LIFE Lynx

Preventing the extinction of the Dinaric-SE Alpine lynx population through reinforcement and long-term conservation

LIFE16 NAT/SI/000634

Surveillance of the reinforcement process of the Dinaric - SE Alpine lynx population in the lynx-monitoring year 2019-2020

Action C.5

Including data collected within Slovenian national large carnivore monitoring scheme, Interreg Central Europe 3Lynx project and Public Institution National Park Plitvice lakes

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1. INTRODUCTION

The main goal of the LIFE Lynx project (LIFE16 NAT/SI/000634) is preventing extinction of the Dinaric – Southeastern Alpine lynx population through reinforcement. Monitoring of the reinforcement process is fundamental in order to be able to fine-tune the process over the years and to choose and implement the optimal solutions for the upcoming lynx releases. It will be also essential in order to gain a good understanding of the process, upon which long-term lynx conservation strategy will be based, and to share experiences from our project with the broader expert community involved in lynx conservation efforts across the species range. Within the C.5 action, we are annually surveying the lynx population over its entire project range in Italy, Slovenia, and Croatia, using methods that allow us to assess its size, distribution, genetic structure, and several other important population and ecological parameters.

However, we could not have presented such a detailed status of the lynx population if we had not also included the data obtained through funding from other international or national projects (see the acknowledgments). Combining different resources allowed us to produce a result that surpasses any of the individual project's or program's goals. For example, roughly half of the camera traps were purchased and maintained by sources beyond LIFE Lynx and thus significantly contributed to the level of accuracy of the data collected and presented in this report. In Slovenia, the field design used for camera trapping surveillance even allowed us to collect data which can be used to make conclusions about the status of the lynx population at a national level. Consequently, all these additional data importantly support the decisions made regarding future lynx translocations which is the core activity of the LIFE Lynx project.

This report describes the current progress of the reinforcement of the lynx population in the Dinaric Mountains and Southeastern (SE) Alps. This action builds on the preparatory project action A.3, where baseline demographic and genetic status of the Dinaric SE-Alpine lynx population was described (Skrbinšek et al. 2019, Slijepčević et al. 2019). There were at least 71 adult lynx in the Dinaric-SE Alpine lynx population in the beginning of 2019, and 16 reproduction events were confirmed (Slijepčević et al. 2019). While a majority of adult animals were detected in Croatia (n=51), where the distribution area of lynx is the largest, only one female was detected in Italy, with the closest lynx in the pre-Alpine area in Slovenia being almost 100 km aerial distance away. Despite this, it is encouraging that these hard data prove lynx presence north of the highway Ljubljana-Koper, which represents an important barrier for wildlife (and especially lynx) movement between the Dinaric and Alpine regions. The genetic analysis showed that the effective population size calculated for the period 2011-2019 was dangerously small (N_e=13.4) in the last three years before the start of reinforcement and the inbreeding coefficient reached F = 0.316, which is very critical, considering that this is expected to correspond to a drop in fitness of 85% (Skrbinšek et al. 2019). These results undoubtedly underpin the necessity of the reinforcement activities taking place since 2019. We expect the long-term improvement of genetic status to start showing only after translocated animals will continue to transfer their genes into the population. In this report, we describe the first events that have a great potential to reinforce and prevent the extinction of the Dinaric SE-Alpine lynx population.

The baseline demographic survey of lynx year 2018/2019 allowed us to detect areas which were the most suitable for the first releases and accordingly released the first lynx into the inbred population in Slovenia and Croatia (Slijepčević et al. 2019). We have been monitoring the progress of the reinforcement with several complementary methods already used for the baseline population status assessment, i.e. camera trapping, non-invasive genetic sampling, GPS telemetry and collecting mortality records, however, we adjusted some aspects of their implementation according to the previous experiences to increase the efficiency of monitoring. We also continued with collecting opportunistic data and categorizing them into SCALP categories (Molinari-Jobin et al. 2003), which gave us additional information about lynx population distribution and helped to fine-tune camera trapping and non-invasive genetic sampling. Furthermore, collaboration with hunters was enhanced and expanded into the new hunting grounds not included in the systematic lynx surveillance in previous years.

