened birds, reptiles, and insects.

The Crau Plain Grasshopper (CPG) *Prionotropis rhodanica* (Figure 2) is endemic to this coussoul habitat. It is listed as Critically Endangered on the IUCN Red List of Threatened Species (Hochkirch and Tatin 2016), on the European Red List (Hochkirch et al., 2016) as well as on the French and Regional Red Lists (Sardet & Defaut 2004, Bence 2018). It is protected under French law.*P. rhodanica* was the first insect species for which a conservation strategy was developed following IUCN standards (Hochkirch et al., 2014). Research on population size and threats have been conducted in recent years, and an experimental breeding program was initiated by the Conservatoire d'espaces naturels Provence-Alpes-Côte d'Azur (CEN PACA). While the actions taken since 2015 have prevented further declines of the species and led to increases of one population, the conservation status of *P. rhodanica* remains alarming. Therefore, a LIFE project was started in 2021, to improve habitat management and breeding actions which should allow to start first reintroductions/translocations in 2024 and to prevent its extinction.

Figure 1. Dry grassland in the Crau plain © Lisbeth Zechner - CEN PACA.

LIFE SOS Crau Grasshopper

1. Context and LIFE project

1.1. Critically endangered Crau Plain Grasshopper

Four main threats to the Crau Plain Grasshopper have been identified:

1. Small population size

The small population size and population fragmentation of *P. rhodanica* makes it highly vulnerable to extinction risks associated with small populations, such as inbreeding depression, loss of genetic diversity, or demographic fluctuations. In the 1990s, the species was still recorded in most remaining steppe patches. However, an estimated loss of more than 90 % of the known distribution was recorded in the last 25 years. Currently, only three populations remain: Peau de Meau (6.5 ha), BMW testing centre (50 ha) and Calissane/Parc à ballons (220 ha).

2. Destruction and deterioration of steppe habitat

Crau dry grasslands have declined dramatically in the 20th Century: 75 % of coussoul areas have been converted into farmland and industrial areas. In the last 12 years, 1000 hectares have been lost outside the NNR (map B2d2). Habitat loss has led to increased fragmentation of the remaining populations, a major threat to the flightless *P. rhodanica*, which is unable to recolonise isolated patches. Changes within the remaining coussoul areas are understudied, but it has been suggested that changes in the sheep grazing regime may have caused the decline of the species in the remaining habitats (Bröder et al., 2019).

3. Climate change may have reduced steppe resilience to traditional grazing

40,000 sheep graze the coussoul from March to June. Extensive grazing is viewed as essential to the preservation of the steppe vegetation. *P. rhodanica* has coexisted with grazing flocks for hundreds of years. Only one of the remaining sites on which the species occurs (the BMW site) is not grazed. Available data does not suggest significant changes in grazing practices in the last 30 to 40 years. However, increasing temperatures also appear to impact plant communities. Increased mortality of dominant plant species is observed after extremely dry summers. Although not thoroughly documented, a progressive degradation of steppe vegetation driven by climate change may be occurring in the Crau, limiting its ability to recover from traditional grazing (CERPAM 2010).

4. Predation by gregarious insectivorous birds

P. rhodanica is a large species (adults 31-45 mm), and its life cycle starts much earlier than in other orthopterans: nymphs emerge already in early April. It therefore stands as a very conspicuous species for predators, available when other orthopterans have not yet emerged. *Bubulcus ibis* and *Corvus* spp. stay with sheep flocks and can be seen preying on flushed arthropods. They are therefore considered as efficient *P. rhodanica* predators (Bröder et al., 2023). Because the populations of *B. ibis*, *C. monedula*, *C. corone* and *C. frugileus* have strongly increased during the last 30 years, they potentially may have caused or amplified the decline of *P. rhodanica*. A field experiment showed that 94 % of predation events on large orthopterans were caused by birds (Bröder et al., 2023). Another known predator is *Falco naumanni*. Its numbers have artificially increased in the Crau thanks to conservation programs. Providing nestboxes facilitated its recovery from a one pair in 1985 to 230 pairs in 2020. *P. rhodanica* became extinct in the late 1990s in the southern part of the Crau where the main *F. naumannii* colonies have developed. During the implementation of the conservation strategy (Hochkirch et al., 2014), one site with occurrence of *P. rhodanica* (Peau de Meau) was partly excluded from sheep grazing since 2015 to maintain an adequate vegetation cover and height and avoid predation by flock-dwelling birds. Simultaneously, most of nearby nestboxes of *F. naumanni* were closed. *P. rhodanica* numbers increased following these actions, from 43 individuals in 2015, to 251 in 2019 (Bröder et al., 2019, 2020) but are still fluctuating.

Figure 2. Female of Crau Plain Grasshopper P. rhodanica © Lisbeth Zechner.

1.2. Objectives and actions of the LIFE project

The LIFE project **"SOS Crau Grasshopper" 2021 -2025** aims to improve the conservation status of the critically endangered *P. rhodanica*. The main long-term aim is to reinforce and reconnect remaining subpopulations by increasing population size and distribution area. It is articulate on 4 key objectives (related to different threats):

1. Increase favourable habitat by adaptive grazing management (threat 3),

2. Reduce predation by colonial insectivorous bird species (threat 4),

3. Improve breeding success in captivity and start reintroduction programme (threat 1),

4. Communicating, education and raising awareness among local stakeholders, the general public and institutions (threat 2).

The main objectives of the project as well as the planned actions are summarised on Table 1. A detailed description of the actions and expected results can be found in the "TECHNICAL APPLICATION FORMS Part C – detailed technical description of the proposed actions" of the LIFE-project SOS Crau Grasshopper (CEN 2020).

Table 1. Main objectives and project actions.

PROJECT OBJECTIVE	ACTION	ACTION CODE
1. **Increase favourable habitat by adaptive grazing management**	**Analysis of the links between grazing management, vegetation and habitat of *P. rhodanica***	A1
	Pre-study for the restoration and management of the Coussoul	A2
	Management of *P. rhodanica* habitat	C1
	Monitoring the impact of grazing on vegetation and *P. rhodanica* populations	D1
	Evaluation of socio-economic impact	D5
2. Reduce predation by colonial insectivorous bird species	**Pre-study of insectivorous predatory birds of *P. rhodanica***	A3
	Adaptive grazing management	B1
	Adaptation of breeding bird colonies	C2
	Monitoring of insectivorous bird species	D2
3. Improve breeding success in captivity and start reintroduction programme	**Preparation of the breeding programme for *P. rhodanica***	A4
	Strategy for the reintroduction of *P. rhodanica*	A5
	Breeding and reintroduction of *P. rhodanica*	C3
	Monitoring of breeding programme	D3
	Population monitoring	D4
4. **Communicating, education and raising awareness among local stakeholders, the general public and institutions**	**General project-related communication**	E1
	Creation of awareness-raising tools:	E2
	Awareness-raising of local populations	E3
	Training, exchanges, and dissemination of technical results	E4
5. Project management	**Project management**	F1
	External audit	F2
	After-LIFE plan	F3

1.3. LIFE project beneficiaries

1.3.a. Conservatoire d'espaces naturels CEN PACA

CEN PACA has been working on the conservation of *Prionotropis rhodanica* for more than 10 years and wishes to intensify its effort and extend partnerships to improve the conservation status of this endemic and highly endangered species. Further information: www.cen-paca.org

1.3.b. Chambre d'agriculture des Bouches-du-Rhône CA13

In the framework of the LIFE project, CA13 pilot actions A2 and D1. It will also participate in public relations actions, e. g. stakeholder communication concerning landowners, sheep farmers and herders. Further information: https://paca.chambres-agriculture.fr/la-chambre-dagriculture-des-bouches-du-rhone

1.3.c. La Barben zoo

Its proximity to the Crau plain (20 km as the crow flies) is a major asset for the LIFE SOS Crau Grasshopper project: great similarities in climate and biotope making it possible to try complete ex-situ breeding, and short transport distances for the transport of grasshoppers and egg pods. La Barben zoo also participates in LIFE public relations actions. Further information: https://www.parcanimalierlabarben.com

1.3.d. Besançon zoo

The Insectarium of Besançon zoo is one of the most complete and interesting in Europe, both for the general public and for scientists. Its extensive experience will contribute to improving ex-situ breeding in the framework of the LIFE SOS Crau Grasshopper project. In addition, the Besançon museum also participates in LIFE public relations actions. Further information: https://www.citadelle.com

2. Working team and workshop

The translocation study is led by CEN PACA, with the support of CA 13 and other institutions:

- Coordination and analyses: Lisbeth Zechner (CEN PACA), and Axel Hochkirch, (IUCN SSC Grasshopper Specialist Group),
- Data analysis: Catherine Godefroid, CEN PACA, Perrine Turiez, CA13 and Ghislaine Dusfour, CEN PACA,
- Support concerning breeding data of *P. rhodanica* and health criteria: Cathy Gibault (external assistance),
- Organisation of workshop: Lisbeth Zechner, CEN PACA,
- Additional cooperation: Scientific Advisory Board Coussouls de Crau NNR, National Council for the Protection of Nature CNPN, IUCN SSP Grasshopper Specialist group and French public authorities (DDPP, DREAL, DTTM),
- Data and proofreading Coussouls de Crau NNR: Axel Wolff and Cynthia Gidoin, CEN PACA.

This document was elaborated based on current knowledge on the Crau Plain Grasshopper and publications (ex. Foucart, 1995) as well as on the outcomes of the first conservation strategy, developed in 2014 (Hochkirch et al., 2014) which were analysed in 2020 (CEN PACA 2020). Preliminary results of the breeding programme within the LIFE project were also considered. Finally, the entirety of this data and accompanying documents were shared with a panel of 10 experts (Table 2) during a workshop held on 20 et 21 March 2023 in Saint-Martin-de-Crau with the aim of drafting the translocation strategy.

Table 2. Participants of the expert workshop in March 2023.

Participants	20-mars	21-mars	Absent
Antoine Foucart, CIRAD/UMR CBGP	x	x	
Axel Hochkirch, MNHN Luxembourg and Trier University	x	x	
Axel Wolff, CEN PACA	x		
Cathy Gibault, coordinator of CPG breeding programme	x	x	
Eric Sardet, Insecta	x	x	
Laurent Tatin, independent expert		x	
Lisbeth Zechner, CEN PACA	x	x	
Stéphane Bence, CEN PACA		x	
Tony Sainsbury, Zoological Society of London (per zoom)	x	x	
Yoan Braud, Entomia (per zoom)	x		
Linda Bröder, Trier University,			x
Paul Pearce-Kelly, Senior Curator of Invertebrates and Fish at Zoological Society of London.			x

Translocation strategy

1. Objectives

The main objective of translocation is to increase the number of subpopulations and improve the conservation status of the species.
In LIFE action C3 (breeding translocation), expected results are defined as follows:

- Obtain a yearly total of 80 to 150 egg pods across all ex-situ breeding sites.
- Transfer 80 egg pods annually, laid in captivity, to in-situ aviaries (n=2) for in-situ incubation.
- Attain at least 150 nymphs hatchings per year.
- Translocate 80 to 100 individuals to each of the 2 or 3 reintroduction sites.
- by Achieve a 10% expansion in range throughout the duration of the LIFE project.

This document (LIFE action A5) serves as the basis for the selection of translocation sites and the implementation of the translocation.

2. Basic biological knowledge and habitat

2.1. Population distribution and size

The historically documented distribution of *P. rhodanica* is illustrated in Figure 3. The map cumulates observations made by biologists since the 1990's. While the species was still recorded in most remaining steppe patches in the 1990's, an estimated loss of more than 90 % of its known distribution has been recorded over the last 25 - 30 years.

Figure 3. Historically known (green) and current (red) geographical range of *P. rhodanica*. Information regarding the year of observation and period of disappearance is also provided (see legend).

The systematic mapping of Crau Plain Grasshopper in the remaining Coussoul areas started in 2012 with the aim of improving knowledge of the species' distribution. 90 % of the original Crau stone steppe was surveyed, covering 364 out of the 406 existing sampling circles. However, the mapping approach proved to be time consuming, hindered by the low detection probability of the species, and as a result, has not been continued since 2015 (Tatin, 2017).

The mapping project had confirmed the presence of subpopulations at four sites. Among these, two (Calissane, Grand carton/Couloubris) were known for several years while the Peau de Meau population was newly discovered during the mapping initiative (Figure 4). At the BMW site, two geographically distinct subpopulations were known in the north-west and in the centre. However, access to the BMW site is contingent upon BMW authorization and the access to the central area is currently prohibited (CEN PACA, 2020). The Couloubris subpopulation has not been confirmed since 2012.

Presently, only three subpopulations of the species persist: at Peau de Meau (PdM) within the National Nature Reserve NNR, on the military site and CEN PACA property Calissane (CAL; 175 ha), partly protected by the NNR, and at the private BMW test centre (50 ha).

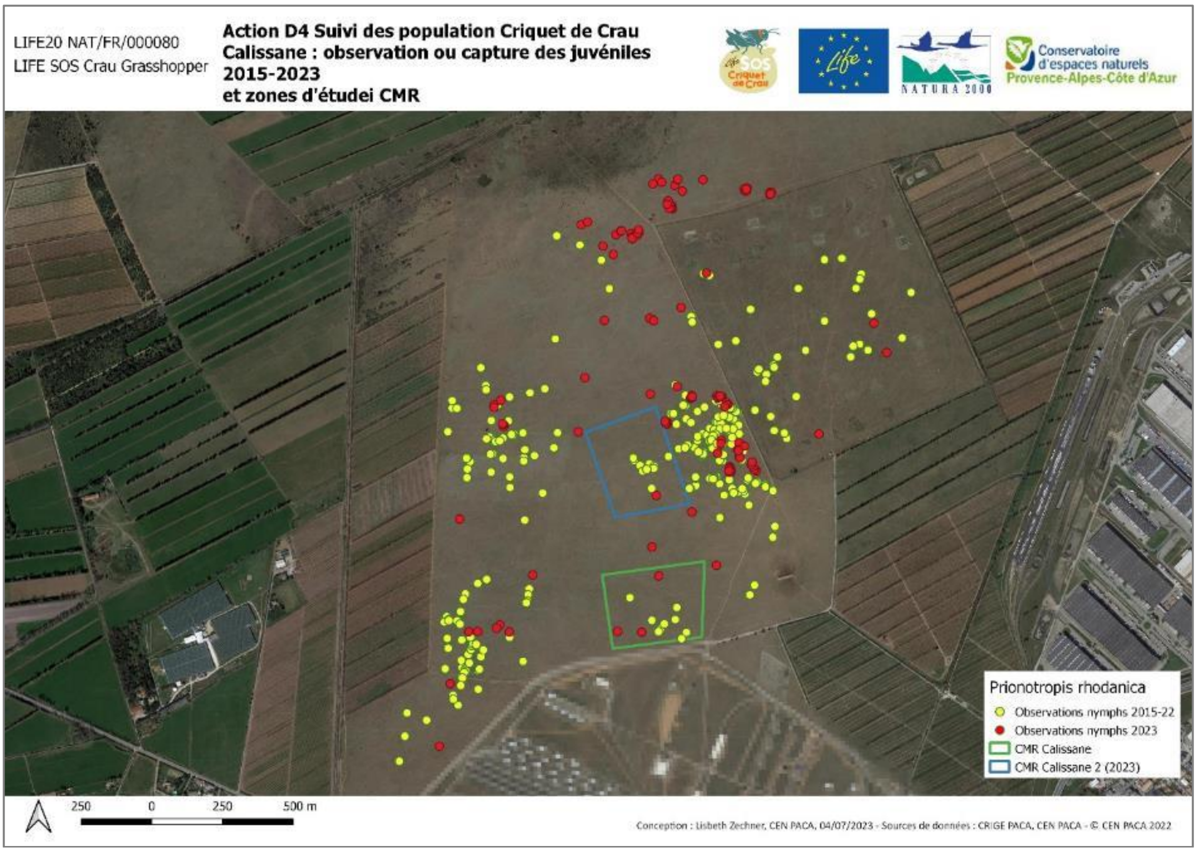


Figure 4. Observations of *P. rhodanica* nymphs between 2015-2023 (yellow dots 2015-22, red dots 2023) with 2 CR study areas.

Table 3 provides a summary of the data sources detailing the spatial distribution of the remaining subpopulations of the Crau Plain Grasshopper (completed after CEN PACA, 2020).

Table 3. Overview on data sources on spatial distribution of remaining subpopulation of the Crau Plain Grasshopper (updated from CEN PACA, 2020).

Subpopulation	Spatial data sources	Year
Calissane	- prospection by humans	2010, 2011
	- locations of juveniles for breeding	2015-2022
	- occupancy studies (humans and detection dogs)	2018-2019
	- mark-recapture sampling	2013, 2017, 2018, 2021, 2022
	- search by detection dogs	2018, 2019, 2022, 2023
	- prospection by humans	2023
Parc à ballons	- search by detection dogs	2022
	- prospection by humans	2015, 2022, 2023
Peau de Meau	- mark-recapture sampling	2015, 2017, 2019, 2021, 2023
	- prospection by humans	2015-2020
	- search by detection dogs	2019, 2022
BMW – North-west	- mark-recapture sampling	2016, 2022
	- prospection by humans	2015, 2016, 2020
	- occupancy study	2020
BMW – centre	- prospection by humans	2015, 2020

Peau de Meau subpopulation

This population, situated in the centre of the Crau plain, is very small, both in terms of occupied surface and population size. In 2023, approximately 6.4 ha of the 8.5 ha fenced area are occupied by the Crau Plain Grasshopper. Since 2015, there has been observed spatial expansion (Figure 5 and Figure 6). In 2022, a detection dog was employed to search outside of the fenced area, but no individuals were found. However, in 2023, as in previous years, some individuals were once again found outside the fence. A future expansion of the fenced-in area (such as in Figure 6) should be considered and agreed with the sheep breeders. After a population decrease in 2021, an increase was recorded in 2023. Maximum population estimates over recent years suggest that 200-300 individuals may be present. Given the very small population size, only small numbers should be captured to prevent negative impacts on the population.

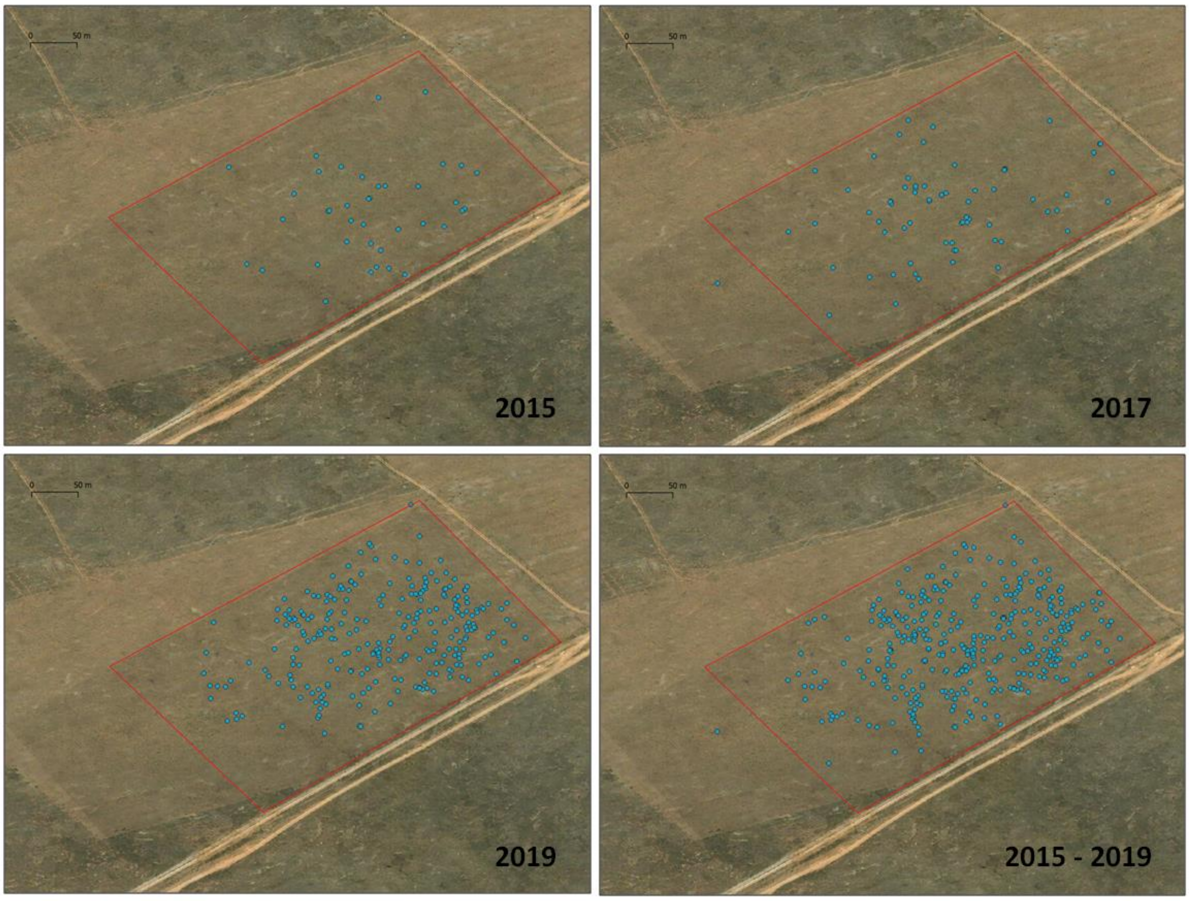


Figure 5. Dispersal pattern of the subpopulation Peau de Meau from 2015 to 2019 (red line indicates mark recapture study area, CEN PACA, 2020).

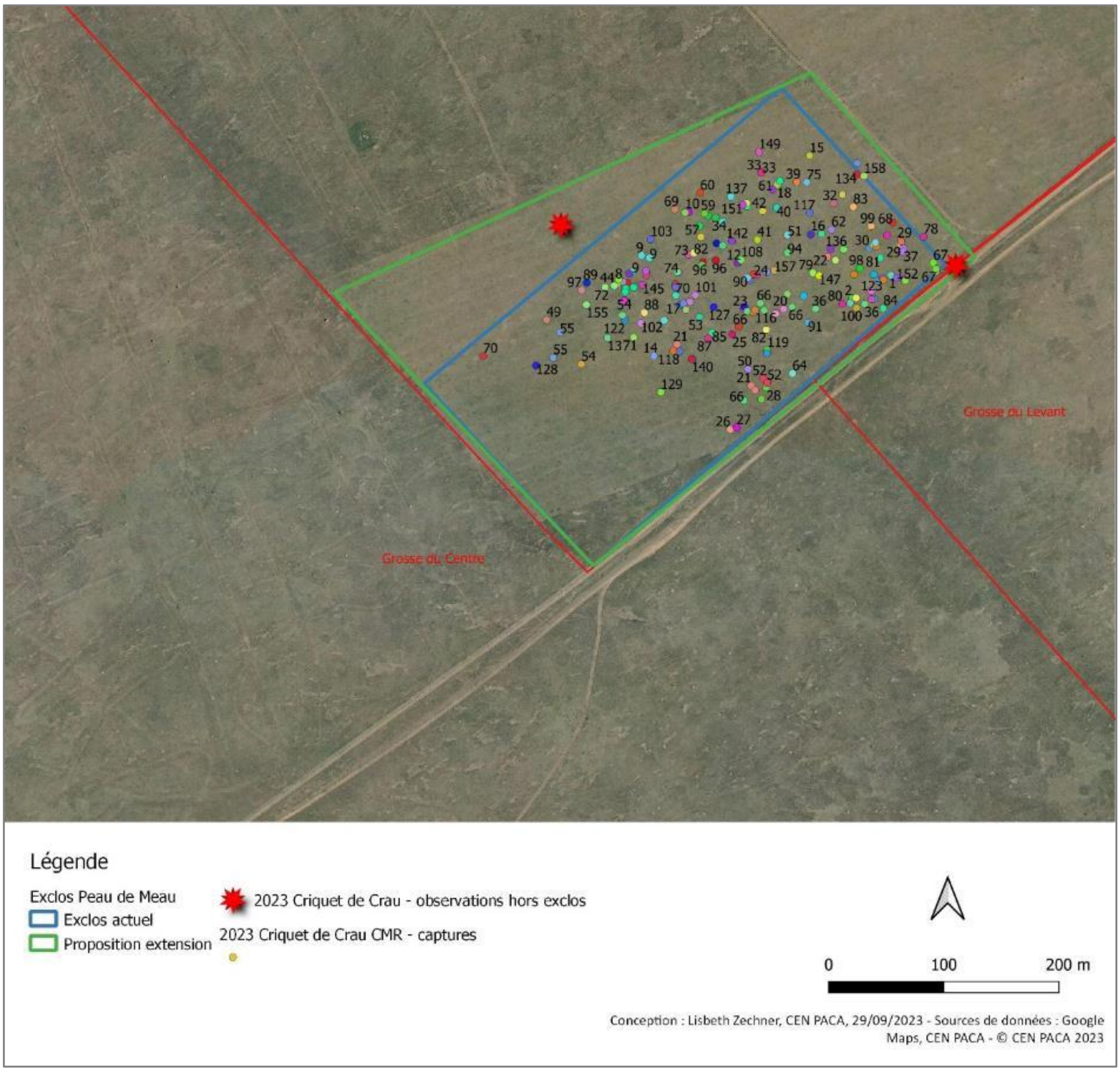


Figure 6. Distribution of CPG population at Peau de Meau in fenced area in 2023 (blue) and hypothetical extension of fenced (green).

BMW subpopulation

The population size of this site is not well-known due to irregular surveys. CR results in 2016 and 2022 suggest a relatively stable or slightly positive trend. Population size estimates from these two studies suggest a low population size. Therefore, only small numbers should be captured to prevent any negative impact on the population.

Calissane and Parc à ballons subpopulation

This population, known for being the largest both in terms of area and number of individuals encompasses approximately 220 hectares based on observations in Parc à ballons and the northern part of Calissane in 2022 and 2023 (Figure 4). The population size estimates from CR studies were derived from only a relatively small area of the entire population. The population dynamics show interannual variation, but no negative trend is observed. It may be reasonable to assume that the total population size is large enough to allow a larger number of captures compared to other subpopulations. Thus, this population may be regarded as a major source for the translocation.

The population of Parc à Ballons was discovered only recently, in 2022, with the help of a detection dog team (Ritas Santos and her dog Hera) and entomologists (Figure 4). The species was detected in small numbers across nearly the entire fenced area. Despite the presence of a gravel track and a wire fence, which represent a certain, though not absolute barrier) it is reasonable to consider Parc à Ballons and Calissane individuals as part of the same population. Although not definitely confirmed, it is possible that the cluster has originated from the transfer of egg pods in 2015 (L. Tatin, personal observation).
In 2023, the northern area of Calissane underwent further exploration with entomologists, revealing the species extending even further north than previously documented, particularly in the northeast area. This further confirmed that the Calissane population is the largest and encompasses a broader area than previously recognized.

Capture-recapture sampling (CR) was employed to estimate the population size of the three remaining subpopulations within the designated study areas: Calissane (9 ha), BMW (7.5 ha), Peau de Meau (8.5 ha) (Table 4). The study area in Peau de Meau encompassed nearly the entire subpopulation allowing the estimates to be interpreted as approximate population sizes for the total subpopulation. In contrast, studies in Calissane and BMW were conducted on a smaller area of the total subpopulations. The estimates for Calissane are considered as a control for global population trends of the subpopulations given that Calissane was considered the most natural site (Bröder et al., 2020) as opposed to Peau de Meau which are subject to different degrees of pastoral management. Based on the estimates and in relation to the spatial distribution of the three subpopulations, Peau de Meau is presumed to be the smallest, BMW a moderate and Calissane the largest subpopulation.

Table 4. Results of mark-recapture surveys in Calissane, Peau de Meau, and BMW. Population estimates according to the closed model are given in column 3; standard error and confidence intervals are given in column 4, 5, and 6.

Site	Year	Population estimate	Standard error	Lower confidence interval (95%)	Upper confidence interval (95%)
Calissane	2013	282,7	19,2	251,3	327,4
Calissane	2017	83,9	5,9	75,4	99,4
Peau de Meau	2015	40,1	3,6	35,5	50,6
Peau de Meau	2017	79,4	6,0	70,7	95,1
BMW	2016	48,4	5,6	40,6	63,4
Calissane	2018	233,9	13,4	212,6	265,9
Peau de Meau	2019	274,6	16,6	247,8	313,6
Peau de Meau	2021	48,7	6,1	40,0	64,9
Calissane	2021	83,3	7,1	72,7	101,4
Calissane	2022	52,9	5,2	45,7	66,9
BMW	2022	76,2	6,2	67,3	92,4
Peau de Meau	2023	216,0	12,6	196,1	246,4
Calissane 2	2023	121,5	8,4	108,8	142,4

The 2015 estimate of the Peau de Meau subpopulation was notably low, but a positive trend emerged in the following years, likely associated to site-specific management practices implemented since 2015 (such as temporal fencing during Crau Plain Grasshopper presence to exclude grazing, and the closure of Lesser Kestrel nest boxes in the immediate vicinity).

Although the trend reversed in 2021, there was a significant increase in numbers again in 2023 (Figure 7).

Strong fluctuations in population size were also observed in the Calissane subpopulation with a stable to negative trend in 2021 and 2022 (Figure 8). However, a higher number was recorder on a second study area in Calissane (cal2) which has the same shape and surface as the main Calissane area, in 2023. The establishment of this second CR study area in Calissane was decided in the context of the modernisation project of the adjacent ammunition depot, which envisions constructing a fence to the north of the existing fence in 2025-2026. Indeed, the presence of this new fence will result in the fragmentation of existing pasture and consequently in significant changes to the grazing management of the site. Thus, the second CR area in Calissane, was established north of the future fence, in order to study the potential influence of future changes in grazing practices on the CPG population, by comparing CR results from this area to those of the other CR site, located south of the fence. The first parallel CR campaigns involving both areas are planned for 2024.

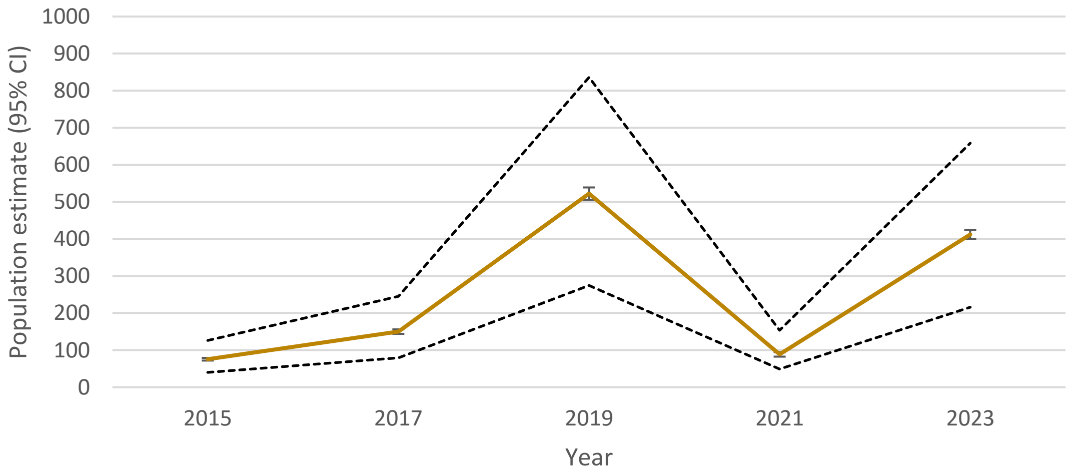


Figure 7. Results of mark-recapture surveys (closed model) in Peau de Meau. Solid lines represent estimates populations, dotted line represent 95% confidence intervals, and vertical bars represent standard errors.

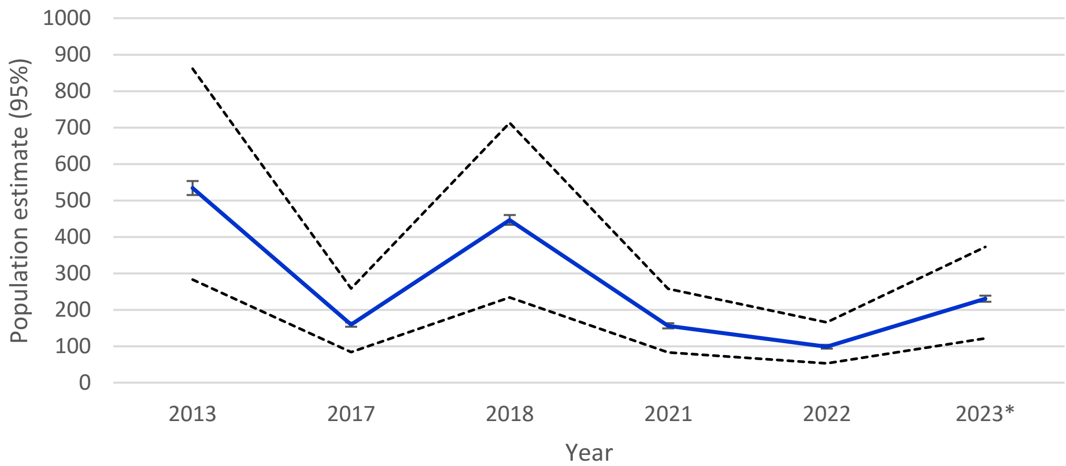


Figure 8. Results of mark-recapture surveys (closed model) in Calissane. Solid lines represent estimates populations, dotted line represent 95% confidence intervals, and vertical bars represent standard errors. *N.B. In 2023, CR was conducted on a different site than in previous years.

Overall, the outcomes of the CR indicate significant annual fluctuations. While temperatures and precipitation during early egg development might play an important role, initial analysis do not reveal any correlation. Further investigation is required to develop more deeply into the relationship between these parameters.

2.2. Habitat

Bröder et al. (2019) studied microhabitat preferences and structural differences between currently and previously populated at the scale of 30 cm-diameter circles. Habitat conditions of the most pristine subpopulation (i.e., Calissane) has been considered as the most suitable for the Crau Plain Grasshopper (CPG) and correspond to 60 % ± 2.1 % vegetation cover, 27 % ± 1.8 % stone cover, 14 % ± 1.7 % bare ground cover, maximum vegetation height of 28 cm ± 2.1 cm, and mean vegetation height of 10 cm ± 0.5 cm (Bröder et al., 2019; Figure 9).

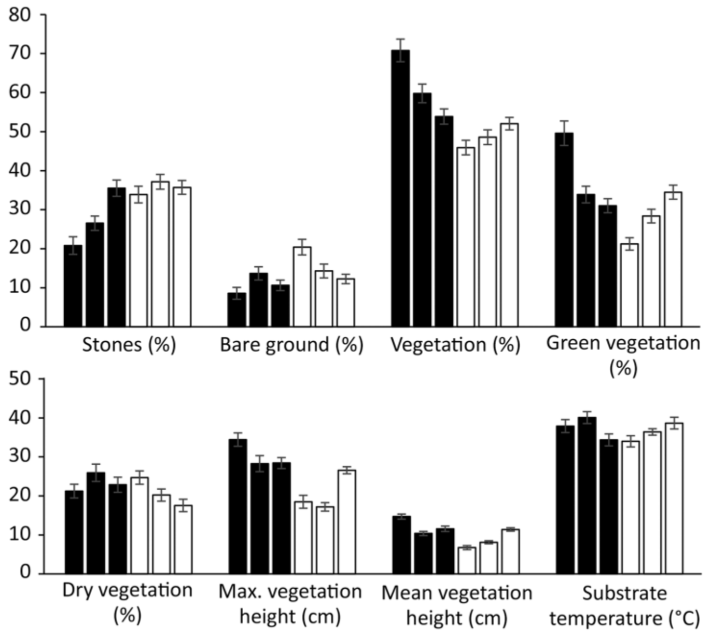


Figure 9. Means ± SE of the microhabitat variable of populated sites (black): BMW, Calissane, Peau de Meau (in this order from left to right) and former habitats (white): EX-Peau de Meau, EX-Grosse du Levant and EX-Couloubris), Bröder et al. (2019).

In addition, the study of vegetation structure of sites located with and without CPG was conducted in the framework of a Masters trainee in 2022 (LIFE action A.1). The study was conducted over a larger spatial scale compared to Bröder et al., (2019), corresponding to 1 m² plots. Results show that grazing places where grasshopper presence has been recorded are not necessarily characterised by high vegetation cover and phytovolume. Thus, vegetation cover is not significantly related to the presence of grasshoppers (Meyer 2022). However, the analysis of data collected only during the second survey period, corresponding to the adult phase of CPG, in 2022 (Meyer 2022), by Larissa Brandl (Trier University) confirms results by Bröder et al., (2019), which show the importance of the vegetation cover. In Calissane and BMW (presence of CPG), an affinity of CPG to areas with large phytovolume of *Thymus* sp. has been suggested, although it was not confirmed by more recent studies (Schaan, 2023).

In 2022, another study was conducted within a Masters trainee (LIFE action A.1) with the objective of developing a tool for vegetation monitoring using remote sensing (Ndim 2022).

Results show a strong correlation between the NDVI (Landsat Normalized Vegetation Index) and the height of *Brachypodium retusum*, a common and widely distributed grass species (Figure 10). NDVI is a metric for quantifying vegetation density using satellite spectrometric data from red and near-infra-red bands. NDVI in summer could therefore serve as a proxy for grazing pressure (Piry et al., 2018). Its evolution in time and space potentially reflects the heterogeneity of grazing practices in the Coussoul. NDVI appears thus to be a reliable indicator for grazing in the Crau steppe offering potential assistance in studying both recent and historical grazing dynamics. This could help in adapting grazing management to meet the species' requirements and identifying potential reintroduction sites. The findings of 2022 were used in the selection of reintroduction sites.

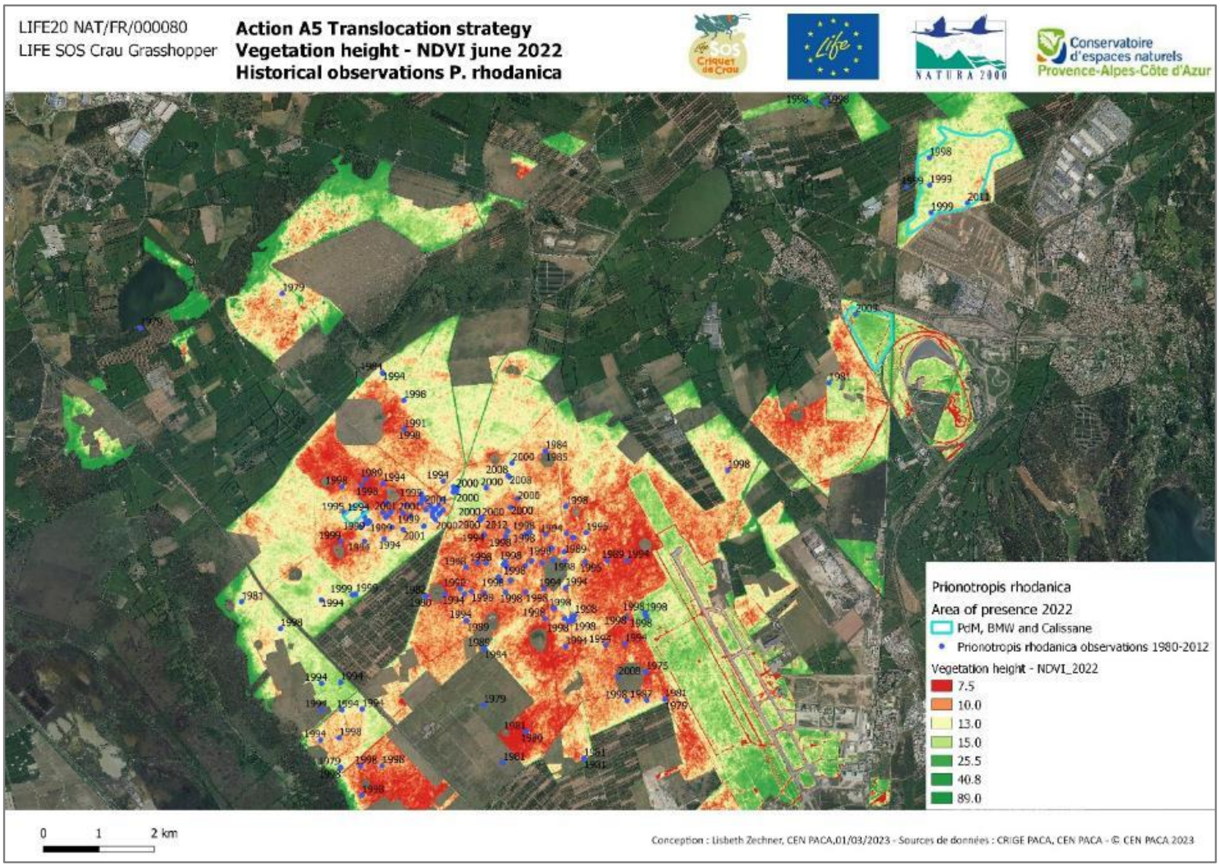


Figure 10. NDVI (vegetation height) mid-June 2022 in the Crau (Ndim 2022).

The remote sensing study of the spatio-temporal evolution of vegetation on the Crau plain from 2018 to 2022 has revealed significant changes in plant production during this period, indicating variations across various scales. Although the results point towards a gradual decline in plant production over the years (Bernard 2023, **Erreur ! Source du renvoi introuvable.**), a comprehensive analysis over a larger timeframe is essential for a better understanding of temporal Crau plain vegetation dynamics. Bernard (2023) also carried out an initial investigation into the distinctions among grazing sites chosen for the reintroduction of the Crau Plain Grasshopper, with the aim of offering valuable data that could guide the selection of the most favourable future translocation sites. However, the findings were only partially conclusive. Currently, statistical tests have only recognised one factor, temperature, as significantly influencing the vegetation of the Crau Plain. Further analysis, incorporating additional factors, will enable a more thorough characterization of variations between sites (Bernard 2023), thereby aiding in a more informed selection of future translocation sites.

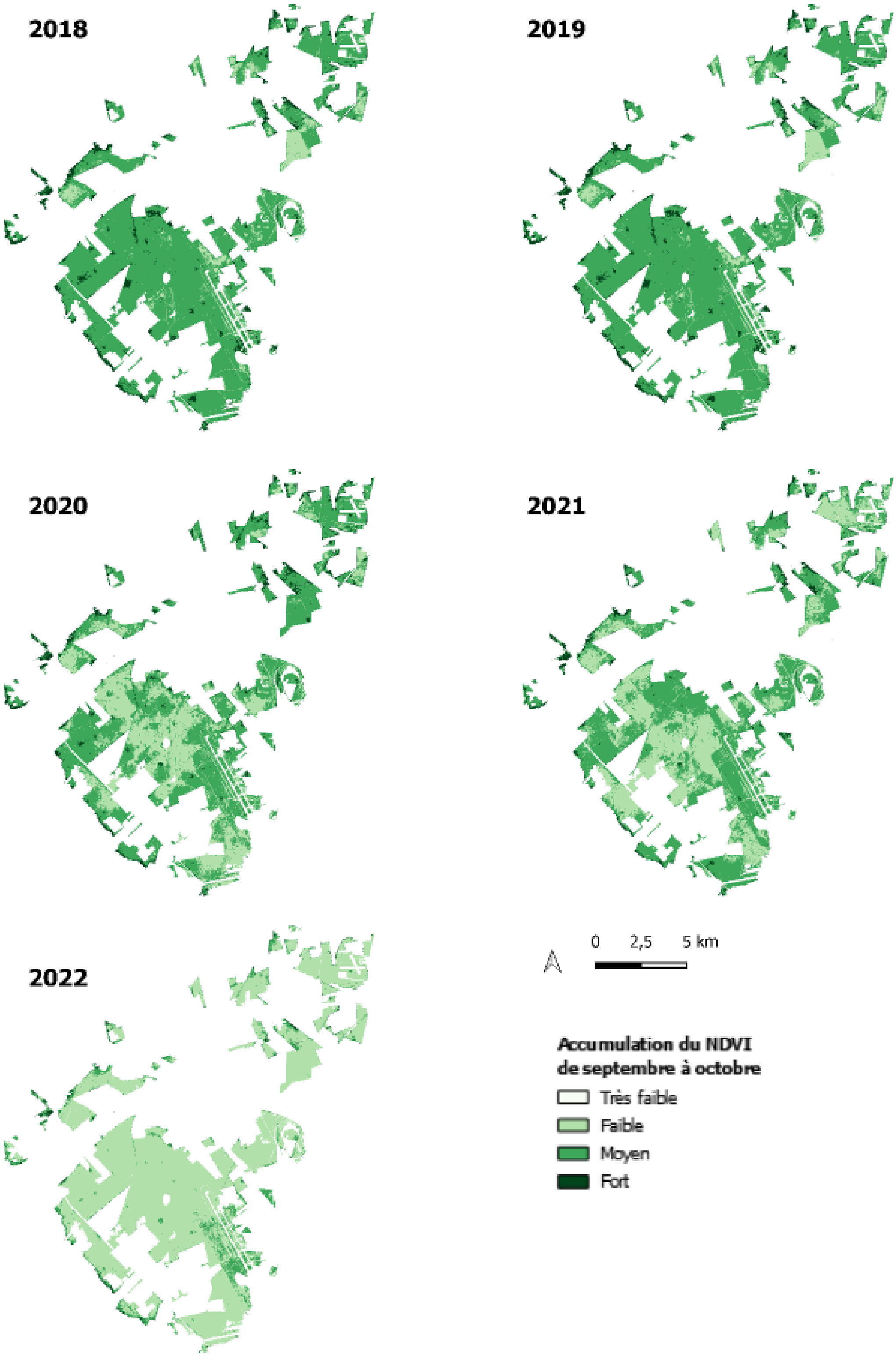


Figure 11. Mapping of cumulative NDVI from 2018 to 2022 (Bernard 2023).