In Slovenia, we are complementing the resources available for lynx surveillance from the LIFE Lynx project with other finances available for lynx monitoring (Interreg 3Lynx and national funding from the large carnivore monitoring scheme), which allowed us to survey the entire area of confirmed lynx presence with sufficient quality and to consequently increase the precision of the population parameters obtained (for further details, see Fležar et al. 2019). Similarly, LIFE Lynx is not the only funding source for surveillance of the lynx population in Croatia and Italy. In Italy, the majority of the camera trapping activities were funded via the 3Lynx project (Molinari-Jobin and Molinari 2020). In Croatia, where lynx is most abundant and widely distributed within the project area, surveillance of lynx was mainly funded by LIFE Lynx, although in Public Institution National Park Plitvice lakes (NP Plitvice lakes) camera trapping is ongoing since 2014 (within project "Space ecology of lynx in national park Plitvice lakes" coordinated by prof. Josip Kusak from Faculty of Veterinary Medicine University of Zagreb) and the project coordinator and the public institution kindly shared their data for lynx year 2019-2020 to be included in this report. Additionally, an environmental impact assessment study by Geonatura Ltd. for WF Lički medvjed, used camera trapping to assess local lynx population in the area south of Mala Kapela, and while they kept the data about camera trapping locations confidential, the records of lynx were also kindly sent to be included in this report. Since understanding current population status and planning further reinforcement activities can be best done using all currently available information, we describe here all the results of lynx surveillance regardless of the funding source, but explain how different funding affected the surveillance effort.

With this report we fulfil the first objective of the C.5 action by providing the information about the basic demographic parameters (e.g. lynx distribution, minimum number of adult individuals and minimum number of reproductions) and the key parameters describing the genetic status (e.g. inbreeding coefficient) of the lynx population during the first phase of reinforcement process, as well as detailed information about the history and current status of all the translocated animals after their release. We report the surveillance results for “lynx-monitoring year” 2019-2020 (i.e. 1st May 2019 until 30th April 2020), which is in accordance with the SCALP methodology as an international standard for assessing and reporting the lynx status (Molinari-Jobin et al., 2020). However, we also reported here some of the data collected outside this time frame (i.e. collected after 30th April 2020), when they were relevant for the further release plan and to obtain a fuller picture of the current situation. Whenever this was done, we noted the extended surveillance period of the shown data.

Chapters of this report are structured so that we first describe the effort and the data obtained with each of the method used (chapters 2.1-2.5) and then we synthesise and interpret the current status of the lynx population for each specific region within the population according to combination of all data obtained from all methods (chapters 3.1-3.7). Based on the results of the surveillance, we also provide recommendations for further lynx releases at the end of the report. Because the plan for the releases in the next year (2021) is to focus on the Alpine region, where almost no lynx are currently present (Figure 1, Figure 30), recommendations for further lynx releases are more general for this year.