2.3. Threats

2.3.a. Habitat modification and grazing activities

The microhabitat study of Bröder et al. (2019) showed that the Crau Plain Grasshopper requires between 50% and 70% vegetation cover, primarily for feeding (given their herbivorous nature) and potentially for seeking shelter from adverse weather conditions and predators. A comparison of microhabitat conditions between currently and formerly occupied sites (three of each) showed that occupied sites exhibit denser and higher vegetation, while former sites displayed higher cover of stones and bare ground. The observed disparity in vegetation structure between currently and formerly occupied sites is attributed to varying grazing pressures suggesting a potential negative impact of local (and possibly temporary) increases in grazing pressure on the grasshopper, hereafter defined as intensive grazing. Conversely, the abandonment of grazing (as observed in the BMW subpopulations, might also pose a threat to the species (Bröder et al., 2019). Piry et al. (2018) found that population density and gene flow are both strongly and positively correlated to habitat quality (higher productivity of grasslands and/or lower sheep grazing). The spatial scales of interaction between these variables were estimated to be highly similar, ranging from 812 to 880 meters. This outcome suggests that *P. rhodanica* is highly sensitive to the quality of the grasslands it inhabits.

Given the high importance of habitat quality for the Crau Plain Grasshopper (Bröder et al., 2019; Piry et al., 2018), adapting habitat management to the species' requirements is a current priority within the project. Due to the distinct contexts of the remaining subpopulations, site-specific habitat management is necessary. For instance, local and temporary fencing or refining from grazing is required in areas with intensive grazing, particularly in the central areas of the Crau steppe. Conversely, grazing must be implemented in areas where it has been abandoned, such as the BMW sites. The complexity of adaptation grazing management in the Crau steppe arises from socio-economic implications, but the proposed management is only temporary and local, impacting specific areas rather than the entire grazing system of the Crau steppe. Priority areas for adapted management are those with the presence of the Crau Plain Grasshopper or identified as potential future reintroduction sites. The successful management implemented in the smallest subpopulation, Peau de Meau serves as a good example for rapid population recovery resulting from site-specific management, and it demonstrate that such actions are feasible when co-organized with sheep breeders. The adaptation of grazing is fundamental for the conservation of the Crau Plain Grasshopper and may also be beneficial for other species. Nevertheless, it is essential to acknowledge gaps regarding the historic fluctuations in vegetation structure, grazing pressure, and the historical response of the Crau Plain Grasshopper to such fluctuations.

The Peau de Meau subpopulation likely represents a remnant of a once extensive population (Grosse du Levant, now extinct) situated in a degraded part of the Crau that was cultivated from the 1960's to the early 1980's. Surprisingly, as a microhabitat study (Bröder et al., 2019) showed that the vegetation structure in the Peau de Meau subpopulation is today similar to areas where the species went extinct, suggesting marginal habitat quality. However, the population size of CPG was very small on this site. In the past, habitat quality must have been better as recolonization occurred after habitat degradation due to cultivation (Dutoit et al., 2011). Following temporal fencing between April and June since

2015, there have been changes in vegetation cover. Initial results from vegetation surveys in 2023, comparing habitat structure of populated sites (Calissane and Peau de Meau) with three potential translocation sites (Grand Carton, Petit Carton, Poitevine) are in Annexes 1 and 2.

2.3.b. Predation

Several synanthropic bird species which are potential predator of the grasshopper, such as the Cattle Egret (*Bubulcus ibis*), Lesser Kestrel (*Falco naumanni*) and corvids, have significantly increased in abundance over the past few decades in the Crau region. This coincides with the widespread disappearance of the Crau Plain Grasshopper in large areas during the same period. It has been suggested that these birds may have an impact on the grasshopper population (Figure 12 et Figure 13

Figure 13).

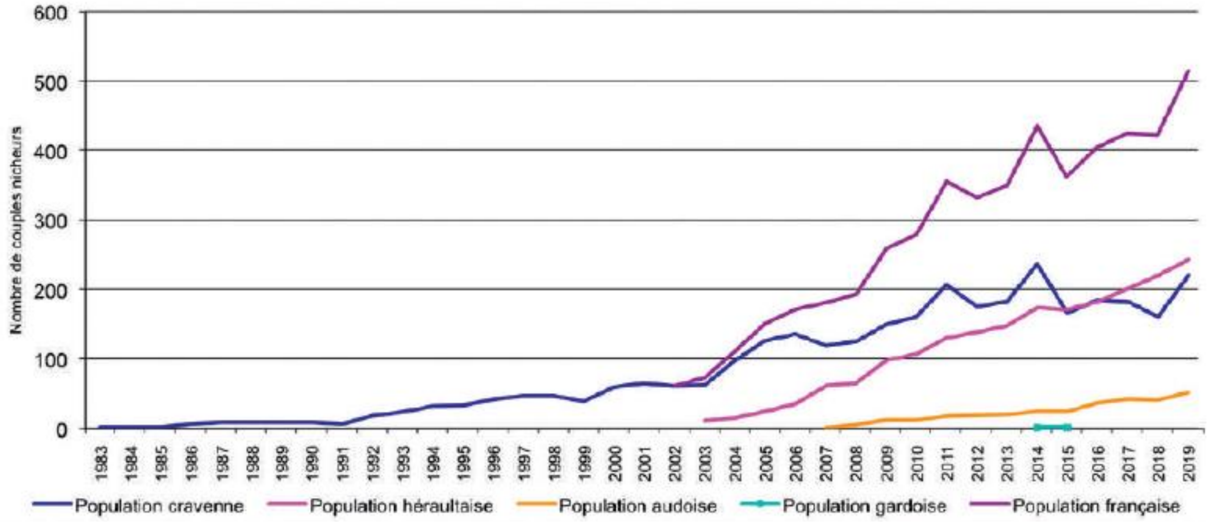


Figure 12. Lesser Kestrel: Evolution of number of breeding pairs in France. Blue = Crau plain population (Pilard 2021).

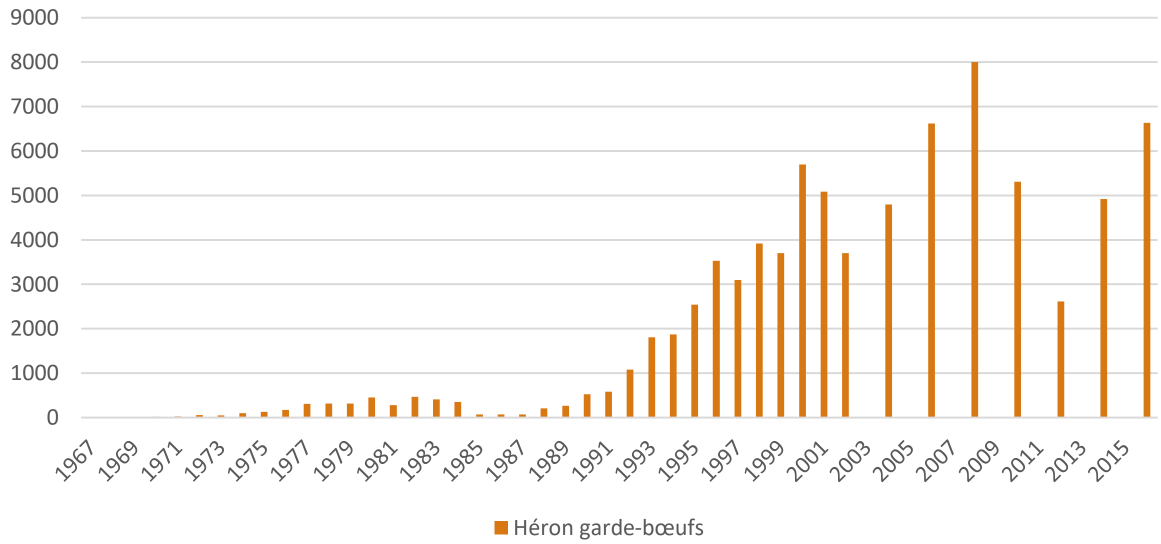
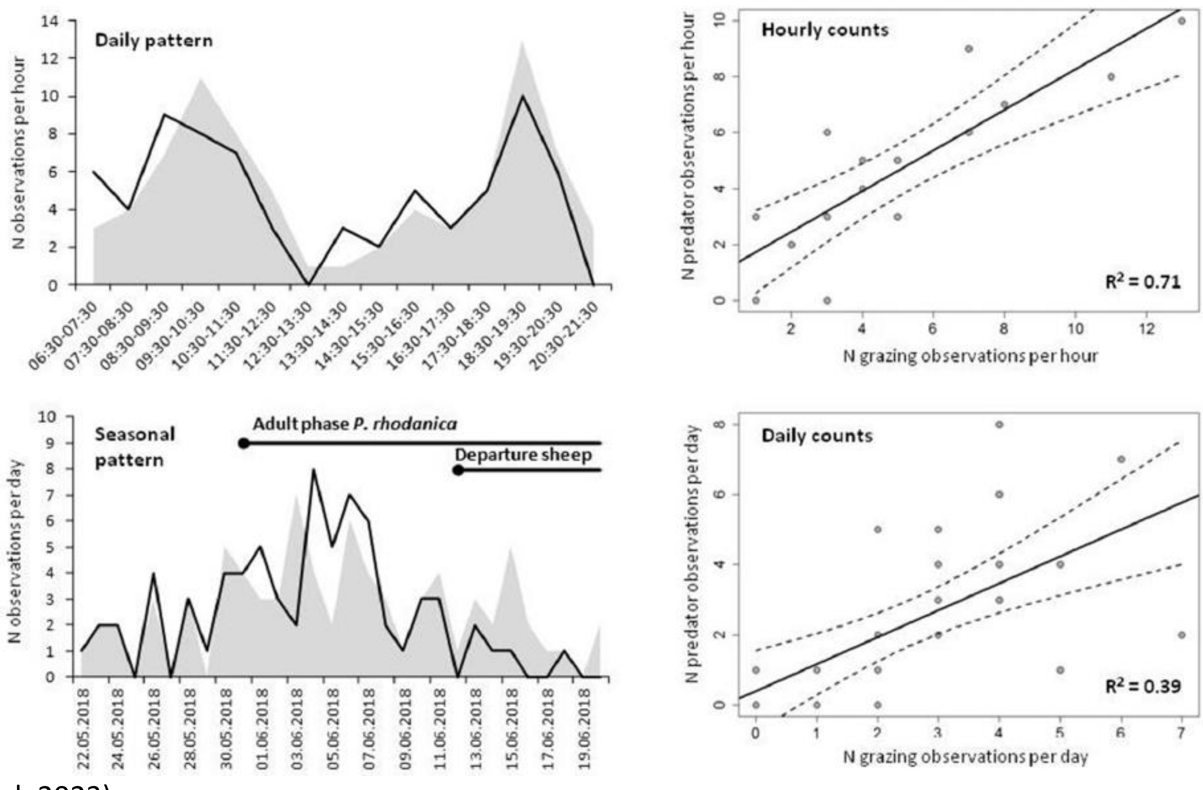


Figure 13. Cattle Egret: Development of number of breeding pairs in the Camargue close to the Crau plain (Kayser et al. 2003, 2008, 2014, in press.).

In 2016, the study of CPG predation through camera traps was initiated (Tatin, 2017). For this purpose, in 2017 migratory locusts (*Locusta migratoria*) with clipped wings were attached to fishing lines and used as baits. They were monitored using camera traps (Tatin, 2017; Hermes 2018). Eight predators were identified through the camera traps (5 in the Calissane and 3 in the Couloubris) with four instances of predation (3 in the Calissane, 1 in the Couloubris) by the Lesser Kestrel. Additionally, one wild boar, one badger, one spider and one sheep were recorded. In addition several individuals of *L. migratoria* were also marked with reflecting foil were releases on two Crau sites (Coloubris and Grosse du Centre) and monitored to study survival rates. This experiment proved the reflection foil methodology to be effective given the high recapture rates (99,2%).

A more comprehensive experiment took place in 2018 using camera traps to identify the main predators feeding on large grasshoppers, as well as reflecting foil marking to investigate grasshopper survival during both periods of presence and absence of sheep herds and of potential predators (i.e., insectivorous birds associated to herds), (Bröder at al. 2023). Crows, in particular Rooks (*Corvus frugilegus*), were identified as the main predators. Results also indicate a positive correlation between the presence of crows and Cattle egrets and grazing, while they showed a negative correlation between the presence of crows and insect survival. Indeed, this result indicate the presence of crows has a remarkable impact on insect survival (Figure 14**Erreur ! Source du renvoi introuvable.**). Furthermore, the presence of crow and Cattle Egret was highest during the early reproduction period of the Crau Plain Grasshopper, suggesting a significant impact on its population. Based on these findings, the simultaneous colonization and increase of these synanthropic predators, namely crows and Cattle Egrets, poses a plausible additional threat for the Crau Plain Grasshopper (Bröder at



al. 2023).

Figure 14. Relation between Insectivorous birds and grazing. Left: Daily and seasonal variation in frequency of grazing (grey area) and crow + cattle egrets visits (black line); Right: positive correlation between these variables (linear regressions with 95% confidence intervals; for hourly counts: R2 = 0.71, t1,13 = 5.66, p < 0.001; for daily counts: R2 = 0.39, t1,28 = 4.24, p < 0.001); the line "Adult phase P. rhodanica" illustrates the period of presence of adult Crau Plain

Grasshoppers, the line "Departure of sheep" represents the period when sheep number was reduced by ≥ 25% (compared to the beginning of the study period) because of transhumance (Bröder et al., 2023).**

According to available monitoring data and available information on the life cycle of other steppe species native to the Crau (such as the Eurasian Stone-curlew, *Burhinus oedicnemus*, the Little Bustard, *Tetrax tetrax*, and the Ocellated Lizard *Timon lepidus*), it is reasonable to consider that the predation pressure they exert, on the CPG, compared to the black crow, is negligeable (Bröder at al. 2023).

In 2022, the presence of potential bird predators was studied both within and outside the mobile fence in Peau de Meau (Godefroid and Dusfour 2022). Six camera traps were placed from mid-April to the end of June at the fence, grouped in pairs covering 1 ha inside of the fence and 1 ha outside the fence (Figure 15). The cameras captured an image every 10 minutes resulting in the analysis of a total of 43,524 photos (LIFE action D2).

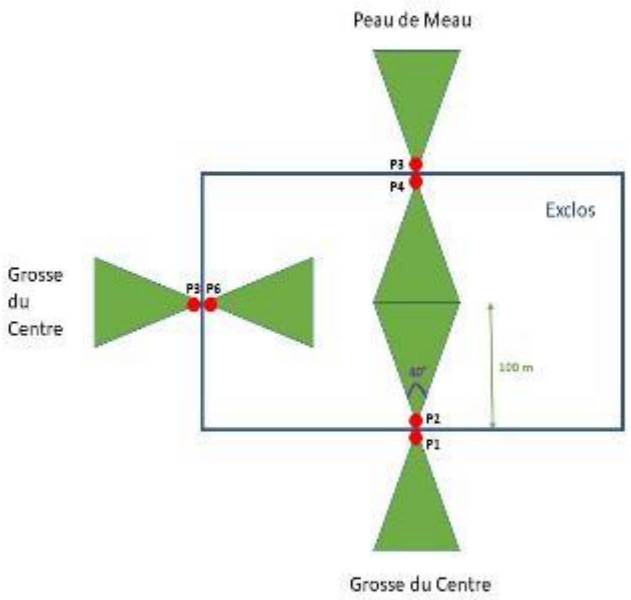


Figure 15. Installation of 6 camera traps around the mobile fence at Peau de Meau in 2022 (Godefroid and Dusfour 2022).

In summary, only rare sightings occurred for **rooks**. The presence of the fence might have a positive effect on this species. Indeed, Bröder et al., (2023) showed a strong correlation of rook presence and grazing suggesting that the fence effectively prevents predation by rooks. Nevertheless, it is important to note that Bröder et al., (2023) studied different grazing places (Calissane and Couloubris) reporting that rooks are more frequently observed in high number in Couloubris.

There was no significant influx of *jackdaws* into the fenced area, confirming the effectiveness of the fence in preventing their influx.

Black crows seem to be attracted to the fenced area from mid-April to the end of May, with 80% of observations having occurred during this period. The presence of large numbers of juveniles and the first adults of CPG may explain this influx.

Cattle egrets show a clear preference for areas outside the fenced area (72% of observations). The data confirm the effectiveness of fencing, with less than 3 herons per day observed in the fenced area. This suggests a reduced risk of predation of CPG by 2 or 4 depending on the period.
Yellow-legged Gulls' presence was recorded only in April, with a preference for the fenced area.
Lesser Kestrels display a net preference for the fenced area in late May (75% of observations). It is worthwhile noticing that this species is known for using fence posts as perches.
In 2022 a study on the hunting ranges and areas of the Lesser Kestrel was conducted as part of the LIFE action A3. Fifteen birds were equipped with GPS transmitters and their movements were monitored using telemetry (LPO France, Philippe Pilard). In addition, data from the MigraLion project involving 47 birds equipped between 2017 and 2022, were also used. The dataset comprised approximately 450,000 geolocated points gathered from seven Lesser Kestrel colonies. Following a selection process (locations > 100 m colony location, between 8 am and 8 pm, during April/May/June, wind speed < 10 m/s), 122,000 data points were analysed accounting for the number of equipped birds and colonies sizes (Godefroid 2022). Figure 16 illustrates the presence of Lesser Kestrel in the different parts of the Crau, which was used as a criterion for the selection of translocation sites. A 2013 study of preys transported by Lesser kestrels to nests for chicks feeding showed that CPG represented a small proportion of prey, only 0.31% at Peau de Meau, a site where CPG populations were present at the time along with several Lesser kestrel nest boxes (Pilard & Tatin, 2014).

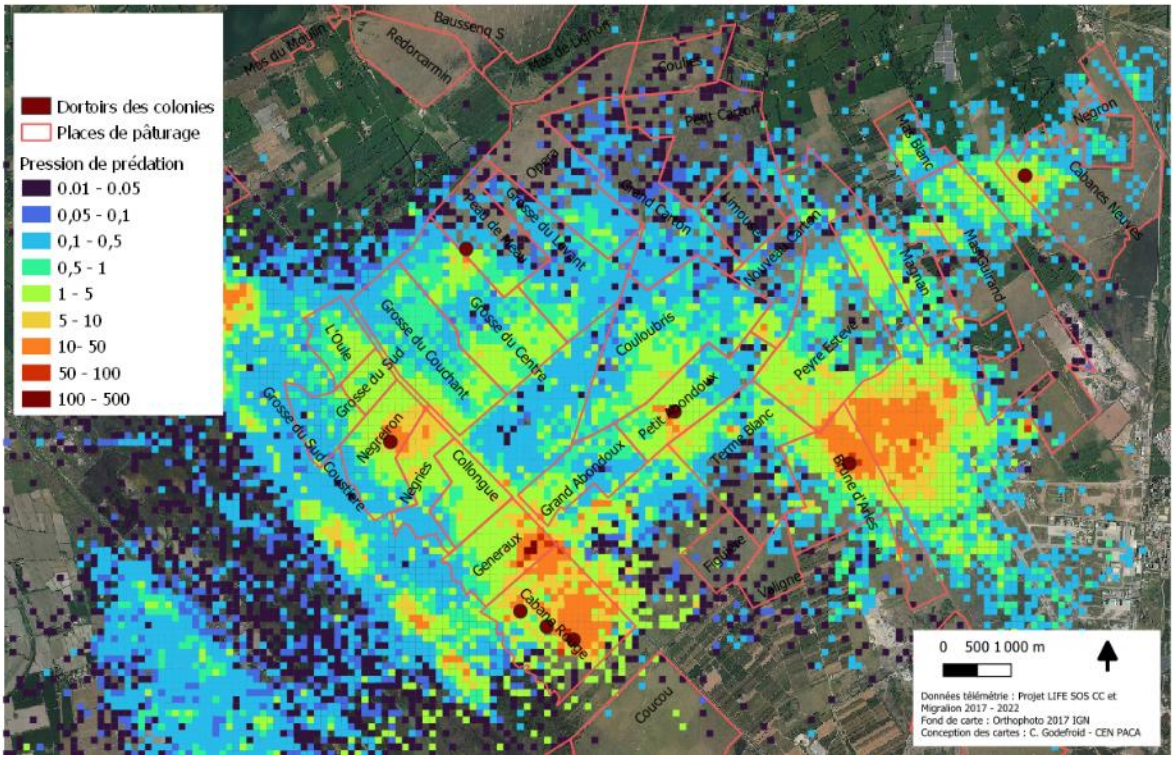


Figure 16. Presence (hunting pressure) of Lesser Kestrel (data of GPS transmitter), Godefroid (2022).

2.4. Breeding programme

Since the initial breeding trials in 2015, the full development cycle has not been successfully accomplished in captivity. Consequently, a breeding programme has been established with two complementary approaches:

1. **Ex-situ breeding**: This involves captive breeding outside the natural habitat, focusing on rearing wild adults and producing egg pods.
2. **In-situ breading**: This takes place in aviaries within the natural habitat, focusing on egg pods development and optimal hatching conditions.

The combination of ex-situ and in-situ rearing is being pursued. Captive ex-situ oviposition allows a large number of egg pods to be produced (up to 14 egg pods per female compared to 3 to 4 expected under natural conditions; Foucart, pers. comm.). However, to date, incubation in-situ remains the sole method for completing the developmental cycle of the eggs (Figure 17).

On the one hand, collaboration with the two La Barben and Muséum de Besançon zoological parks has allowed the expansion of ex-situ breeding stations. Ongoing tests in artificial incubation aim to identify the necessary conditions for the successful completion of the entire life cycle in captivity. On the other hand, the establishment of a second in-situ station at Cabanes Neuves in the Crau, has effectively doubled the capacity for egg pods incubation.

The coordination and management of breeding activities under the LIFE project are overseen by Cathy Gibault, Doctor of Veterinary Medicine specialized in species breeding (holder of a “certificate of competence”). The CEN PACA team has been led until the end of 2023 by Lisbeth Zechner, Doctor of zoology, who also holds a “certificate of competence”. The new LIFE project manager, Camilla Crifo (Doctor of Biology) will be trained by C. Gibault to obtain a “certificate of competence” as well. It is important to note that the breeding efforts are solely for conservation purposes.

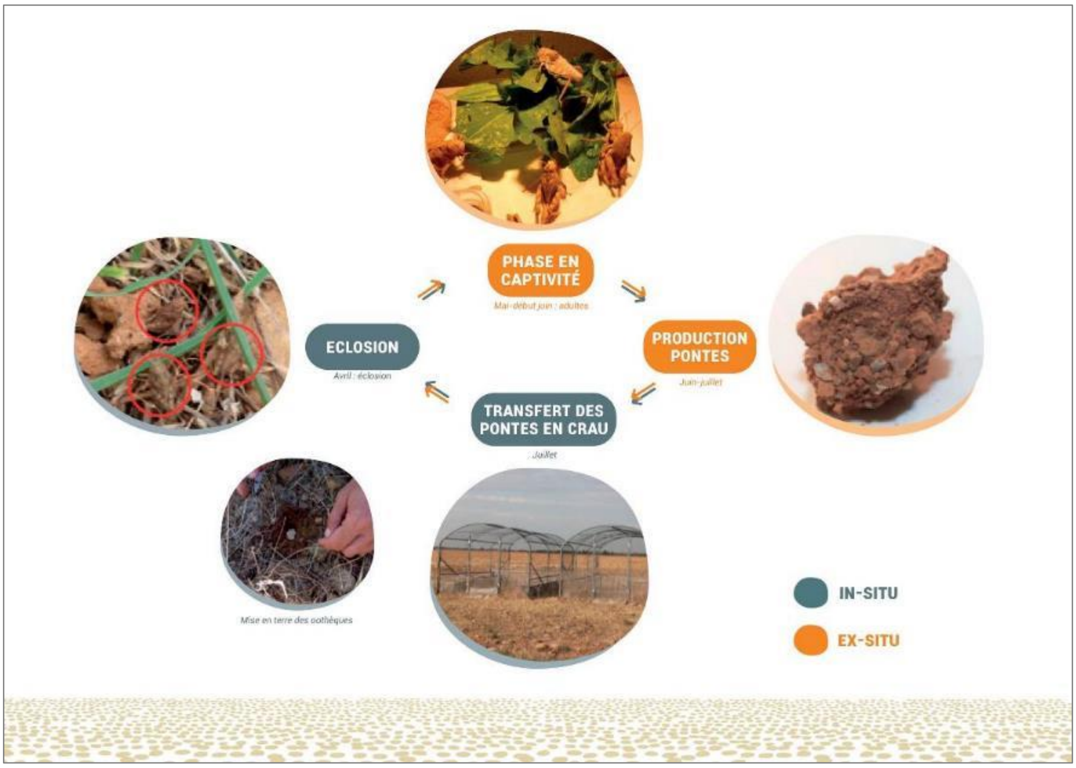


Figure 17. Diagram of in-situ and ex-situ rearing of *Prionotropis rhodanica* (Audrey Hoppenot, CEN PACA).

Ex-situ breeding is currently carried out at two breeding sites: the Besançon Museum (Figure 18) and the La Barben Animal Park (Figure 19). However, the first ex-situ breeding site was established in 2015 at the Thoiry animal park (Paris) by Cathy Gibault and Paul La Panouse. In 2016, following a change of owner, who did not express the desire to pursue the programme, C. Gibault breeding was managed by C. Gibault in her own facilities in Corrèze until 2022. Dedicated breeding rooms for the Crau Plain Grasshopper, equipped with terrariums, are present at each site. The La Barben Animal Park also features an outdoor aviary. Every year, in early May, individuals in the juvenile stage are transferred to the ex-situ breeding stations. These individuals, originating from the aviaries of in-situ breeding stations (cf. In-situ Breeding) or captured in the wild (maximum of 50 individuals per year; in accordance with the prefectoral decree of 3 May, 2021; DDTM, 2021), reproduce in the terrariums. In June, the females lay eggs in egg pods.

Figure 18. Breeding room at Besançon. © Lisbeth Zechner, CEN PACA.

Figure 19. Breeding room at La Barben. © Lisbeth Zechner, CEN PACA.

In-situ breeding takes place at two breeding sites: the Calissane site, where two aviaries measuring 3 x 6 m are located (Figure 20), and the Cabanes Neuves site, which features an aviary of 32 m^2 (Figure 21).

Figure 20. Calissane aviaries. © Lisbeth Zechner, CEN PACA.

Figure 21. Aviary at Cabanes Neuves. © Lisbeth Zechner, CEN PACA.

Between June and July, the oothecae laid in the terrariums of ex-situ breeding stations are transferred to the aviaries of in-situ stations (Figure 22) and marked. In-situ incubation continues until April of the following year.

Figure 22. Transfer of egg pods to the aviaries. © Lisbeth Zechner, CEN PACA.

These oothecae undergo two different destinies:

1. Primarily, they are left in the in-situ aviaries until the eggs hatch. The juveniles resulting from hatching are then captured and transported to replenish ex-situ breeding (production of new egg pods). Ex-situ, they are intended for terrariums (in the breeding rooms in Besançon and La Barben) or the aviary in La Barben (Figure 23).
2. A minor portion of the egg pods is quickly transferred ex-situ to achieve the complete developmental cycle (from hatching to the adult stage) in captivity, in the breeding rooms in Besançon (Figure 18) and La Barben (Figure 19).

Figure 23. Outdoor aviary at La Barben. © Lisbeth Zechner, CEN PACA.

It is noteworthy that the La Barben aviary, unlike those in the Crau (in-situ), is not supplied with oothecae laid in the breeding room but only with egg pods produced within the aviary itself, where the entire development cycle takes place.
To minimize the risk of contamination of *P. rhodanica* by other insect species, each site is equipped with a facility meeting specific biosafety standards dedicated to the breeding of the Crau Plain Grasshopper. A health protocol outlining standards for terrarium cleaning and the feeding and monitoring of grasshoppers has been established by C. Gibault. Sanitary risks are negligible in the aviaries as the juveniles feed on plants naturally growing within the aviary. Nevertheless, any handling of grasshoppers and oothecae in the aviaries is conducted in accordance with established safety and health protocols.

2.4.a. Results

A comprehensive review of the breeding programme from 2015 to 2021 is documented in Gibault (2022).
Up to now, the complete developmental cycle of the species has been successfully accomplished within a single year, employing a combination of ex-situ and in-situ rearing phases (i.e., captivity and semi-captivity phases). Ongoing enhancements to the protocols are anticipated to enable, in the long run, the achievement of the goal of completing the species' entire developmental cycle in captivity, without dependence on the wild population to sustain the cycle.

- **Capture and survival**

Since the beginning of the breeding programme, 195 individuals were captured in the wild mainly at the nymph stage (Table 5) for breeding in captivity. Over time, the implementation of ex-situ breeding has allowed to reduce the number of captures in the wild as adults are predominantly generated from egg pods laid in captivity and subsequently transferred to in-situ aviaries.

Table 5. Number of individuals (captures of wild individuals and individuals from aviaries). * In 2023 transfer of egg pods to breeding stations before hatching, therefore less egg pods left in the aviaries in comparison to previous years.

Year	Number of individuals captured the wild	Number of individuals captured in the aviaries	Proportion
2015	26 (survival rate)		
2016	22		
2017	22		
2018	28	25	
2019	37		
2020	30		
2021	**0**	17	
2022	30	13	
2023	**0**	**16***	
Total	**195**	**71**	26,6

Breeding in captivity has the advantage of increasing individuals' life expectancy (CEN PACA, 2020). In 2015, for instance life expectancy was of 103 days in captivity while life expectancy in the wild has been observed to be of 67 days (Foucart, 1995). High lifespan (and thus higher egg pod production) was recorded since the beginning of the breeding programme in 2015, up to 2017. However, iridovirus infections in 2018, and nematode infestations in 2019, caused a sharp increase in mortality among juveniles and adults, reducing their average life expectancy (CEN PACA, 2020).

- **Egg laying and hatching rates**

From 2015 to 2018, the higher lifespan of the captive populations, compared to the wild populations lead to high average production of egg pods by the females (Figure 24). Then, egg production declined following the spreading of iridovirus infections in 2018 and of nematode infestations in 2019 and remained low during the following years due to additional disease outbreaks. In 2023 the overall number of egg pods per females remained average (6.86) compared to previous years but exhibited variations across the breeding station (5.4 in Corrèze; 6.4 in La Barben; 9 in Besançon).

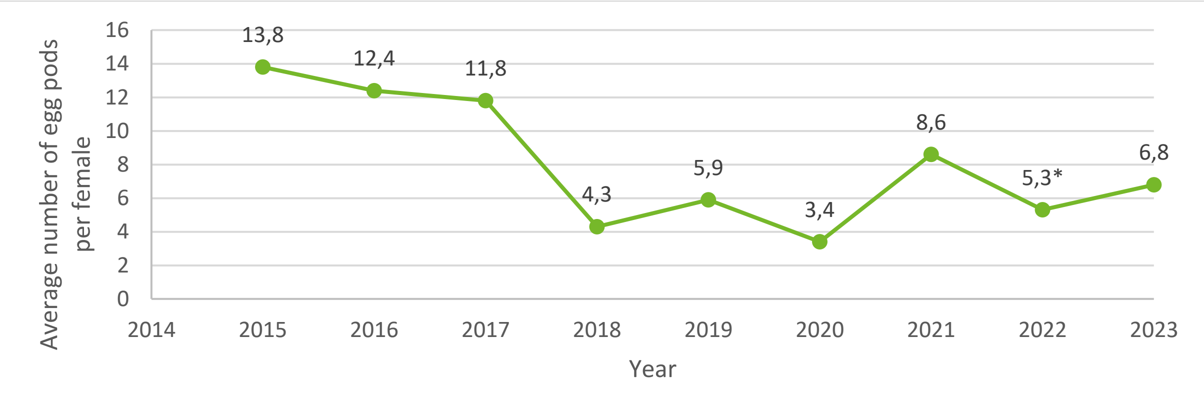


Figure 24. Number of egg pods per adult female per year. *The value for 2022 represents a mean between two ex-situ breeding stations (Corrèze and La Barben).

Since 2015, higher hatching rates have been observed when egg pods are incubated in the wild compared to captivity due to incomplete embryonic development in captivity (CEN PACA, 2020).
Thus, in 2018 egg pods started to be transferred into in-situ aviaries shortly after being laid. However, the number of nymphs obtained on a given year compared to the number of egg pods transferred the previous year in the Crau aviaries (Table 6) indicates that hatching rates remain low compared to the potential number of eggs available.

Table 6. Number of egg pods and juveniles in the aviaries at Calissane. In 2022 most of the egg pods were transferred to the breeding station before hatching, only 23 were left for hatching at Calissane.

Year n	Number of egg pods (year n)	Number of juveniles in the Crau (year n+1)
2018	69	
2019	74	0
2020	45	20 (17/04)
2021	65	17 (03/05)
2022	103 among which 90 in the Crau (23 transferred on 29/03/2023)	13 (02/05)
2023	(149)	16 (02/05)
Total	277	56

Indeed, an egg pod can contain up to 18 eggs (16.88 ± 4.42 eggs on average). This would correspond, between 2018 and 2023, to a total of 4,986 eggs (from 277 egg pods), which is more than 80 times the number of juveniles hatched during this time frame (57). Thus, it is likely that significant loss occurs across the whole cycle from egg laying to hatching as well as further stages. Although some losses occur during the incubation period (parasitism on eggs, for example, by beetles), predation become more prevalent after nymph hatching. For instance, in 2017 juvenile mortality was 70% (CEN PACA, 2020). Indeed, while after hatching, numerous juveniles at stages 0 and 1 are continuously observed, successive stages become much rarer over time. Despite natural survival rates of juveniles are unknown, this suggests an increased predation pressure from invertebrates (harvestmen, spiders, insects, etc.).
Each year (except for 2021 and 2023), the low production of individuals in the aviaries designated for ex-situ breeding, has been supplemented with individuals captured in the wild. Furthermore, starting in 2021, juveniles were captured 2 to 3 weeks earlier in early May, and transported to the rearing stations to maximize their chances of survival. Rearing from nymph stage 2 onwards was conducted with minimal losses achieving a survival rate ranging from 82 to100% (Table 7).

Table 7. Survival rates of all individuals captured in the wild (juvenile stage 2 or 3) and from the in-situ aviaries in the Crau and transported to the ex-situ breeding stations between 2015 and 2022. No data are available for 2023 as monitoring of individuals transferred to La Barben from Calissane was not carried out. No other transferred from Calissane to other ex-situ stations, and no captures in the wild occurred in 2023.

Année	Taux de survie jusqu'au stade adulte
2015	96.2 %
2016	100 %
2017	95.5 %
2018	86.8 %
2019	100 %
2020	93.3 %
2021	82.3 %
2022	86,3%

In order to enhance our understanding of egg development and improve breeding success, several experimentations have been carried out starting in 2015. These include egg dissections, ex-situ egg pod and egg incubation experiments, as well as ex-situ egg pod hatching.

Dissections have been carried out since 2015. The first observations of embryonic development have pointed to a delayed development in eggs incubated ex-situ compared to those incubated in the Crau, suggesting that local climatic conditions play a major role in embryonic development (CEN PACA, 2020). This trend has been confirmed in the following years. In 2022, dissections were performed by A. Foucart on eggs issued from two egg pods which were incubated in the Crau to investigate embryonic development and diapause, (Figure 25).

07 Août 2023 **14 Novembre** 2022 **28 Février** 2023 **28 Mars** 2023

Figure 25 . Images of dissections showing embryonic development © Antoine Foucart, CBGP - Continental Arthropod Collection.

Ex-situ egg incubation tests were carried out by C. Gibault in Corrèze using the eggs left in the egg pods after dissection. Two sets of eggs were individually incubated on sand in small boxes (one box per egg pod) following two treatments: on at room temperature, and the other in a refrigerator (Table 8). The egg hatching rates for the two treatments were relatively high, reaching 48% and 57% respectively.

Table 8. Number of "loose" eggs incubated in Corrèze and Crau.

Incubation site	Incubation mode	Number of eggs	Number of hatchings	Percentage of hatched eggs
Corrèze (14/11/22)	Crau then boxes + refrigerator sand	23	13	56,5 %
Crau (14/11/22) - Corrèze (22/03/23)	Crau then boxes + sand outside temperatures / unheated room	29	14	48,3 %

Ex-situ egg pod incubation was conducted, by C. Gibault in Corrèze, by the Besançon team at their breeding station, as well as by Dr G. Köhler, in his laboratory at Jena University. Three egg pods were incubated in Corrèze (where they were also laid), eight were sent to University of Jena, and eight to Besançon. The hatching rate was very low (Table 9), ranging from 0 (in Corrèze) to 12.5% (at Besançon and the University of Jena). The low hatching rates may be attributed to prolonged storage of the egg pods in the refrigerator (Table 9).

Table 9. Ex-situ incubation 2022/23 with number of egg pods and hatching rate.

Incubation site and date of transfer from Crau	Incubation mode	Number of egg pods	Number of egg pod hatchings	Percent of egg pods hatched (%)
Corrèze (egg-laying in	Unheated room	3	0	0 %

Corrèze)				
Jena (25/10)	Fridge (5-7°C)	8	1	12,5 %
Besançon (21/11)	Fridge (3-8°C) in a moist substrate non saturated with water	8	1	12,5 %

Figure 26. Egg pod incubation in the refrigerator at University of Jena. © G. Köhler, University of Jena.

In addition to the dissections and incubation experiments, ex-situ hatching experiments were conducted at the breeding stations of Corrèze and La Barben. At La Barben, the experiment took place both in the breeding room and in the outdoor aviary. Egg pods were transported to the two breeding stations just before the hatching date (Table 10) with the aim of preventing losses at the first nymph stages in the in-situ aviaries. The hatching rate was relatively high, ranging from 50% to 80%. Dissection of the remaining egg pods revealed that some had been damaged by predators or parasites, or that the eggs had dried out.

Table 10. Transfer of egg pods to ex- situ rearing stations at the end of March 2023.

Incubation site and date of transfer from Crau	Incubation mode	Number of egg pods	Number of egg pod hatchings	Percent of egg pods hatched (%)
Corrèze (27/03)	Terrarium: room temperature, unheated (from 15°C at night to 23°C during the day) and manually sprayed twice a week.	12	7	58,3 %
La Barben (29/03)	Breeding room (ambient temperature, unheated room)	5	4	80 %
La Barben (29/03)	Outdoor aviary	13	6-7	50 %

Finally, it is interesting to note that embryo development, hatching and survival of juveniles up to stage 2 from eggs incubated over a two-year period were observed in 2016, 2017, 2023 and 2020 respectively (Gibault, 2022; Tatin, pers. comm.). This observation needs to be confirmed but potentially provides new important information regarding egg development and population dynamics of *P. rhodanica*.

- **Juvenile survival**

In captivity the lifespan of individuals is significantly higher than in the wild, but their survival rates decline even more so as breeding in captivity starts at an early developmental stage.

In 2023, a high hatching rate of egg pods in breeding rooms in Corrèze (Figure 27) and La Barben, was followed by high mortality at the first stages 0 and 1 (La Barben: 78%, Corrèze: 67%), (Table 11).

Figure 27. Juveniles of *P. rhodanica*, just after hatching © Cathy Gibault, Corrèze.

Table 11. Number of juveniles, adults and egg pods observed at different sites in 2023. *Exact monitoring not possible in the aviary (vegetation).

Incubation site	Number of stage 0 juveniles	Number of stage 1 juveniles	Number of stage 2 juveniles	Number of stage >2 juveniles	Number of adults	Number of egg pods
Jena University	?	7	1	0	1 F	(4)
Besançon	10	6	0	0	0	0
Corrèze total	92	80	37	33	Total: 33 Corrèze: 9 M, 8 F Besançon: 9 M, 7 F	Corrèze: 41 Besançon: 63
La Barben breeding room	?	37	8	Crau: 6 + 16 transfer on 02/05/23	7 F 7 M	45
La Barben ex-situ aviary		28*		max 37 individuals observed* 24/04/23	Total: 14 11 F** 13 M**	?

The cause of high mortality rates in the early stages is difficult to ascertain, whether it occurs in La Barben, Besançon, or Corrèze. Various hypotheses are considered for all three stations:

- Are the captivity conditions partially unsuitable (temperatures, light, humidity, food)?
- Is there significant natural mortality in the early stages (as observed in other Orthoptera species)?
- Could the mortality in the early stages be linked to a health problem? Although the few cadavers tested for iridovirus yielded negative results, the sample size should be increased in the coming years to rigorously examine this hypothesis.
- Could mortality in the early stages be related to an environmental issue in the natural habitat: pollution, climate change, or other factors?

This list of potential causes is not exhaustive, and further investigation into other possibilities should be undertaken in the future.

2.5. Diseases and parasites

Two important diseases have been identified in *P. rhodanica*: A viral disease (Iridovirus Cr-IV) confirmed in captive as well as wild populations, and a parasitic disease (Nematodes of the family Mermithidae) found in captive individuals only. These diseases are believed to be the cause of the collapse in reproductive success observed in individuals raised in captivity starting from 2018 (Figure 24). In fact, Iridovirus infections lead to early and increased mortality in juveniles and/or in adults resulting in fewer egg pods being laid while Nematode infestations cause early mortality in adults, leading to reduced reproduction, with some males no longer mating at all (observed through colour marking). This results in fewer egg pods being laid.

2.5.a. Tests on dead individuals

Between 2015 and 2020, deceased individuals were not systematically tested for the presence of iridovirus and nematodes. Therefore, the prevalence of these diseases *in P. rhodanica* populations is poorly documented. Nevertheless, some data (absolute numbers) are provided in Table 12. All tested individuals came from the Calissane population and were captured either from aviaries or from the wild and subsequently transferred in captivity at a breeding station in Thoiry. Iridovirus and nematode presence were first detected in 2015 and in 2019 respectively. A review of the impact of these diseases on breeding success from 2015 to 2021 is documented in Gibault (2022).
Starting in 2022 individuals were systematically searched for nematode infestation as well as tested for iridovirus by PCR. In 2023 individuals from the ex-situ breeding stations (Corrèze, Besançon, and La Barben) continued to be tested for the presence of iridovirus infection and nematode infestation (see Table 12).

Table 12. Summary of test results for iridovirus infections and nematode infestations on deceased individuals since 2015.

Site	Year	Number of tested individuals	Number of iridovirus infections	Number of nematode infestations
Thoiry	2015	n.a.	1 juvenile killed in captivity	None
Thoiry	2017	n.a.	Several juveniles deceased in captivity	None
Thoiry	2018	n.a.	Several juveniles and adults, all captured from the wild or from in-situ aviaries, deceased captivity.	None
Calissane	2018	n.a.	1 wild individual	None
Thoiry	2019		n.a.	Many adults
Thoiry	2020		Several young adults captured in the wild or from aviaries, deceased in captivity	
Thoiry	202	Several eggs from a positive female	None	n.a.
Thoiry	2021	3 adults	None	Several individuals
Corrèze	2022	17 deceased individuals	1	None

Corrèze	2022	Eggs of two egg pods	None	n.a.
La Barben	2022	7 deceased individuals	1	n.a.
Corrèze	2023	12 (7 juveniles and 5 adults)	0	2 (1 adult male and 1 adult female)
Besançon	2023	9	2 (+ 2 possible viral inclusions observed)	2 (perhaps resulting from contamination due to transport from Corrèze)
La Barben	2023	20	0	n.a.

During the expert workshop held in March 2023, particular emphasis was placed on the health status of wild populations and individuals at breeding stations. Thus, to better evaluate the presence of the iridovirus and its impact on individual mortality, it was deemed necessary to examine the three wild populations (20 individuals per sub-population) before translocation to decide on the origin of the individuals to be released.

Stricter capture and transport protocols employed since 2022 have allowed to reduce although not to eliminate the cases of iridovirus infection Despite the infection has no caused acute mortality in there may be major effects on longevity and reproductive success.