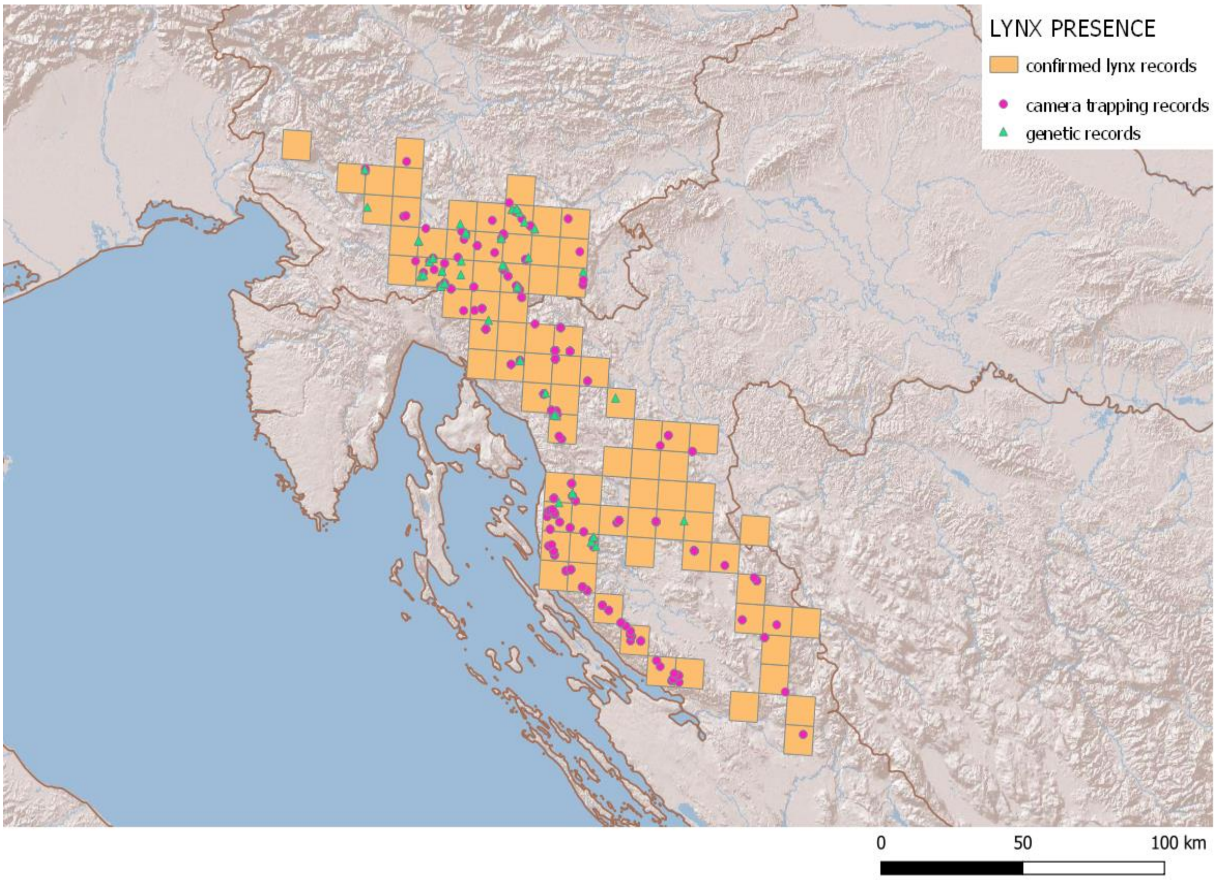

Figure 1. Confirmed lynx distribution in the Dinaric-SE Alpine area in 2019-2020 projected on the basis of confirmed records of lynx in a standard European 10 × 10 km grid net. Three types of data were considered as confirmed lynx records: opportunistic data categorized as C1 or C2 record, camera trapping and genetic records (the latter two are also highlighted as data points). See Figure 3 for the distribution of camera-traps deployed as part of systematic photo-trapping.

2. METHODOLOGY AND RESULTS

2.1 Opportunistic data collection

Opportunistically-collected data represents the basic information available for lynx presence across the three countries (Italy, Slovenia, Croatia) and is an important guide for all further efforts aiming to evaluate lynx population parameters in a more coordinated, systematic way. The categorization of opportunistic data about lynx presence follows an international standard (Molinari-Jobin et al. 2003), making these data comparable over different habitats, regions, and countries. It recognizes three levels of opportunistic data reliability, so called SCALP ("Status and Conservation of the Alpine Lynx Population") categories: unconfirmed records (C3), records collected or verified by lynx experts in the field (C2) and hard facts with material evidence (C1). The data is usually presented in a grid with 10×10km cells, e.g. (KORA 2017, Molinari-Jobin et al. 2020). Traditionally, SCALP reports were produced on an annual level following calendar years, but since 2017 it has been agreed that the data is summarized per biological lynx year (i.e. from 1st May of the given year till 30th April of the following year), which is also a standard used in this report.

In North-Eastern Italy, there was only one unverified opportunistic data record (prey remains) about potential lynx presence collected in the past year (Table 1, Figure 2), which suggests that lynx are possibly locally extinct in this region. In the Slovenian Alpine region (Julian and Karavanke/Kamnik-Savinja Alps) there were only a few unconfirmed records about lynx presence available, suggesting that presence of remnant lynx (i.e. descendants of the lynx reintroduced to Slovenia in 1973) in this region is questionable, but possible. However, in December 2020 one of the translocated lynx from Slovakia reached Slovenian Julian Alps (see telemetry chapter for details). We expect to gain more reliable information about the potential presence of any remnant lynx in the Julian Alpine region in the 2021/2022 monitoring year, when camera-trapping monitoring will be expanded into this region. In the pre-Alpine region (on Hrušica plateau and Trnovski gozd), we were again able to confirm presence of remnant lynx also in this monitoring year with collected photo and non-invasive genetic evidence (Figure 2, see also Figure 3).