A stricter sanitary protocol for food selection at the Corrèze breeding station has allowed to reduce the number cases of infestations by nematodes although a few cases were still observed. Although no acute mortality was reported, the potential effects on longevity and reproductive success remain poorly known and need further investigation.

2.5.b. Tests on live individuals

- **Iridovirus tests on *P. rhodanica* regurgitate**

In 2023, in collaboration with the Laboklin laboratory, a new method of testing for iridovirus has been experimented to avoid the capture and destruction of live individuals of this highly endangered species. Thus, the presence of iridovirus was tested on the regurgitate of several individuals from the three sub-populations in the Crau and from La Barben zoo (Table 13).

All the 22 individuals tested gave negative results, apart from one female from the Peau de Meau subpopulation. These results should be interpreted with caution. Indeed, while a positive PCR confirms the presence of the pathogen in the sample, typically indicating evidence of infection, a negative PCR does not completely rule out an infection. Thus, all PCR results must be interpreted in the light of clinical and epidemiological information. Further, uncertainty about the method's true ability to detect the virus was raised by Doctor Katharina Kerner, from Laboklin (e-mail communication). Doctor Kerner, believes that "*[]… either the virus is not in the populations tested or the samples were not the right ones…*", and that "*this is a new sampling method (regurgitate from grasshoppers), so we don´t know yet if that is really working…*".

Table 13. Number of live *P. rhodanica* per site tested for iridovirus in 2023; the number of positive test results is given in the parenthesis.

Site with subpopulation of *P. rhodanica*	Number
BMW	3 (0)
Calissane	5 (0)

La Barben (3 in terrarium, 4 in aviary)	7 (0)
Peau de Meau	7 (1)
Total	**22 (1)**

- **Iridovirus tests on regurgitates of other orthopteran species**

In 2023, Iridovirus test on regurgitates were also performed on other grasshopper species. A total of 64 individuals were tested at the 3 sites where *P. rhodanica* was present (Table 14). All individuals tested negative. As for the CPG, there is some uncertainty with respect to the validity of these results.

Table 14. Number of live individuals per genus and per site tested for iridovirus in 2023. All test results were negative.

Genus	BMW	Calissane	Peau de Meau	Total
Calliptamus	11	14	19	44
Euchorthippus	11	2		13
Oedaleus		4	2	6
Oedipoda		1		1
Total	**22**	**21**	**21**	**64**

In conclusion, to validate this new testing methodology, comparisons of regurgitate, faeces and tissue samples from the same individuals are needed. These are planned for 2024 on other orthopteran species to avoid any impact on the *P. rhodanica* population.

3. Regulatory compliance

The objectives and actions of the LIFE project are already familiar to local and national conservation authorities. Authorisations for breeding and transport have already been obtained. CEN PACA is tasked with requesting permits, and the following institutions are monitoring partners: DDTM13, DREAL, INPN, Scientific Advisory Board of the nature reserve RNNCC.

Once the reintroduction strategy has been finalised and validated, a new request will be submitted at the end of 2023 to DDTM13 to obtain authorisations for reintroduction/translocation.

4. Risk assessment

4.1. Minimising risk to source populations

The exact population size of the three subpopulations remains uncertain, even for Peau de Meau where nearly the entire subpopulation is under study. Due to low catch and recapture numbers, finding suitable analysis models is challenging. Closed models assume that the population is sealed, meaning that no individuals enter or leave during the study period. In contrast, open models allow for individuals to enter or leave the population during the study period (Young and Young, 1998). Estimates of CPG population size vary significantly depending on the CR analysis model used (Table 15). Results from open models indicate larger population sizes compared to closed models, albeit with high confidence intervals. Thus, closed models were used to elaborate the translocation strategy. A revision of models currently used is ongoing with the objective of improving the robustness of population estimates.

Table 15. Estimates of population sizes 2022 and 2023 (CR open and closed model).

Population and year	Area of CR study site (ha)	Estimate pop POPAN - open model	Estimate pop Closed model	Size of total distribution area of subpopulation (ha)
BMW 2022	7.5	270 (±117)	76 (±6)	50
Calissane 2022	9.0	198 (±124)	53 (±5)	220
Calissane2 2023	9.0	466 (±185)	122 (±8)	200
Peau de Meau 2023	8.5	810 (±228)	216 (±13)	6.5

To minimize the risk for the remaining populations, the plan is to primarily obtain individuals from the breeding programme. In 2023, after modifying the breeding methods, around 60 adult individuals were available at the beginning of the adult season. In 2024, however, higher numbers are anticipated, as approximately twice as many egg pods were produced in 2023. Furthermore, with anticipated improvements in breeding success in the open-air aviaries at La Barben and the construction of a new aviary in Calissane mirroring the one in La Barben, higher numbers could be attained. If necessary, captive individuals can be supplemented by catching wild individuals in Calissane, BMW and Peau de Meau. The removal of nymphs from the native population must be conducted in a way to minimize the impact on the wild population.

Unfortunately, there is limited experience regarding the reintroduction or translocation of grasshopper species, resulting in a paucity of data (see annex 3). Insights obtained from *Decticus verrucivorus* reintroduction projects in England suggest that approximately 10 % of the population present during the early adult stage could be safely extracted within a single year (Cheesman, unpublished data). This is contingent upon the population size being large, and the removal of females being delayed until a portion of their eggs has been laid. Similarly, the extraction of 213 nymphs showed no significant effect on the population of a field cricket (*Gryllus campestris*) estimated at approximately 2,000 individuals. Insects generally experience heightened mortality during egg and nymphal development. Based upon the numbers of egg pods produced by female grasshoppers, it can be inferred that a mortality of less than 95% before reaching adulthood would result in a population decline. For the Crau Plain Grasshopper, the average number of eggs per egg pod is 16.88. Assuming a female produces an average of five egg pods, this would yield a total of 84 eggs, only two (2.3%) needing to reach adulthood to secure population stability.

A permit has been issued for the capture of 50 nymphs per year in Calissane for the CPG breeding programme, valid until April 30, 2026. However, in recent years, lower numbers have been captured. To assess the potential impact of nymph removal, a conservative estimate of the total population size for each population was conducted (Tab. 13). Considering the results of the CR, using the conservative outcomes of the closed model with significantly lower estimates than the open POPAN model and extrapolating them to the total areas of the three subpopulations, it is suggested that the removal of 50-60 nymphs in total should not have any adverse effects under current conditions. This is particularly applicable for Calissane, which is likely to sustain over 1,000 individuals, even if we assume that unstudied areas have a lower density than the CR study areas (e. g. only 30 %; see Table 15 and Table 16). A 2010 study in parts of Calissane illustrates the different densities in the study area (Tatin 2010, Annexe 4). Assuming that the outcome of the open model is accurate, there should be no detrimental impact even in years of low-density (e.g. 2022, Figure 5). Nevertheless, to mitigate potential negative consequences in unfavourable years, priority should be given to nymph removal from the largest population (Calissane).

Table 16. Estimates of total population size of subpopulations (open model) as base to estimate impact of capture of wild individuals for translocation.

Population and year	Size of CMR study area (ha)	Estimation of population size POPAN - open model	Size of total distribution area of the subpopulation (ha)	Estimation of total population size - POPAN open model		
				100% (= same density than CMR study area)	30% of density in CMR study area	10% of density in CMR study area
BMW 2022	7.5	270 ±117	50	1800	540	180
Calissane 2022	**9.0**	**198 (±124)**	**220**	4840	1452	484
Calissane2 2023	**9.0**	**466 (±185)**	**220**	11391	3417	1139
Peau de Meau 2023	8.5	810 (±228)	6.5	810		

4.2. Ecological risk

The translocation of *P. rhodanica* in confined to the original range of the species (the Crau Plain) and only to sites where its historical presence has been documented. Thus, no adverse ecological impacts are anticipated. This is particularly true given the relatively low number of individuals being released. The CPG is not known to engage in any potentially negative interactions with other biota and may, in fact, serve as an important food source for birds, reptiles, and other predators. Competition with other grasshoppers is unlikely, as the CPG reaches adulthood earlier than most other grasshoppers. Furthermore, the risk of hybridization in negligible, as related species are absent from the area. While there could be negative effects on the vegetation in the event of mass propagation, this scenario is unlikely based on the observed population dynamics in recent years and decades. Indeed, no such event was recorded since 1951.

4.3. Disease risk assessment

Prior to initiating translocation, it is imperative to conduct a Disease Risk Analysis (DRA) to address the considerable disease risks associated with translocation IUCN, *Guidelines for*

Reintroductions and Other Conservation Translocations. Version 1.0. Rather than aiming for risk elimination, wildlife DRA focuses on risk reduction, allowing for solutions that mitigate risks while aligning to stakeholders' goals.

Detecting disease-carrying agents in many invertebrates is difficult due to the limited knowledge on the symbiotic microorganisms they may carry. While screening "healthy" wild-caught individuals could provide background information, practical constraints often hinder this approach. In the case of *Prionotropis*, the populations are too small to conduct such research effectively.

The DRA process follows the guidelines published by OIE and IUCN in 2014 (Jakob-Hoff et al., 2014) as well as incorporating the methodology proposed by Sainsbury and Vaughan-Higgins (2012). The DRA will undergo annual updates based on results of new screenings (conducted on captive and wild individuals) and the findings from the reintroduction in the previous year.

4.3.a. Hazard identification

Hazard identification relies on information gathered from the literature, as well as data obtained from sampling and screening captive and wild Crau Plain Grasshoppers (CPG) and other orthopteran species living in the same habitat. The main sources of information for hazard identification are:

- Screening of captive CPGs from 2015 to 2023.
- Screening of wild CPGs in 2023.
- Screening of other Orthoptera species in 2023.

It is important to note that much of the literature on Orthoptera pathology involves publications focused on finding and testing biopesticides in laboratory settings rather than in the wild, often using exposure doses greatly different from captive breeding or wild conditions.

Consideration must extend beyond known pathogens to include apparent commensal parasites, as the pathogenicity of many parasites of free-living wild animals remain unknown. Translocations and/or captivity could disrupt normal host-parasite dynamics through stressors, potentially resulting in disease (Sainsbury and Vaughan-Higgins 2012). Loss of commensal parasites may disrupt host immune regulation (Dargent et al., 2013) and alter host population dynamics. Conversely, the accidental gain or spillover of parasites or pathogens can lead to epidemics and host extinctions.

In addition, captive rearing in an environment with other (particularly non-native) insect species poses a risk of contamination by pathogens or parasites.

Depending on the type of reintroduction planned (captive or wild animals) hazard related to travel, and transport must also be considered. Biosecurity measures will be defined for wild-to-wild translocations not crossing geographical or ecological barriers; The latter theoretically reduce disease risk compared to captive-to-wild translocation. Since Orthoptera are not known carriers of zoonotic diseases (diseases transmitted from animals to humans), zoonotic hazards can be excluded. Two potential non-infectious hazards were identified: pesticides and residues of anthelmintics in the stool and/or urine of grazing sheep. Captive trial showed that Crau Plain Grasshoppers does not seem to consume sheep faeces thus the risk of exposure to anthelmintics is negligible. Pesticides are not used inside the Crau nature reserve RNNCC, but they are used in cultivated land around the Reserve. Hence, it would be preferable to select reintroduction sites far from these areas.

Considering this factors, three main potential hazards have been identified in captive and/or wild populations since 2015:

- The grasshopper iridovirus
- A parasitic Eugregarine protozoan (undetermined)
- A parasitic nematode of the family Mermithidae (undetermined)

Characteristics about these three hazards are listed in Table 17.

Table 17. Main infectious hazards identified for the reintroduction of the Crau Plain Grasshopper *Prionotropis rhodanica*.

	Iridovirus (virus)	**Parasitic Nematodes (Mermethidae)**	**Parasitic Eugregarine protozoan**
Target populations	Captive Wild (it is not clear if it is pathogenic because healthy carriers have been observed)	Captive	Captive Wild (likely as commensal parasite)
Target stages	Juveniles (all stages) and adults	Adults	All
Risk of contagion	High	None	Medium to high
Mortality risk	High despite the presence of healthy carriers	High (captivity)	Low (wild) to medium (captivity)
Other effects	- Decrease in reproduction - Reduced longevity	- Decrease in reproduction - Reduced longevity	-Decrease in reproduction - Weight loss - Reduced longevity.
Healthy carriers/commensalism	Healthy carriers	None	Commensal parasite
Mode of contamination	Oral contamination via food (including cannibalism)	Oral contamination via food	- Oral ingestion of oocysts - Transmission along with egg laying.
Methods of screening	Post-mortem: - PCR on fresh cadavers without preservative. Risk of false negatives. - Histology: Risk of questionable results.	Post-mortem: necropsy.	- Live animals: coproscopy Post-mortem: histology
Treatment	None	None	None
Means of prevention	- Prevention of transmission by other susceptible species (grasshoppers, drosophila, etc.): strict sanitary protocols. - Tests (PCR, histology) on other susceptible species. - Reduction of stress and reinforcement of the immunity of captive populations.	- Selection of food harvesting sites (not wet) - Systematic meticulous examination (magnifying glass) of harvested plants. - Cleaning plants with white vinegar.	- Eugregarines can cause negative effects if CPG are under suboptimal conditions, e. g. stress of transport, overcrowding … Efforts should be made to reduce stress factors.
Remarks	- Several strains of varying virulence. - Thermolabile virus whose replication is inhibited at over 30°C. - Known « cocktail effect », ex. with varoa in bees.	- These nematodes can carry iridovirus. **- Strictly related to captive populations with non-adapted food**	Possible « cocktail effect » with other pathogens when reduced immunity of the host.
Research needed	- Survey on other orthoptera species sharing CPG habitat. - More analysis on captive eggs. - Develop a means of detection on live animals - Study the potential role of thyme (cf. chemotype).	- Develop alternative ways to eliminate eggs of Mermithidae on harvested food.	- Study the presence of Eugregarine in wild population (coproscopy; collect of faeces during CPR sessions).

4.3.b. Disease risk management

The Disease Risk Management (DRM) is designed to minimise the risks associated with disease during the reintroduction of CPG *P. rhodanica*.Information regarding the management of the three identified hazards listed in the previous paragraph, is provided in Table 17, outlining methods of screening, potential treatment, means of prevention, and future research needs. It is noteworthy that one of these three hazards, the infestation of nematodes of the Mermithidae family, exclusively occurs in captive populations. Biosecurity measures are already in place for the capture and captive breeding processes, and these measures undergo a thorough review and adaptation at least once a year. The DRM will undergo annual updates based on the outcomes of new screening and the results from the reintroduction in the previous year. This adaptive approach ensures that the management strategies remain effective and up to date in addressing potential disease risks during the reintroduction efforts.

4.4. Gene exchange

Streiff et al. (2002) studied genetic differentiation of *Prionotropis rhodanica* based on six microsatellite loci. The findings revealed a high level of genetic diversity. Streiff et al. (2005) investigated the demographic status (through a genetic survey) of both *P. rhodanica* and *P. azami*. Results indicate strong genetic drift, with minimal gene flow at the regional scale, consistent with the limited dispersal of this flightless species and the patchy configuration of its habitat. Further, Piry et al. (2018) conducted a study of CPG density and genetic variation based on 11 microsatellite markers on a small area of about 3 km². They found a strong correlation between both gene flow and population density, and habitat quality (high grassland productivity and/or low sheep grazing pressure) pointing to a high sensitivity of the Crau Plain grasshopper to the quality of its grassland habitat.

In the short-term, genetic exchange between the relocation sites and the three subpopulations is hindered by significant spatial distances. The likelihood of that wild CPG still existing in the immediate vicinity of the translocation sites is very low or zero. However, genetic exchange between the remaining populations is more likely to have positive effects on fitness rather than negative ones, especially for the small populations. The absence of related species in the area eliminates the possibility of gene exchange with other species. To facilitate gene exchange the reintroduction can involve specimens from more than one population and high habitat quality should represent an important criterion for the selection of translocation sites.

4.5. Socio-economic risks

No negative socio-economic impact is anticipated for the translocation project. The translocation sites are spatially limited, occurring on relatively small areas (7-8 ha). Furthermore, sheep farmers receive compensation for the suspension of grazing between April and June on the translocation areas (LIFE and/or CAP funds).

In the medium to longer term, considering the context of climate change and drier, hotter years, adapting grazing practices (reducing grazing intensity) on the translocation sites may have a positive impact on vegetation. Less intensive grazing can contribute to maintaining vegetation. Thus, the adjustment of current grazing practices might be necessary to sustain forage resources in the long term.

4.6. Financial risks

There is no financial risks associated with breeding and translocation actions as they are covered by the LIFE project. The continuation of these actions will be financed within the framework of the nature reserve management (RNNCC) and the after-LIFE conservation plan. An action plan at the department level (département Bouches-du-Rhone 13) will be developed within the LIFE project, and potential financiers for further actions include the State (DREAL, MTE), the Region (Conseil regional CR), and the Department (Conseil départemental CD13).

5. Release and implementation

5.1. Selecting release sites and areas

5.1.a. Translocation sites

In the planning phase of the LIFE project, several grazing places were pre-selected in the central region of the Crau, primarily based of the timing of the last observations of *P. rhodanica* (Figure 3). The following places were considered:

- Grosse du Levant and Grosse du Centre: Potential reintroduction sites in a sector where *P. rhodanica* has recently disappeared, with proximity to the Peau de Meau site in 2001 (landowner: CD13);
- Petit Carton et Grand Carton: Potential reintroduction site in a sector where *P. rhodanica* has recently disappeared in 2008 (landowner: CD13);
- Couloubris: Potential reintroduction site in a sector where *P. rhodanica* has recently disappeared in 2012 (landowner: Conservatoire de Littoral);

Two sites at the periphery of the Crau plain were also selected:

- Cabanes Neuves: Landowners are CEN PACA and CD13,
- Poitevine: Features favourable vegetation type and experiences low predation pressure from bird species, with a sheep breeder who is supportive.

On the selected grazing places, sheep farmers were consulted to identify sectors with low grazing intensity that could be suitable for the establishment of a new population of CGP This consultations, part of the LIFE project’s action A2, involved interviews by Perrine Turiez (CA13) (Turiez et., 2023).
Sheep farmers were also queried about their willingness to participate in a reintroduction project and adjust their grazing management on the reintroduction site. Apart from Couloubris and Grosse du Centre, one or more areas were proposed at each site, and an analysis was conducted to identify the most suitable locations for translocation.

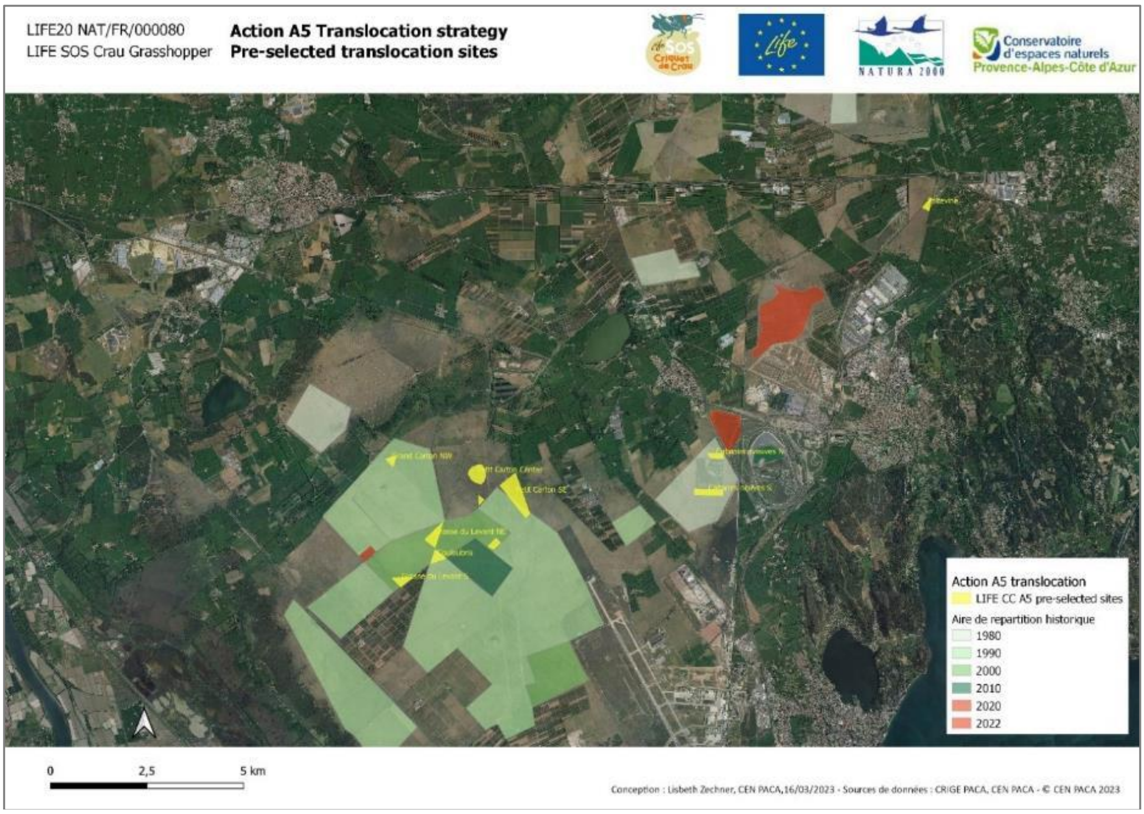


Figure 28. Proposition of translocation sites and historical distribution area of CPG

5.1.b. Selection criteria

To analyse, rank and select translocation sites, information on the following criteria was gathered:

- **Stakeholder**
  - Landowner
  - Support of farmers and shepherds – LIFE action A2
  - Area of the site (ha)
- **Grazing management (CA13, LIFE action A2)**
  - Total surface of grazing place (ha), number of grazing tours
  - Grazing period 2022
  - Grazing pressure 2022 (*JPB, Journées de Pâturage Brebis)*
  - Days of Grazing per ha and year
  - Degree of uniformity between years
  - Development of grazing pressure
- **Vegetation**
  - Vegetation high and density, stone, and bare soil cover, etc. (Bröder et al., 2019), complemented with vegetation surveys in 2023 (Hauprich, 2024)
  - Vegetation height June 2022 (average per site) – LIFE action A1

- **Predation risk by bird species (LIFE actions A3 and D2)**
    - Distribution of birds with sheep flocks (*B. ibis*, etc., Bröder et al., 2023) - analysis of observations on 08/05/22 = predation pressure (see map)
    - *F. naumanni*: average of species presence GPS data, Kernel 50 – GPS data, number of breeding pairs 2022
    - Sheepfold with *Corvus monedula*: number of nesting places
- **Crau Plain Grasshopper (LIFE actions A4, C3, D3 and D4)**
    - Health risks (iridovirus, parasites, etc.): distance to subpopulation
    - Historical presence of *P. rhodanica*: if present, period of disappearance of the species in the sector - important if two sites have same conditions, the site with a more recent observation could be preferred.
    - Dispersal possibilities (0 – 360 degree) = suitable habitat
- **Pollution and other disturbance**
    - Pollution by pesticides - distance of orchards and compass direction (mainly wind direction: NW -> SE)
    - Vermifuge treatments: no recent data
    - Presence or planned construction of infrastructures, such as highways, pipelines or high-voltage power lines in the vicinity of the translocation site.