Due to a widespread and good collaboration with hunters, they have provided the majority of the opportunistic data collected in Slovenia. What is more, within the framework of the national large carnivore monitoring scheme, we sent out questionnaires about lynx presence to more than half of the Slovenian hunting grounds which cover areas with suitable lynx habitat. The questionnaires that were first sent out within action A3 (Slijepčević et al, 2019) proved to be a cost effective and easy-to-implement method which gave valuable and reliable information about lynx presence in a certain area. We received responses that overlap other opportunistic data collected almost perfectly. Therefore, we assume that indications about lynx presence provided through questionnaires are also an important source of information to be considered in defining potential lynx presence in Slovenia. In Croatia, the majority (75%) of opportunistic records were photos from different sources which reliably indicate the lynx presence in Croatia. One potential lynx photo (it is unclear whether the animal in the photo is indeed a lynx) was obtained from Pelješac peninsula in Dalmatia, however, the following camera traps set in the area were not able to confirm the lynx presence there.

Table 1: Opportunistically-collected data about signs of lynx presence, categorized according to SCALP criteria, in lynx-monitoring year 2019-2020.

	Slovenia	**Croatia**	**Italy**	**All countries**
C1	57	94	0	152
C2	20	15	0	35
C3	25	13	1	38
total	102	122	1	225

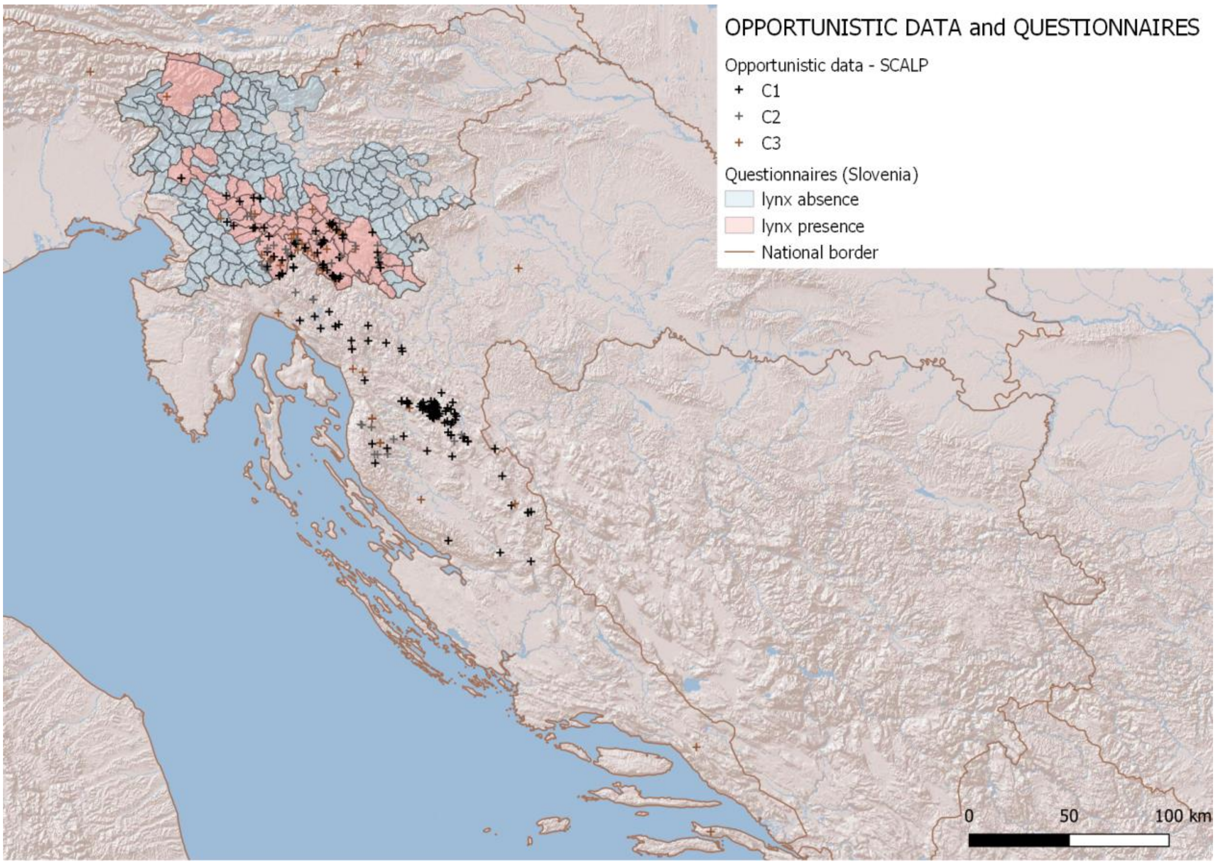

Figure 2. Opportunistically-collected data categorized in three SCALP categories shown together with responses received from the questionnaires sent to Slovenian hunting grounds.

2.2 Camera trapping

Camera trapping is currently recognized as the most effective method for monitoring lynx abundance and distribution in Europe (Hočevar et al. 2020). It often allows individual identification based on the distinctive coat pattern of each individual animal (although the identification process can be more difficult in case of lynx pelage with rosettes or no spots). It allows a straightforward and robust estimation of minimum number of individuals in the study area or with appropriate data also an estimate of abundance based on (spatial) capture-recapture analysis (Royle et al. 2014). We used an extensive network of camera traps (Figure 3) over the core area of lynx distribution in Slovenia, Croatia and Italy, covering roughly 10600 km^2 (Figure 3). The main population parameters that we obtained through camera trapping are the minimum number of adult lynx and the minimum number of reproduction events with the number of kittens per such event (Table 2). The sex of the animal could not be determined in all cases.

In Slovenia, most of the camera trapping involved help provided by the hunters from hunting grounds managed by Slovenia Forest Service (LPN) or by local hunting clubs (LD). Exceptions to this are three camera-trapping sites, where other volunteers are operating the cameras and retrieving the data and some of the sites that were operated by the project staff. The majority of surveillance in LPNs (56% of all camera trapping activities) was funded by the Interreg 3Lynx project (minimum 35 locations monitored) and national large carnivore monitoring scheme (minimum 69 locations monitored), while the funding in LDs was funded by LIFE Lynx project (44% of all camera trapping activities; minimum 82 locations monitored Table 2). Due to the sufficient funding to implement camera trapping surveillance over the entire permanent lynx distribution area in Slovenia (Figure 1 and Figure 2), we can conduct it in a systematic way on a national level.

In Italy, about 66% of all camera trapping activities was funded by Interreg 3Lynx project and the rest (34%) by LIFE Lynx so the efforts for detecting lynx with high-density camera-trapping were comparable to Slovenia (Table 2), while in Croatia the lack of additional funding did not allow such an approach. In Croatia, LIFE Lynx provided the majority of the funding for lynx surveillance with camera traps (77%) while NP Plitvice lakes monitored and funded additional 21 locations (23%). Therefore in Croatia, where surveillance is also limited in some areas due to land mines, logging activities and high disturbance due to movements of illegal migrants, camera trapping was adjusted over space and time to certain areas which were believed to be potentially best for lynx translocations and where it was safe to conduct the fieldwork.

We placed one or two (exceptionally three) camera traps per location (i.e. camera trap station), either at the same locations that we already surveyed in the 2018-2019 lynx-monitoring year (Fležar et al. 2019, Slijepčević et al. 2019), or at new ones. This year we expanded the camera trapping to a pre-Alpine area N of the highway Ljubljana Koper, which is a strong barrier for the lynx, involving 9 additional hunting grounds, where we established 11 new camera trap stations.

In Slovenia, we collaborated in total with 5 LPNs and 42 LDs, their hunting grounds covering almost a third of the entire country. In total, we set up 81 camera trap stations in LPN and 100 sites in LDs. For the new locations, we followed the same approach as in the first survey year: we held informative meetings with hunters or other camera trap operators and discussed the potential best locations for lynx camera trapping and visited them together on the field, together choose the micro-locations using criteria described in Stergar & Slijepčević (2017) and finally, set up the camera(s). Afterwards, most of the camera traps were operated (i.e. regular maintenance and changing of SD cards and batteries) by hunters and other non-project staff. We also held informative meetings with 67 hunters (10 in areas not included in the previous year). In Croatia, 54 such meetings were organized, and the camera trapping was conducted in 30 different hunting grounds and 3 national parks. In Italy, the grid covered 9 hunting grounds, and two meetings were held to build on existing connections with hunters.

A vast majority (n=157) of the camera trap stations in Slovenia were set up at locations where we expected lynx to move through, i.e. ridges, stone walls, logging trails, or animal paths. The remaining stations (n=28) were set up at potential or confirmed lynx scent-marking sites, which included active or abandoned forest cabins, ungulate feeding structures, bunkers, conspicuous trees or rocks, and other similar objects frequently used by lynx for territorial scent-marking in this region (Allen et al. 2017). The cameras were mostly set to take one photo and one video when triggered by movement. One type of the camera traps used (StealthCam G45NGX) could not record both photo and video, so we set it to only record photos to standardize data collection with the other two types of camera traps used (Moultire M40-i and Cuddeback X-Change). Also the Cuddeback camera traps with white flash illumination only collected photos during the night, while also videos were recorded during the daytime. In addition to recordings made upon detection of movement, camera traps in Slovenia were programmed to take additional one photo per day using 'time-lapse' function for operability check-up.