Detailed information on each criterion and the translocation sites can be found in Annex 5.

5.1.c. Selection and ranking of selection criteria

During the expert workshop, a thorough review and discussion of the individual criteria and information took place, leading to the ranking of criteria in order of importance (Table 18). Experts in attendance identified the following criteria as particularly significant (the top three are highlighted in bold):

- **Stakeholder/landowner**
- Support of farmers and shepherds
- **Grazing pressure 2022**
- Vegetation height June 2022
- **Dispersal options**

Table 18. Selection and ranking of selection criteria for translocation sites. Selection: 1 = yes, 0 = no; Priority: number of votes (7 participants).

Criteria	Selected	Priority	Arguments
1. STAKEHOLDER			
Landowner	1		
Support of farmers and shepherds	**1**	**7**	
Potential translocation sites : surface per site (ha)	0		
2. GRAZING MANAGEMENT			
Surface	0		number of circuits
Grazing period 2022	0		
Grazing pressure 2022	**1**	**6**	
Degree of uniformity between years	0		
Grazing pressure evolution	0		future more important than past
3. VEGETATION			
Cover + height vegetation - Bröder et al. 2019	0		data not for all sites available, data 2023
Vegetation height June 2022	1	2	national model ? (Dominique Courault, INRAe Avignon)
Thymus sp. degree of coverage	0		
4. PREDATION RISK BY BIRDS			
Distribution of birds with sheep flocks - 08/05/22	0		
F. naumanni : presence	1	3	
F. naumanni : Kernel 50	0		
F. naumanni : number of breeding pairs 2022	0		
Sheepfold - *Corvus monedula* : no of nesting places	0		
5. CRAU PLAIN GRASSHOPPER			
Health risks (iridovirus, parasites, etc.)	0		
Historical presence	0		
Dispersal options (0 - 360 degree)	**1**	**4**	
6. POLLUTION			
Insecticides - proximity to orchards	0		

5.1.d. Final selection of translocation sites

Table 19 illustrates the final selection of translocation sites, with the sites in Petit and Grand Carton grazing places deemed favourable according to the chosen criteria. In early summer 2023, complementary vegetation surveys were conducted by two students from Trier University (A. Hauprich and J. Gröbel) involving 30 randomly distributed points per area. The recording area per point comprised a circle with a diameter of 30 cm and a square of 1 m). These surveys aimed to provide more detailed information on the vegetation at the translocation sites compared to Calissane and Peau de Meau (Hauprich, 2024; see annexes 1 and 2). Initial results show that among the three studied translocation sites, Grand Carton appears to be the least suitable. The area has the lowest proportion of green vegetation, primarily consisting of *Brachypodium retusum,* and thus lucks diversity. In addition, the site has the highest cover of stones. According to Bröder et al. (2019), areas characterised by high stone cover were associated with the disappearance of the decline of the Crau Plain Grasshopper

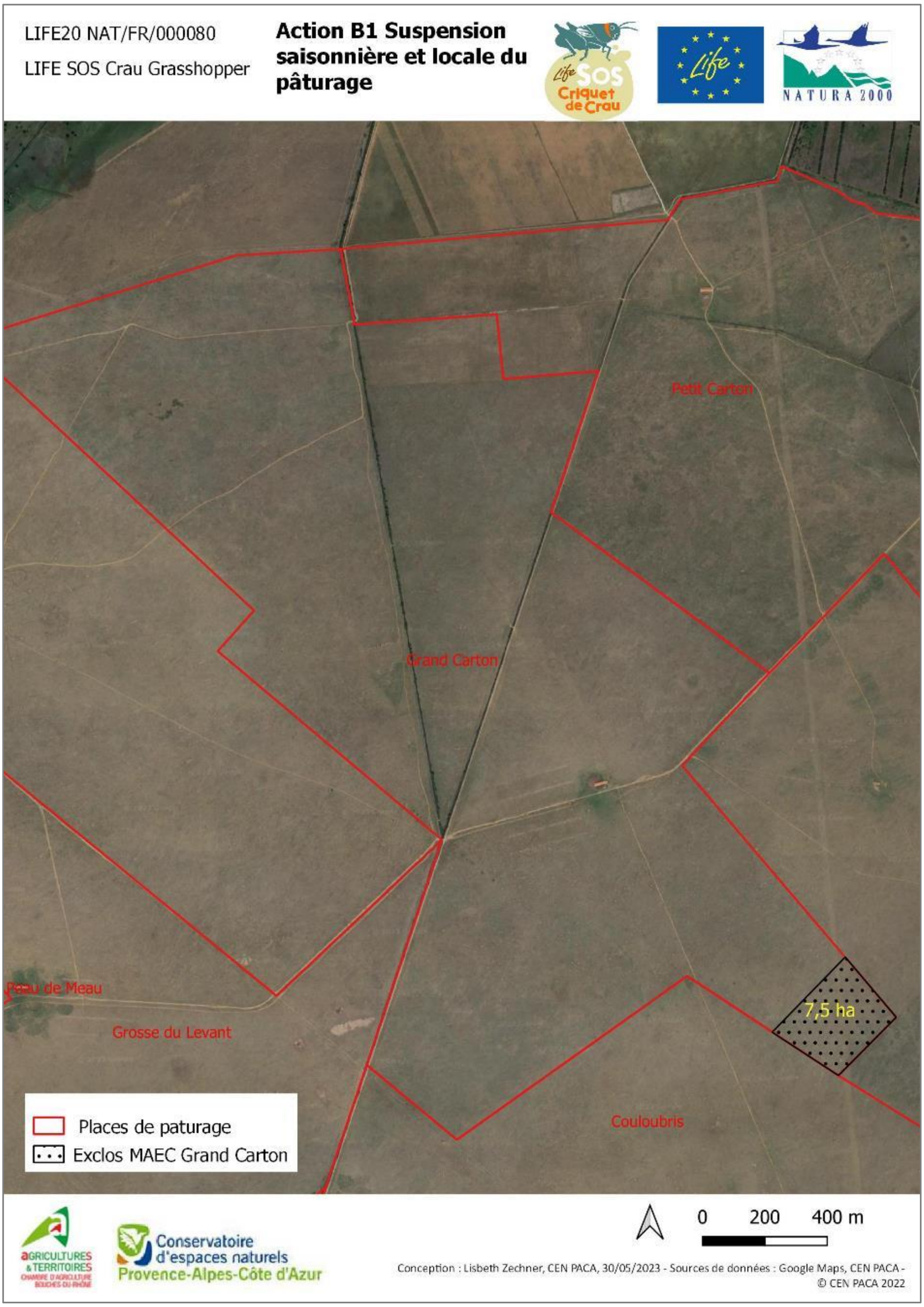
The Petit Carton site is similar to the Calissane reference site in terms of stone and litter cover as well as spring vegetation cover. However, summer vegetation cover (during the CPG adult period) in petit Carton is lower than in Calissane. Additionally, the area is also less diverse than Calissane, with *Brachypodium retusum* dominating the vegetation.

The Poitevine site emerges as highly favourable overall. Among all the sites, it has the highest vegetation cover, nearing almost 50 %, a value indicates good conditions for the grasshopper. Importantly, the vegetation at Poitevine is quite heterogeneous. Despite this favourable vegetation characteristics, it is important to note that Poitevine has been deemed less suitable for translocation by experts (Table 19) based on its location on the periphery of the Crau and its limited size, encircled by a road and a water channel. Indeed, the constraints posed by these features make it impossible for the species to disperse effectively. Further, the site is also threatened by a highway construction project.

Table 19. Final selection of translocation sites according to five criteria. Green = favourable; red = unfavourable.

	GROSSE DU LEVANT		PETIT CARTON		GRAND CARTON				CABANES NEUVES		POITEVINE
Criteria	NE	S	CENTER	SE	NW	E	SE	SW	N	S	E
1. STAKEHOLD-ER			to discuss with sheep breeder and CD13	to discuss with sheep breeder and CD13					military site – is access possible?	military site – is access possible?	sheep breeder wants to participate
2. GRAZING MANAGEMENT	ok in the triangle, but high outside	ok in the triangle, but high outside	ok	ok	ok	ok	ok	ok	high grazing pressure	high grazing pressure	ok
3. VEGETATION	ok	ok	very good, similar to Calissane	very good, similar to Calissane	quite good	quite good	not so good, also surroundings	quite good	not good, see grazing pressure	not good, see grazing pressure	quite good
4. PREDATION RISK BY LESSER KESTREL	quite low	quite high	low	low	low	low	low	low	low	low	no presence of LK
5. CGP DISPERSAL POSSIBILITIES	<180 °	<180 ° dispersal, Grosse du Centre heavily grazed	360 °	290 °	290 °	360°	360°	180°	<180 ° dispersal, surrounding heavily grazed, fenced military site not grazed but access not allowed	<180 ° dispersal, surrounding heavily grazed, fenced military site not grazed but access not allowed	too small, isolated, no expansion possible

In collaboration with shepherds and the landowner (CD13), a specific area was selected within Petit Carton (10 ha) and another within Grand Carton (7,5 ha) grazing places; These areas have been included in the CAP application of the farmers (Figure 29 and Figure 30).


LIFE20 NAT/FR/000080
LIFE SOS Crau Grasshopper
Action B1 Suspension saisonnière et locale du pâturage
Petit Carton
Grand Carton
Peau de Meau
Grosse du Levant
7,5 ha
Couloubris
Places de paturage
Exclos MAEC Grand Carton
0 200 400 m
Conception : Lisbeth Zechner, CEN PACA, 30/05/2023 - Sources de données : Google Maps, CEN PACA - © CEN PACA 2022

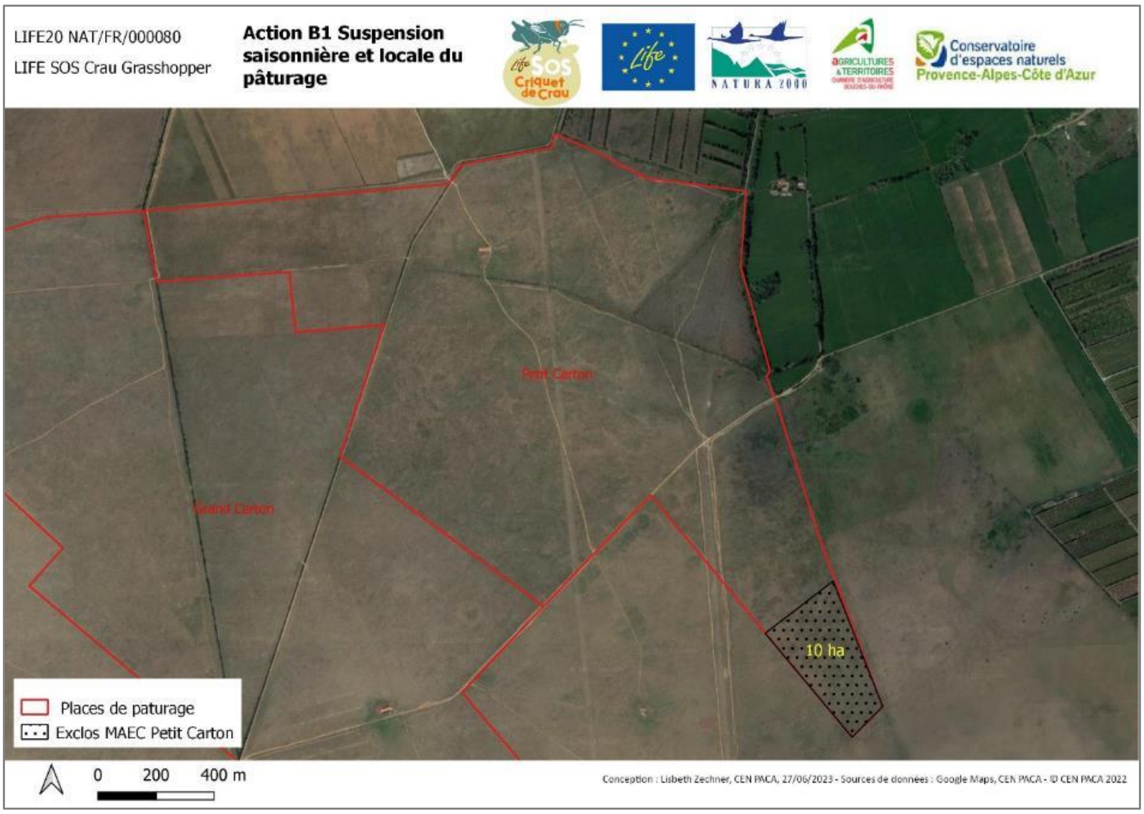


Figure 29. Selected translocation site in Petit Carton (10 ha).

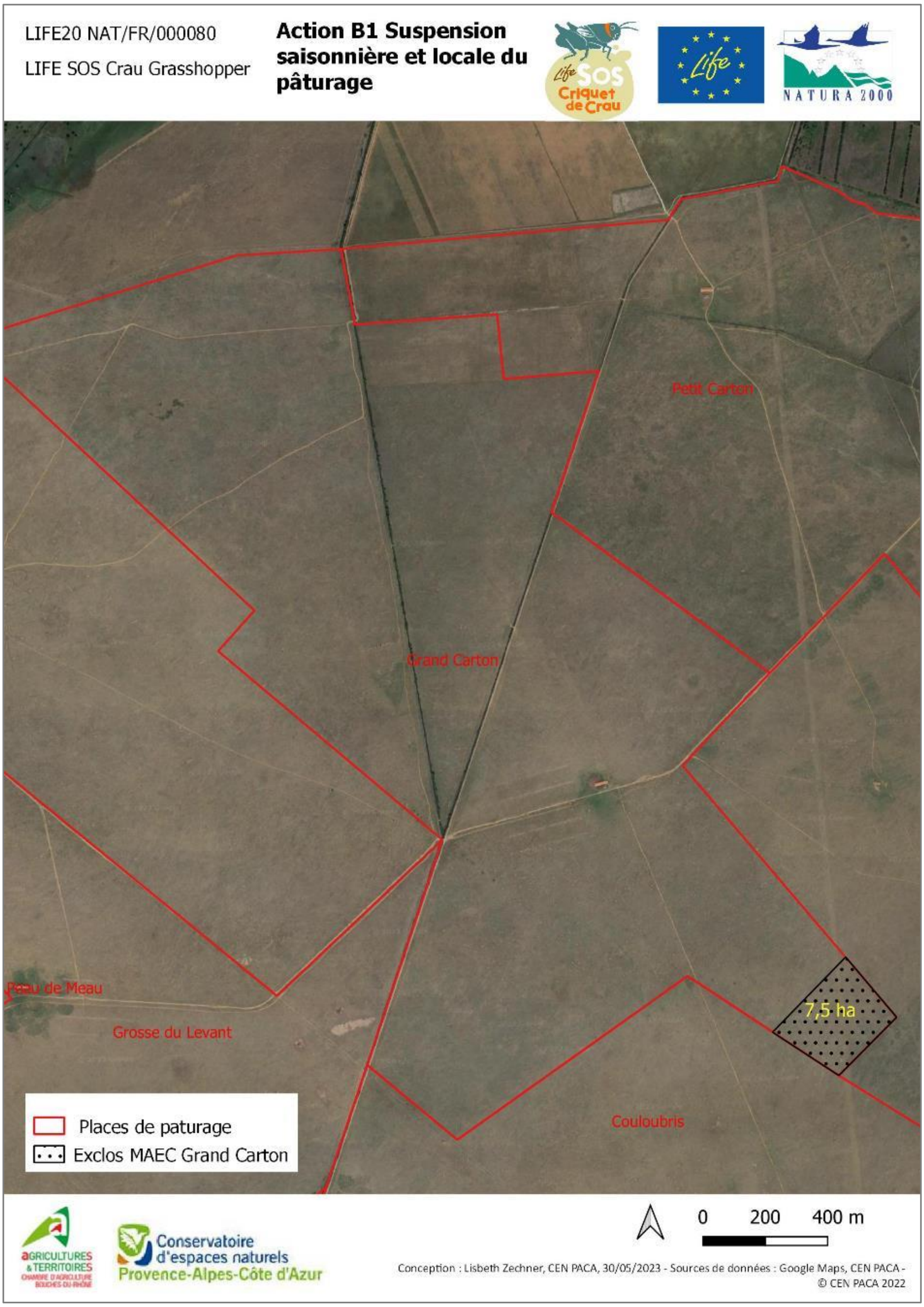


Figure 30. Selected translocation site in Grand Carton (7.5 ha).

5.2. Release strategy

Details of the release strategy were discussed during the expert workshop in March 2023. The advantages and disadvantages associated with releasing different stages are outlined in annex 2. A summary of the decisions is presented in Table 20.

Table 20. Details of the release strategy of the Crau Plain Grasshopper discussed by the expert panel.

Implementation	Questions and discussion	Decision taken during workshop March 2023
No of ind. per release	Release of 30 or, 50 adults?	60 individuals (6 groups of x 10 individuals)
No of sites	LIFE action C3: Number of sites for translocation (2 to 3)	Translocation in 2024 to 1 site only due to the anticipated number of adults available. 1-2 alternative sites should be indicated?
Number of releases / no of years with releases	How many releases per year? For how long (1 or several years)?	Release at least in 2024 (n) and 2025 (n+1) At year n+1 nymphs should be monitored; based on the monitoring outcome, subsequent released may be planned (year n+2 and more)
Sex ratio ♀♀:♂♂	1:1?	1:1
Life stages / season of release	Release of egg pods after laying in summer, juveniles in April/May or adults (end of May?) Information on the advantages and disadvantages concerning the release of different life stages is found in Appendix 2.	Release of young adults from the in-situ breeding programme (aviaries) If necessary, capture of wild nymphs (to be released at adult stage after breeding in the aviaries) as well as egg pods
Genetic diversity	In case of translocation of wild individuals: should they be captured from the 3 current subpopulations?	Yes, pending negative testing to iridovirus
Captive - wild stock		Depending on the results of presence of iridovirus in 3 wild subpopulations
Factors / indicators of success or failure	How is translocation success measured?	Translocation is considered as successful if the released population is stable or increases through time

5.2.a. Release numbers and sex ratio

The initial goal of the LIFE project was to introduce at least 80 to 100 individuals at each of the 2 or 3 reintroduction sites aiming for a 10% expansion in range during the LIFE project.
In light of the challenges encountered during the breeding programme, figures were revised in view of the anticipated low number of available individuals. The adjusted plan involves releasing 60 adults complemented 30 egg pods per sites. Adults egg pods will be released at a single site, for two consecutive years at minima. The release of additional individuals may be considered if hatching and survival rates in the aviaries are higher than expected. A sex ratio of 1:1 was chosen to ensure reproduction of all females.

5.2.b. Use of individuals hatched in the outdoor aviaries for translocation

To minimise the risk of iridovirus transmission and to prevent additional captures in the wild, individuals hatching in the outdoor aviaries exclusively should be used for translocation. These include the aviary in La Barben and the upcoming aviary in Calissane scheduled for construction in March 2024.

Starting this year, the old Crau aviaries (Calissane and Cabanes Neuves) will no longer be used for hatching and translocation. Indeed, all egg pods currently undergoing incubation in these aviaries will be transferred to the new aviary in Calissane (approximately 60 egg pods) and to La Barben and Besancon breeding stations (approximately 15 egg pods each) before hatching. A small proportion (about 35 egg pods) will be left in place to supplement the breeding stations later on, in case of low hatching or high mortality rates in captivity (see Table 21).

Table 21. Destination and use of egg pods currently undergoing incubation in the Crau old aviaries.

Destination	Number	Objective
Calissane new aviary	60	Translocation in May 2024
Cabanes Neuves existing aviary (no transfer)	35	Stock of egg pods for the breeding stations
Indoor breeding station La Barben	15	Continue the developmental cycle: supply of adult for ex-situ breeding and supply of egg pods for in-situ aviaries and future translocation (2025)
Indoor breeding station Besançon	15	
TOTAL	**125**	

Based on the outcomes of in-situ breeding in the La Barben aviary in 2023 (24 adult observed from 13 egg pods), it is anticipated that Calissane will yield around 80 adults or more from the 60 transferred egg pods. Individuals will be released at the stage of young adult (before reproduction) to maximize the number of on-site egg pod laying.

Concerning individuals from the 2024 hatching season in the breeding stations, they will be used exclusively for egg pod production to ensure the continuation of the ex-situ breeding program and to supply in-situ aviaries for a new translocation action in 2025.

It is important to emphasize, that ensuring a substantial stock of individuals in the ex-situ breeding station is crucial for the success of the project, whose long-term objective is to achieve the full developmental cycle of *P. rhodanica* in captivity.

5.2.c. Use of wild juveniles and adults for translocation

Where feasible, the individuals destined to translocation should be supplied by the in-situ aviaries (see above). However, if necessary, the translocation may be supplemented with wild individuals which can be captured annually with a maximum limit of 50.

Thus, two scenarios may be considered:

- The translocation of 60 individuals from the breeding stations with no additional capture of wild individuals.
- The translocation of 60 - n individuals from the breeding stations supplemented by n individuals captured in the wild (with maximum n = 50).

In the latter case nymphs may be captured in the wild and reared in the in-situ aviaries until they reach the adult stage, at which point they would be released again. Alternatively, young adults may be directly translocated from one site to another (although it is assumed that capturing adults has a more significant negative impact on the wild population compared to capturing juveniles). Wild individuals will be captured either from Calissane only or from all sites where populations are presents, with a bigger proportion being captured from Calissane (which hosts the biggest population) and a lower proportion form Peau de Meau and BMW (10 individual each maximum), with the objective of increasing genetic diversity.