The cameras in Slovenia were deployed in August or September when project staff from Slovenia Forest Service (SFS) and University of Ljubljana (UL) joined the camera operators at the initial setup, while camera operators alone checked the cameras, retrieved the SD cards and handed the data to the local coordinators from the project team who then checked the photos collected and saved and annotated them on an internal server (software Camelot). This was mostly done every monthly until January, based on recommendations from Zimmermann et al. (2013). After January the cameras were left recording until April, but they were not maintained nor the data retrieved until the withdrawal of the equipment. In Italy and Croatia, the camera trapping stations were operative over the entire year. In Croatia 58 camera traps are maintained by project personnel, 28 by nature and national park rangers and 10 by hunters from collaborating hunting grounds.

In total, we identified a minimum of 101 different adult lynx from the obtained photo/video material. Only one lynx was detected in two countries (Slovenia and Croatia) and this was accounted for in the summarized data (Table 2). No lynx was detected in Italy so we could not confirm their presence there (see also chapter 2.1). In Croatia, there were considerably more lynx identified in this (2019/2020) survey year (i.e. 83) than in the first (2018/2019) year, when 51 were detected (Slijepčević et al. 2019). In Slovenia, the minimum number of adult lynx detected was similar in both years: 17 in 2018/2019 (Fležar et al. 2019) and 19 in 2019/2020 (also 17, if we exclude the two Romanian males translocated here in 2019). Relatively small number of individuals detected in the first survey as adults or kittens was confirmed during the second lynx survey: 36 lynx in Croatia and 13 in Slovenia. It is too soon to conclude whether the undetected animals are still in the population and only did not get detected or whether they actually disappeared. The trend of individual transience over the years will be subject of the following reports. In Slovenia, all lynx (n=4) wearing a telemetry collar during most of the camera-trapping season were also detected with camera traps.

This year we detected a minimum number of 15 reproductions and 25 kittens. Majority of them (13 reproductions and 23 kittens) were recorded in Croatia, while only two reproductions with at least one kitten each were detected in Slovenia. Whether the lower number of detected reproductions in Slovenia (in the previous year we recorded 6 reproductions with a minimum of 7 kittens; Fležar et al. 2019) is a consequence of imperfect detection or genuine decline in reproduction cannot be assured at the moment.

Table 2: Summary of the photo/video data obtained per country in 2019-2020 lynx-monitoring year. The minimum number of adult lynx in the Dinaric-SE Alpine region takes into account the fact that one animal was detected both in Slovenia and Croatia. The minimum numbers summarize all photographic data collected (camera trapping and opportunistically collected photos/videos, i.e. SCALP C1 data). Sex of some of the animals could not be determined (these lynx are included in the min. no. of adult lynx, but not included in the min. number of males or females).

	Slovenia	**Croatia**	**Italy**	**Dinaric-SE Alpine area**
Total no. of camera trapping sites (funded by LIFE Lynx)	*185 (82)*	*112 (91)*	*51 (33)*	*348 (206)*
Number of km^2 monitored	3700	5200	1700	10600
Density of camera trapping sites per 100 km^2	5	2.2	3	3.3
Min. no. of adult lynx	19*	83	0	101*
Min. no. of females	6	24	0	30
Min. no. of males	9	20	0	29
Min. no. of adult lynx of unknown sex	4	39	0	42
Min. no. of kittens	2	23	0	25
Min. no. of reproductions	2	13	0	15

*includes two translocated lynx from Romania that were released and established their territories in Slovenia in 2019.

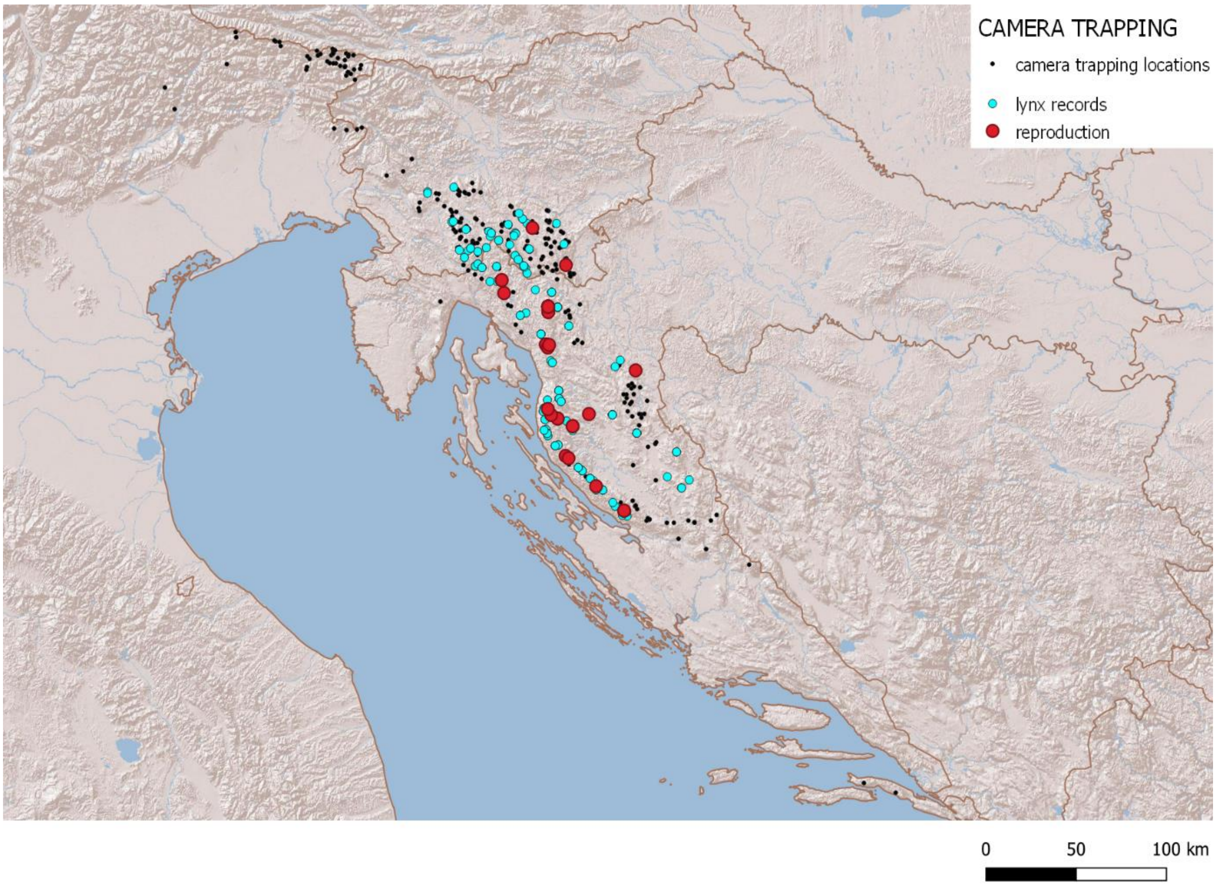

Figure 3. The camera trapping effort and main demographic results from the 2019-2020 survey season. The locations of all camera traps used for camera-trap monitoring are shown as black points. The locations where at least one lynx was recorded are shown in blue and reproduction events marked additionally with red points.

2.3 Non-invasive genetic sampling

For genetic analysis, several types of non-invasive samples were collected: scat samples were stored in 95% non-denatured ethanol, urine samples (collected in snow) were stored in DETs buffer, hair samples were stored in sealed bags with desiccant (silica) and saliva samples were collected with forensic swabs. We also analysed blood in ticks collected from captured lynx (blood non-invasive). Tissue samples were stored in 95% ethanol and blood samples (on WTA cards) were taken from animals captured for telemetry. The number of collected samples is provided in Table 3.

DNA in non-invasive genetic samples is of very low quality and quantity, and contamination (especially with PCR products) is a serious issue. Therefore we used a dedicated laboratory for non-invasive genetic samples for DNA extraction from non-invasive samples and PCR setup. For all non-invasive samples, we used MagMAX DNA Multi-sample Kit (Thermo Fisher Scientific), whole blood protocol. The extraction protocol is implemented on a liquid handling robot (Hamilton Starlet) to achieve reliable, error-free, and fast DNA extraction (Skrbinšek et al. 2017). DNA extraction from tissue and blood samples is done in a separate laboratory, using manual DNA extraction kit (Sigma GenElute Mammalian Genomic DNA Miniprep Kit) following the manufacturers protocols.