5.3. Management of reintroduction sites

The management approach applied to the Peau de Meau area is considered as a model. Implementing exclusion from grazing, combined with the closure of Lesser Kestrel nest boxes appears promising:

Since 2015, the smallest of the remaining subpopulations has been temporarily fenced during the presence of the Crau Plain Grasshopper from April to the end June. Additionally, most the nest boxes of Lesser Kestrel in the surroundings of the subpopulation have also been closed during this period. The fencing serves to enhance habitat quality by reducing grazing pressure (Piry et al., 2018; Bröder et al., 2019) and reduce predation pressure, from predators associated with sheep flocks. These measures have been effective in reducing predation pressure by Cattle, while occasional foraging by crows within the fenced area has been noted (CEN PACA, 2020, Godefroid and Dusfour 2022, Bröder at al., 2023) except for the black Crow which showed a preference for the fenced area. Despite the closure of artificial nest boxes, at least one or two pairs of Lesser Kestrel have continued nesting close to Peau de Meau subpopulation until 2022. Although occasional observation of individuals outside the fence have been made in 2023, these occurrences remain rare, and it is assumed that almost the entire population is still within the fence.

In line with Peau de Meau approach, the translocation areas will be fenced with mobile electric fences from the beginning of April to the end of June (LIFE action B1 and/or CAP funding). Material for this purpose is already available for one site, and additional electric fence equipment should be financed within the LIFE project (LIFE action B1). In addition, proposals for reducing grazing pressure around translocation sites will be presented to shepherds.

Considering the low presence of the Lesser Kestrels, the relocating the nest boxes is not an immediate necessity. Further actions in this regard will be contingent on monitoring results.

5.4. Monitoring programme

5.4.a. Population monitoring

Monitoring of the CPG population dynamics is crucial to ensure the success of the translocation and understand the population size trend. The following monitoring plan, suggested by the experts during the workshop, will be implemented within the framework of LIFE action D4 and the "After LIFE conservation plan":

Year n:

- Before translocation, conduct a search of CPG individual, to check for the presence of the species.
- After translocation, conduct a search of CPG individuals to confirm its presence.

Year n+1:

- Conduct a search of CPG individuals to confirm CPG presence.

Year n+2 and later:

- Conduct a comprehensive capture-recapture study to obtain population size estimates.
- If necessary, perform additional surveys using alternative searching methodologies.

To assess the success of translocation a certain number of individuals could be equipped with transmitters and monitored over time. A study on mark loss conducted in 2007 (Besnard, 2007) did not revealed any increased mortality associated to transmitters. However, some experts caution that transmitters might enhance the visibility of grasshoppers, thereby attracting predators. Additionally, the increased human presence may create a constant disturbance in the field and further attract predators.

5.4.b. Health and mortality monitoring

Post-release health monitoring will be conducted according to the IUCN recommendations (IUCN, 2013), aiming to facilitate the early detection of potential pathologies that might impact the health and survival of both introduced and existing populations. However, monitoring *Prionotropis rhodanica* faces practical challenges primarily due to the difficulty of locating these animals. Their effective camouflaged within the vegetation and stones, coupled with their lack of song makes them exceptionally elusive.

Objectives:

- Ensure that reintroduction sites and endogenous fauna are free from contamination by exogenous agents.
- Ensure continued healthy of reintroduced populations.
- Monitor the emergence of any unknown diseases.

Methodology:

Post-release health monitoring will include clinical examinations, post-mortem examinations, sampling and investigation of any suspected outbreaks of disease. The specific methods employed will depend on the results of the monitoring of population trends.

In addition, other Orthoptera species present at the release sites will also be monitored if deemed necessary, particularly in the event of unusual mortality.
Rigorous hygiene practices will be followed during field surveillance, including hand disinfection upon arrival at the site and between each contact with an individual.

- Clinical examination

Grasshoppers will be captured using transparent plastic boxes to facilitate visual examination and observation of their behaviour.
A standardised examination protocol will be developed, and all relevant information will be recorded on an examination sheet.
Sample size will be determined based on the prevailing context.
Ectoparasites and faeces may be collected in the capture box.
If necessary, spit may be collected to investigate the presence of iridovirus.

- Post-mortem examination

All CPG carcasses found on or near the release site will undergo post-mortem examination. A detailed protocol will also be established, and observations will be recorded on a special form. Depending on these observations, the carcasses may undergo laboratory analysis.
These examinations will be conducted throughout the LIFE project period (action D.3) and as part of the "After LIFE conservation plan".

5.4.c. Monitoring of grazing practices and vegetation parameters

Monitoring of grazing practices and vegetation parameters started in 2023 as part of the LIFE action D1, reported by Gidoin (2023). Monitoring will continue throughout the LIFE project duration and within the framework of the "After LIFE conservation plan".

5.4.d. Monitoring of insectivorous bird species

Monitoring using camera traps will be implemented on the translocation plots, mirroring the approach used in Peau de Meau 2022. The decision on the extent to which camera traps will be used before translocation, as recommended by the participants of the expert workshop, will depend on financial resources and the feasibility of automatic image analysis (LIFE action D2 and the After-LIFE conservation plan).

5.5. Alternative (exit) strategy in case of major problems

If success is not achieved in the first year, efforts will be made to improve the breeding programme to increase the number of individuals designated for translocation.

5.5.a. Breeding success and number of individuals for translocation

If an improvement of the breeding programme is not achieved by the end of the LIFE-project (September 2025), a reassessment will be required to determine the justification for continued investment of resources in the programme, which has been ongoing since 2015. As an alternative, a simpler and more cost-effective option, potentially more successful, could involve the direct transfer of wild individuals (from Calissane, BMW, Peau de Meau) with the capture of nymphs reared until adult stage in well-constructed aviaries in the Crau.

5.5.b. Problems with translocation sites

If it becomes evident that the chosen translocation sites are less suitable than expected, perhaps due to challenges such as unmanageable predation by insectivorous birds, inappropriate vegetation structure, or modifications in grazing management, alternative options will be considered. Conditions may indeed become more favourable in other sites, including those initially excluded from the initial selection of translocation sites. This could result from changes in grazing management and practices, successful habitat restoration actions, and other factors.

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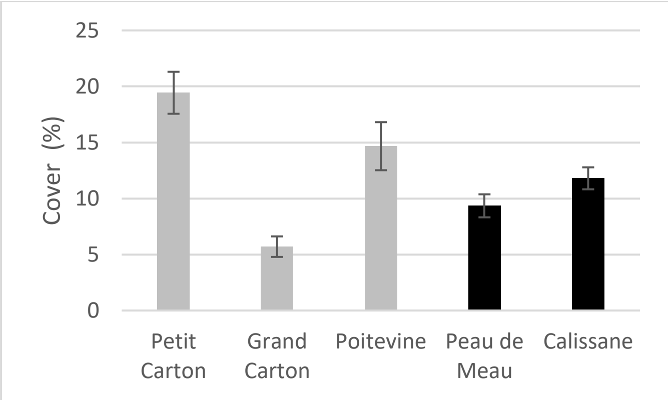
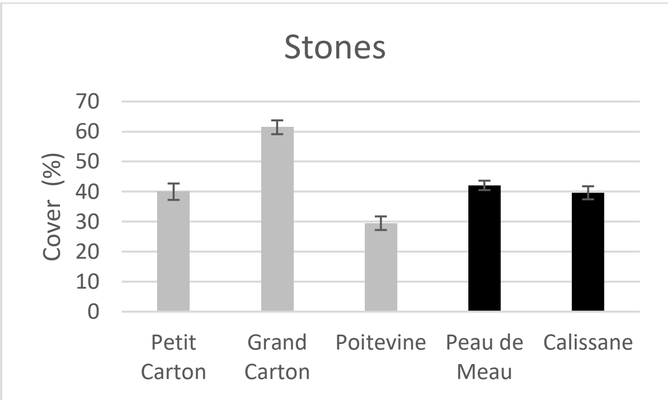
Annexes

Annex 1: Vegetation analysis 2023

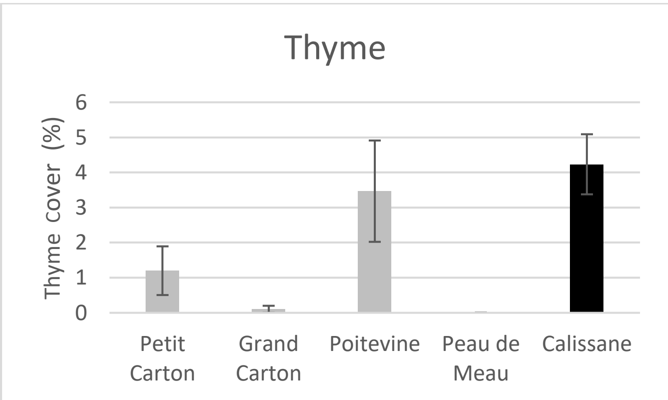
Percentage of bare ground cover per site



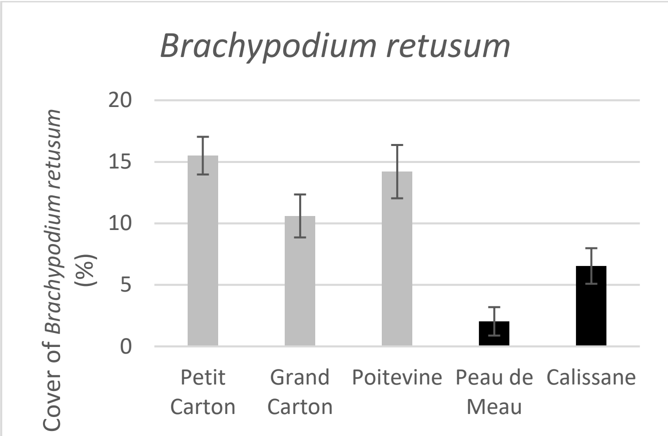
Percentage of stone cover per site



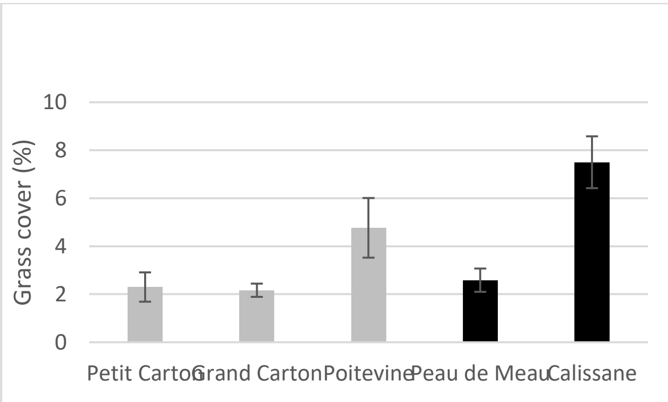
Percentage of Thyme cover per site



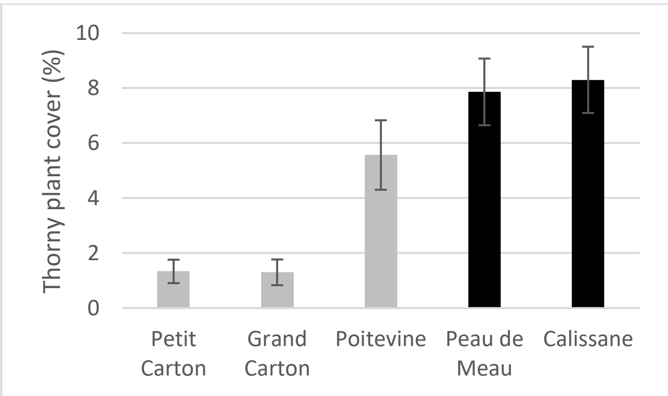
<u>Percentage of cover of *Brachypodium retusum* per site</u>



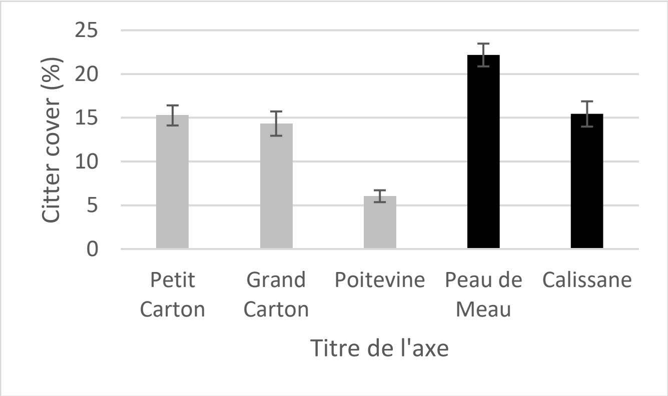
<u>Percentage of grass cover per site</u>



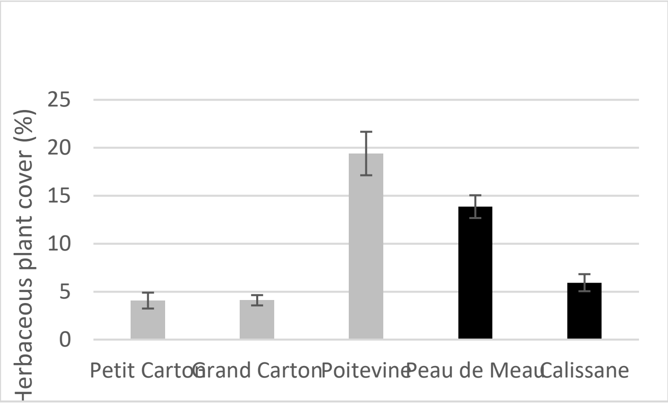
<u>Percentage of thorny plant cover per site</u>



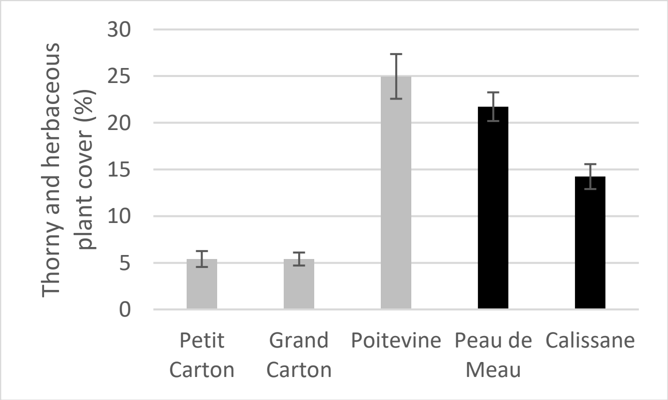
Percentage of litter cover per site



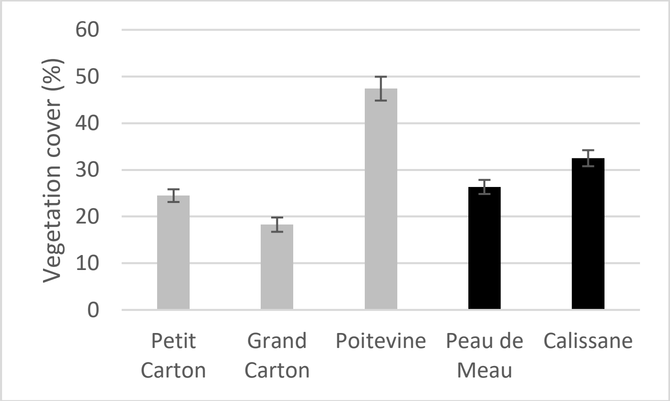
Percentage of herbaceous cover per site



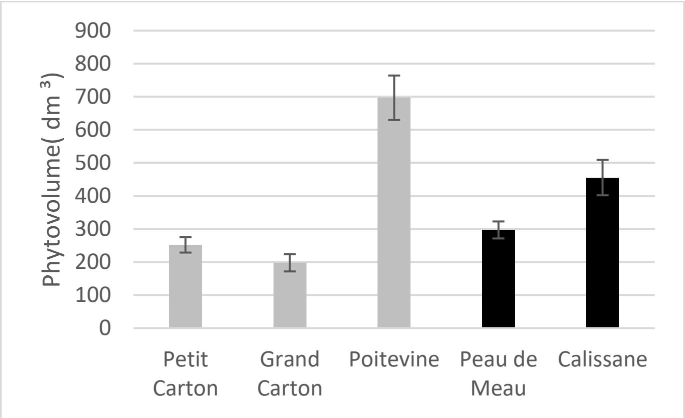
Percentage of thorny and herbaceous plant cover per site



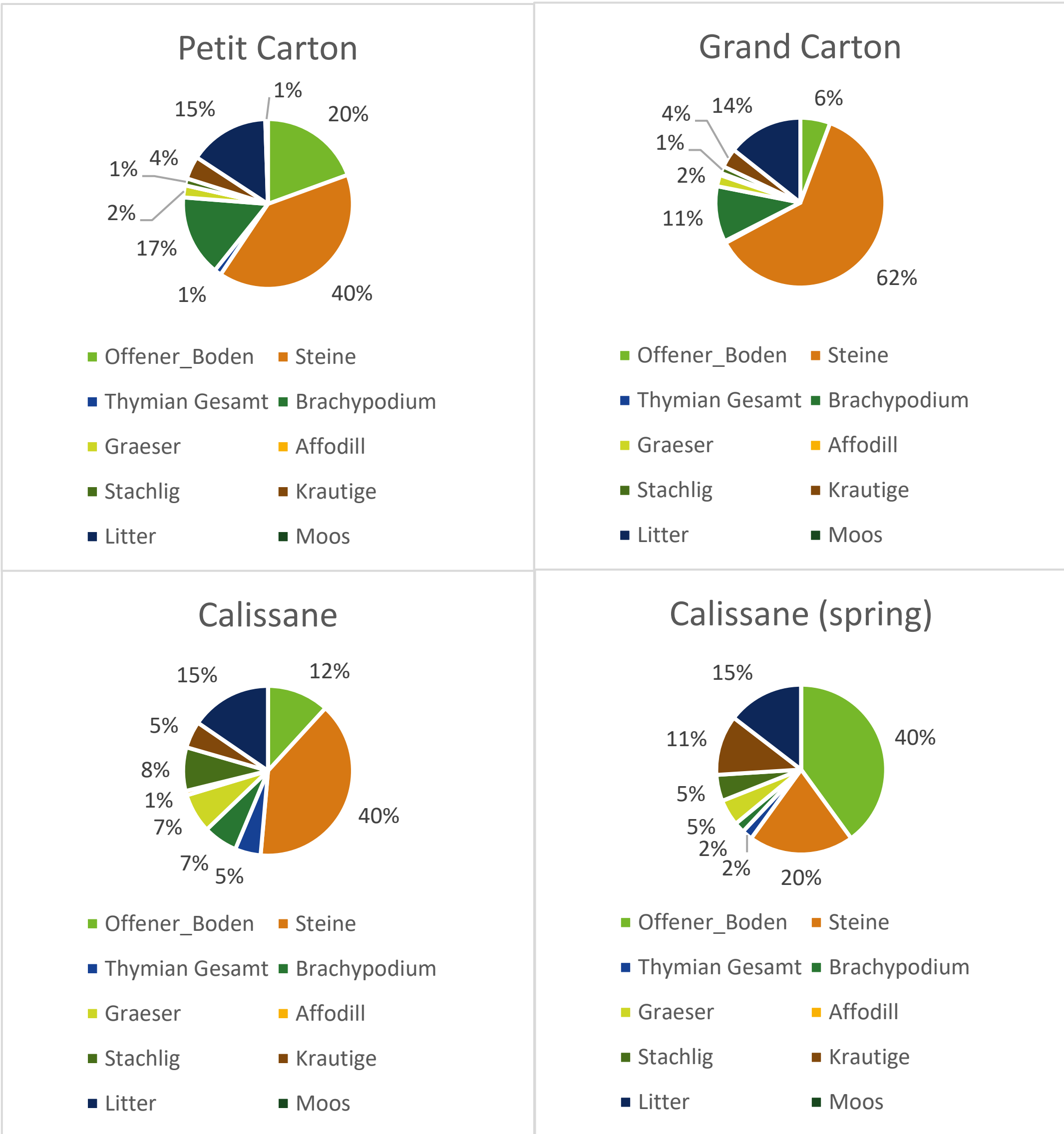
Percentage of (green) vegetation cover per site

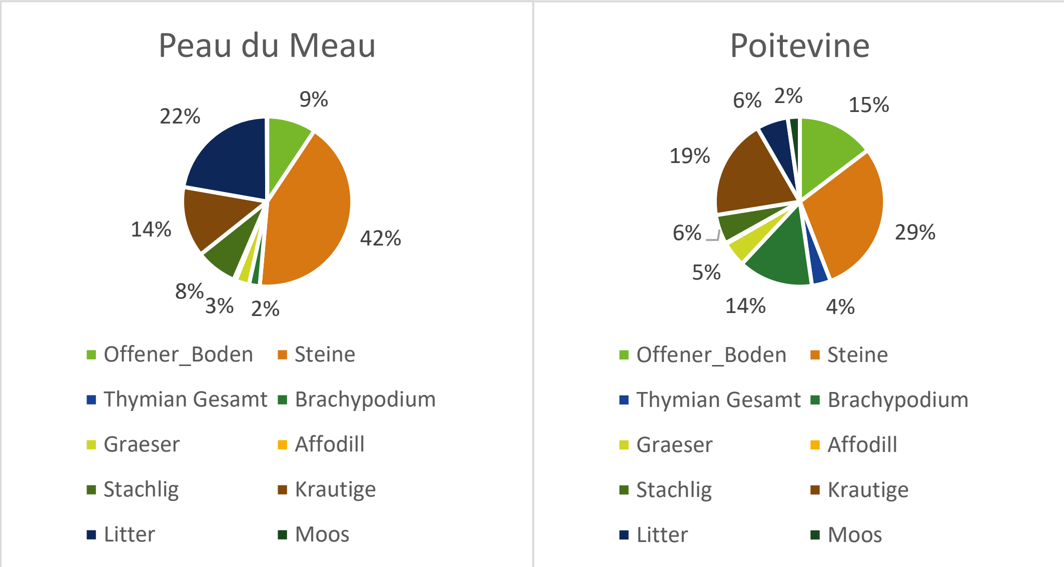


Phytovolume per site

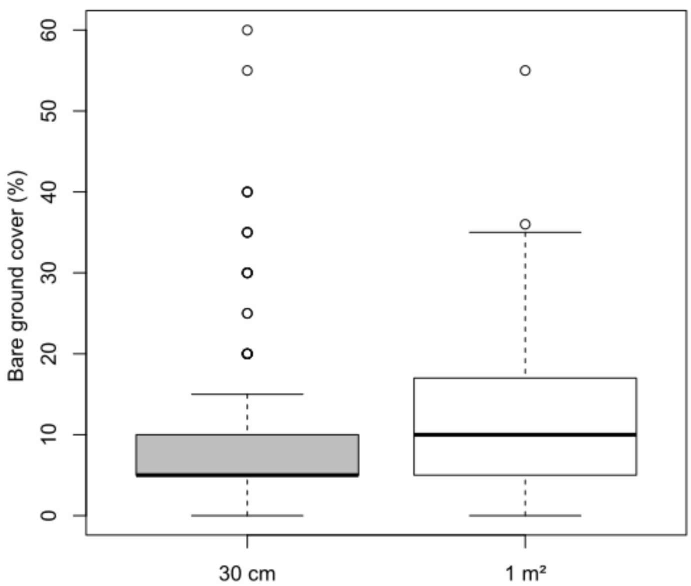


<u>Percentage of bare ground cover (Offener Boden), stone cover (steine), Thyme cover (Thymian Gesamt), *Brachypodium* cover (*Brachypodium*), grass cover (Graese), Affodile cover (Affodile), thorny vegetation cover (Tachlig), herbaceous cover (Krautige), Litter cover (Litter) and Moss cover (Moos) per site.</u>





<u>Comparison of mean bare ground cover in 30 cm and 1m² quadrats</u>



Annexe 2. Vegetation analysis 2023 - Principal component analysis (PCA)

The number of variables is reduced and only the most important ones are retained: This allows me to show well which of the variables are really different between habitats and settlement areas

If I turn the whole thing around, I get an indication for each of the 89 surfaces how well it is explained by the respective component: This is simply far too confusing! If I reduce the data set beforehand by forming the mean values of each surface, I can compare them better and see which surfaces look most similar! Unfortunately, the results were rather ugly, since the first principal component, which explains the most, has negative values for all surfaces.

Linear method, therefore, possibly not as suitable as NMDS

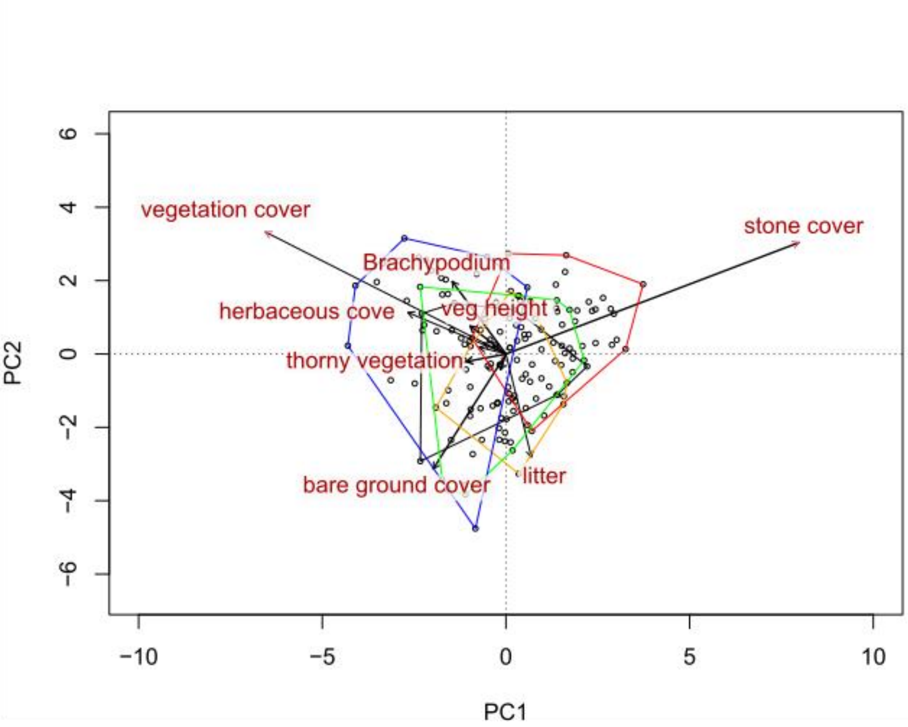
<u>PCA for the variables: Shows redundancies and how much variance is explained between areas.</u>

1st principal component explains 45.12% variance and seems strongly positively influenced by stones.

2nd HK strongly negatively influenced by *Brachypodium.*

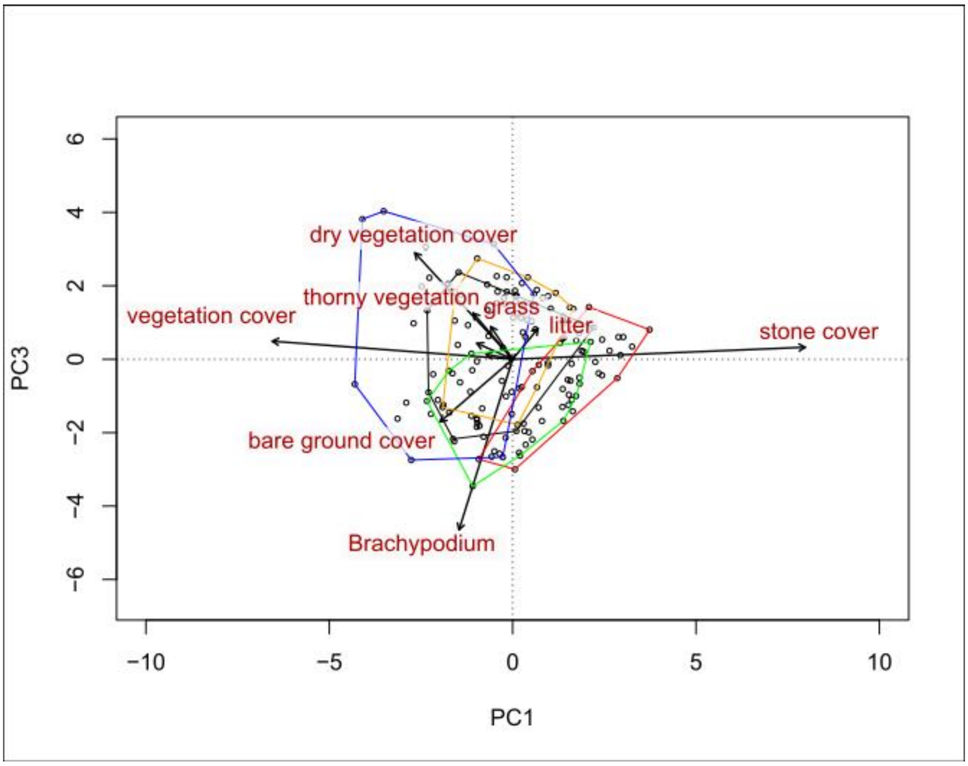
3. HK marked by herbaceous plants (negative) and litter (positive).

<u>Principal component analysis of sampled points according to the measured vegetation variables showing sampled points along PC axes 1 and 2. Sample points distribution in the ordination space is encircled by polygons which are color-coded according to sites as follows: black for Calissane, orange for Preau de Meau, blue for Poitevine, green for Petit Carton, and</u>



red for grand Carton.

Principal component analysis of sampled points according to the measured vegetation variables showing sampled points along PC axes 1 and 3. Sample points distribution in the ordination space is encircled by polygons which are color-coded according to sites as follows: black for Calissane, orange for Preau de Meau, blue for Poitevine, green for Petit Carton, and

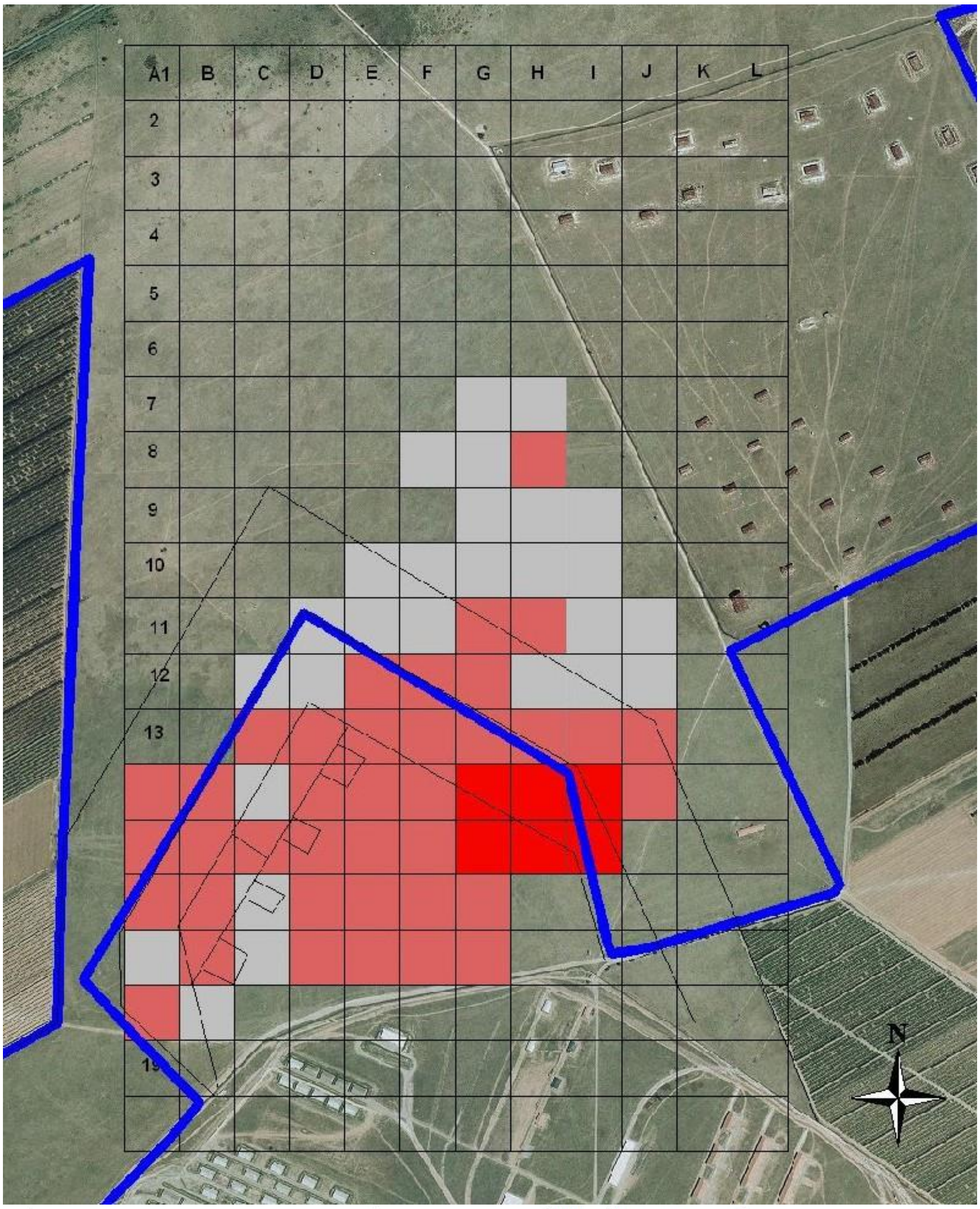


red for grand Carton.

Annex 3: Examples of reintroduction / translocation projects of orthoptera species

EXAMPLES	***Prionotropis azami*** **France (Braud 2020)**	***Epacromius tergestinus*** **Switzerland (Werner 2005)**	***Oedipoda germanica*** **Germany (Köhler 2017)**	***Gryllus campestris*** **Germany (Hochkirch et al., 2016)**	***Decticus verrucivorus*** **LYDDEN England (Cheesman, in prep.)**	***D. verrucivorus*** **MOUNT CARBURN England (Cheesman, in prep.)**
No of sites	1	1	2 (distance 20 m)	2 (distance 20 m)	1	3 (W, SW, SE)
No of years w. releases	1 (2019)	1 (2004)	1 (2002)	1 (2001)	4: 1993-1996 1998	4: 1995, 1997, 1999, 2000
Captive - wild stock	wild	wild	wild	wild	wild and captive	captive
Life stages	adults	nymphs	nymphs (instar 2-5), 1 adult	nymphs (instar 7–8)	late instar nymphs, ad.	mid- to late instar nymphs, adults
No of ind. per release	122 adults on 11/06/19. 70 adults on 19/06/19	37 nymphs 03/07/04	12 ♀♀ and 6 ♂♂ // 8 ♀♀ and 4 ♂♂ on 13/07/02	213 individuals // 31/07/2001	1993: 54 adults (wild) 1994: 121 nymphs/adults (118 captive; 3 wild) 1995: 307 nymphs/adults (captive) & 14 adult ♀♀ (wild) 1998: 1 adult ♀	1995 W: 50 nymphs, SW: 29 nymphs; 1997 W: 28 adults; 1999 W: 25 nymphs/adults; 2000 SE: 45 nymphs
Sex ratio ♀♀:♂♂	?	?	2:1	?	1993: 1:1 bias towards ♀♀ in 1994, small bias towards ♂♂ in 1995	some bias towards ♂♂ in 1995, towards ♀♀ in 1997, 1999 and 2000
Factors of success or failure	20/06: mortality of 8 ind. (underfeeding before release + high temperature?)	habitat quality?	??	habitat quality & heterogeneity, weather conditions, demography of species	extent and quality of favourable habitat (and related factors, such as predation pressure), in interaction with the size of the population, not by population size alone.	
Success / failure	Failure?	Success	Failure	Success	Success	Success

Annexe 4: Spatial characterisation of *Prionotropis h. rhodanica* in the Calissane (Miramas, 13)



Cellule vide = non prospecté

Légende

- Densité faible ou absence
- Densité moyenne
- Densité forte
- Emprise projet 4ème RMAT Miramas
- Limite RNCC

Echelle: 1 cellule = 100 x 100 m

	Effectifs	unités
Prionotropis déctectés	244	individus
Prionotropis géoréférencés	83	individus
Quadrats prospectés	71	ha

Annex 2: Selection criteria for translocation sites

Categories	very good	good	moderate	bad

Criteria	Grosse du Levant		Grosse du Centre	Petit Carton		Grand Carton			Couloubris / GC	Cabanes neuves		Poitevine (Regarde-venir)	Peau de Meau	Calissane / Parc à ballons	BMW
STAKEHOLDER															
Landowner	**CD13**		**CD13**	**CD13**		**CD13**			**Couloubris : CdL**	**MINARM**		**CEN PACA**	**WWF France**	**CEN PACA, MINARM**	**BMW France**
Support of farmers and shepherds	possibility of mobile fencing in the "corner"		sheep breeder not very motivated, but OK for marginal changes	sheep breeder not very motivated, but OK for mobile fencing 10 ha; **uncertainty regarding succession**		translocation of GPG might be possible,selection of site with sheep breeder			sheep breeder not very motivated, OK for translocation if sheep are not removed	OK for translocation of CPG, proposition of translocation sites by the sheep breeder		OK for translocation of CPG, proposition of translocation site by the sheep breeder	sub population of *P. rhodanica*	sub population of *P. rhodanica*	sub population of *P. rhodanica*
Location of potential translocation sites	nord-east	south	0	Center	south-east	nord-west	east	south-east	nord-west	nord	south	east			
Potential translocation sites : surface per site (ha)	NE: 10,85	S: 6,63		Center: 15,93	SE: 34,76	NW: 3,55	E: 1,58	SE: 4,44	6,49	N: 4,46	S: 10,67	3,4	*Prionotropis rhodanica* : subpopulation - mobile fencing	area of presence : 181,9 ha	area de présence : 56 ha
GRAZING MANAGEMENT															
Total surface of grazing place (ha), number of circuits	192 / 2		242 /2	210 / 3 à 4		347:04:00			650 / 4	344 / 2		140 / 3	147	300 + 75	
Grazing period 2022	mid-March - end of June		mid-March - end of June	beginning of March - end of June		mid-February - mid-June			mid-Jan - end of June	beginning of March - mid-June		beginning of March - end of April (exception)	beginning of march - mid- june	mid-april to mid-june	
Grazing pressure JPB (Journées de Pâturage Brebis - Days of Grazing Ewes - sampling over the year) in 2022	396 JPB/ha		237 JPB/ha	288 JPB/ha		287 JPB/ha			310 JPB/ha	537 - 581 JPB/ha		Since 2018: 142 JPB/ha	462 JPB/ha in 2022 (rather long season)		
Degree of uniformity between years	stable		stable since 2014	stable since 2016		stable since 2020			rather stable	stable		rather stable (since 2018)	rather stable	variable	
Grazing pressure evolution	INCREASE since 2007: 354 and 520 JPB/ha and year, since 2013: 434-637 JPB/ha and year		INCREASE before 2014: 330 to 335 JPB/ha and year after 2014: 339 to 486 JPB/ha and year	DECREASE Since 2015 no winter passages (before 2015 415 JPB/ha and year)		DEACREASE since 2020 2008 - 2019: Ø 385 JPB/ha and year Since 2020: Ø 287 JPB/ha and year Small INCREASE in flock numbers planned (100 ewes) to reach 295 JPB/ha and year.			Little data available. Overall, the CI are low, but the durations are long.	STABLE Slight decrease in recent years due to the expansion of the area.		STRONG DECREASE Before 2016: 609 JPB/ha and year Today: 142 JPB/ha and year	stable	INCREASE Before 2014: 245 JPB/ha and year After 2014: 350 JPB/ha and year	
VEGETATION															
Vegetation - Bröder et al. 2019	Presentation														
Vegetation height June 2022 (average per site) - see map	13,62 (n=369)	14,61 (n=215)		12,82 (n=368)	16,69 (n=1143)	13,64 (n=120)	13,93 (n=59)	12,14 (n=127)	13,10 (n=178)	10,48 (n=108)	10,09 (n=287)	15,65 (n=159)	12,01 (n=163)	13,45 (n=4913)	15,615 (n=2289)
Thymus sp. degree of coverage (2022 compared with 2003)	Presentation - MAP														
PREDATION RISK BY BIRDS															
Distribution of birds with sheep flocks - 08/05/22 = predation pressure (see map)	NE: moderate	S: moderate	*scale grazing place: NW: low SE: high*	Center: moderate	SE: no data	NW: low	E: moderate	SE: low	low	N: moderate	S: low	low			
F. naumanni : Ø predation pressure GPS data (see map)	0,271 (n=20)	1,074 (n=12)		0,058 (n=5)	0,205 (n=34)	0,048 (n=3)	0,137 (n=5)	0,353 (n=10)	0,211 (n=16)	0,216 (n=2)	0,398 (n=4)	0	0	1,038 (n=18)	0,37 (n=27)
F. naumanni : Kernel 50	outside	outside	outside	outside	outside	outside	outside	outside	(inside, but resting/sleeping place nearby!)	outside	outside	outside	inside	outside	outside
F. naumanni : number of breeding pairs 2022	-		-	-		-			9	6		0	2	-	-
Sheepfold with *Corvus monedula* : number of nesting places	26		13 (Grosse du Couchant : 35)	0 (Coulies : 1)		5 (Limouse : 10)			6 (Nouveau Carton : 10, Terme blanc : 8)	21		no sheepfold	0 (Opéra : 0)	40	-
Crau Plain Grasshopper															
Health risks (iridovirus, parasites, etc.)	proximity of PdM with présence *P. rhodanica*	proximity of PdM with présence *P. rhodanica*	proximity of PdM with présence *P. rhodanica*	?	?	?	?	?	?	?	?	?			
Historical presence of *P. rhodanica*	2006		1999	no observations known		2008			2012	1981		no observations known	still present	still present	still present
Dispersal option (0 - 360 degree)	180°	180°		360°	290°	180°	360°	360°	180°	290°	360°	90°	360°	0°	0° (mur)
POLLUTION															
Insecticides - proximity to orchards (information: distance and orchards in which direction)	no orchards nearby	0 m, S - SE	no orchards nearby	no orchards nearby		no orchards nearby			85 m - SW	no orchards nearby		no orchards nearby	1150 m - SE	0 m - W and NW	no orchards nearby
SUMMARY	sheepbreeder ok, vegetation ok, predation moderate-low, also no high chance of further dispersion from	sheepbreeder ok, vegetation ok, but predation high, moderate possibility for dispersion from this site		sheepbreeder difficult, vegetation ok, predation moderate-very low, very good option for	sheepbreeder difficult, vegetation ok, predation low-very low, very good option for dispersion	Cooperation with sheepbreeder ok, vegetation ok, predation low, possible dispersal to larger areas to the E	Cooperation with sheepbreeder ok, vegetation ok, predation moderate-low, very good option for dispersion	sheepbreeder ok, vegetation medium, predation moderate-low, very good option for dispersal	Cooperation with sheepbreeder difficult, vegetation ok, predation low, moderate option for dispersion	Cooperation with sheepbreeder ok, vegetation infavourable, predation low, very good option for dispersal	Cooperation with sheepbreeder ok, vegetation infavourable, predation low, very good option for dispersal	Cooperation with sheepbreeder ok, vegetation ok, predation very low, isolated, disperal possibilities very limited			

Annex 3: Advantages and disadvantages concerning the release of different stages

Stages	Advantages	Disadvantages
Adults	- Ready to reproduce/lay eggs	- More susceptible to stress during transport and transfer - Could be infected by pathogens and risk of transmission to wild populations (CPG and others) - Risk of rapid disappearance due to predators - Risk of non-adaptation to the new environment
Juveniles	- More adaptable to a new environment than adults?	- Susceptible to stress during transport and transfer - Could be infected by pathogens and risk of transmission to wild populations (CPG and others) - Risk of rapid disappearance due to predators - Risk of non-adaptation to the new environment
Egg pods	- Easy to transport - Low risk of contamination of eggs by pathogens (iridovirus)?	- Lack of data on the positioning of eggs in the wild

Annex 6: Abbreviations

BMW	Bayerische Motoren Werke
CA13	Chambre d'agriculture 13
CAL	Calissane
CD13	Conseil départemental des Bouches du Rhône
CEN PACA	Conservatoire d'espcaces naturels d'Alpes-provence-Côte d-Azur
CERPAM	Centre d'études et de réalisations pastorales Alpes-Méditerrannée
CPG	Crau Plain Grasshopper
GPS	Global positioning system
CR	Capture-recpature
DDTM13	Direction départementale des territoires et de la mer des Bouches-du-Rhône
DRA	Disease Risk Analysis
DREAL PACA	Direction régionale de l'environnement, de l'aménagement et du logement (DREAL) de Provence-Alpes-Côte d'Azur
DRM	Disease Risk Management
INPN	Inventaire national du patrimoine naturel
IUCN	International Union for Conservation of Nature
IUCN SSC	IUCN Species surviaval commission
LIFE	L'Instrument Financier pour l'Environnement
MTE	Ministère de la Transition écologique et de la Cohésion des territoires
NNR	National natural reserve
PdM	Peau de Meau
RNNCC	Réserve naturelle nationale Coussouls de Crau

Plaine de la Crau © Lisbeth Zechner - CEN PACA

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