We used ten microsatellite markers for individual ID run in a single multiplex: Fca132, Fca201, Fca247, Fca293, Fca391, Fca424, Fca567, Fca650, Fca723, Fca82. The best (reference) sample of each detected animal was amplified using 9 additional markers (F115, F53, Fca001, Fca132, Fca161, Fca369, Fca559, Fca742, HDZ700 (Menotti-Raymond et al. 1999; Menotti-Raymond et al. 2005; Williamson et al. 2002), bringing the total number of studied microsatellites to 19. SRY locus was used to determine sex of the animal. Microsatellites were amplified in 3 multiplexes (detailed protocols are described in Polanc et al. 2012). The SRY sex marker amplifies also in non-felid species and it is used for sex identification also for other carnivores, so prey DNA (like fox) in a scat could cause problems. Also slight contamination from different animals in a sample (urine, hair), can make the sex determination difficult. That is why in some cases we additionally analysed sex of the animal with amelogenin genetic marker (Pilgrim et al. 2005).

Good quality tissue and blood samples were re-amplified twice. For non-invasive samples, we used a modified multiple-tube approach (Taberlet et al. 1996; Adams & Waits 2007) with up to 8 re-amplifications of each sample according to the sample's quality and matching with other samples. In the first screening process, each sample was amplified with the 10-marker panel (multiB panel) protocol twice and analyzed on an automatic sequencer (Applied Biosystem ABI 3130xl Genetic Analyzer). Results were interpreted using GeneMapper v.4.0. software (Applied Biosystems, USA). Samples that provided no specific PCR products at that stage were discarded as were the samples from the non-target species, i.e. the wild cat (*Felis silvestris*). Consensus genotypes were determined using an Access database application programmed by T. Skrbinšek (MisBase, unpublished).

Genetic data were prepared in a laboratory database (MisBase), which we use to keep a record of the field data (T. Skrbinšek, unpublished). All non-GIS analyses were run in R (R Development Core Team 2018).

Table 3: Genetic samples collected from 1.5.2019 till 30.4.2020 and genotyping success.

Sample type	Sampling season 2019/2020	Successfully genotyped	Genotyping success
tissue	1	1	100%
blood	5	5	100%
blood (from ticks)	2	2	100%
scat	34	17 (21*)	50% (62%*)
urine	6	2	33%
hair	70	26	37%
saliva direct	5	3	60%
saliva from prey	7	0	0%
TOTAL	130	56	35%

* additional four samples from identified non-target species (wild cat)

A total of 130 genetic samples were collected in the lynx-monitoring year 2019-2020 in Dinaric Mts. and SE Alps, 124 of them non-invasive. Out of all collected samples, 56 could be used for individual recognition and 15 samples were confirmed lynx, but the sex or individual animal could not be determined. Four scat samples belong to wildcats, the rest were discarded due to poor quality (65).

Genotyping success of non-invasive genetic samples was highest with scat samples and saliva collected directly from a sedated animal. Most of the collected samples were hairs (n=70), which we mostly collected during visits to known marking sites or during snow tracking. The genotyping success of hair samples was lower compared to scats, but similar to urine samples. Several different animals may leave hair on the same spot and because of high inbreeding and low genetic diversity of lynx in our study area, it can be difficult to identify mixed samples. In some cases where we suspected the samples to be from more than one animal and could not resolve it with more reamplifications we only report the sample belonging to lynx. Urine samples are collected from snow and due to limited snow cover in the sampling season 2019/2020 only six were collected.

One tissue sample belonged to a juvenile male that was killed on Slovenian A1 highway Ljubljana-Koper near Postojna, and five blood samples are from animals captured for telemetry (four of these animals were captured within the scope of other projects). Blood samples were not analysed from all captured animals (e.g. when an already known animal was captured) and in some cases we analysed other types of samples, such as direct saliva, hair or blood from a tick collected on the captured animal (two animals).

Since hair trapping did not yield the expected results in the first year (Fležar et al. 2019), we decided to limit it to opportunistic setup during ongoing camera trapping, where we would either increase the chance of collecting lynx hair at already known lynx scent-marking site or at another frequented site where we assumed that the hair trap could attract the lynx. In this manner we set up 7 hair traps, but only one attracted lynx kitten and provided a hair sample – only sex could be determined for that animal (female).

Out of 56 full genotypes 26 individuals were recognized and for more than half of them only one sample was collected (Fig G). Ten animals (7 males, 3 females) were already known from previous years. Genetic analysis confirmed that male lynx captured for telemetry in March 2020 is indeed lynx Mihec, who had been collared already in 2010 as a kitten. There were also three hair samples from a female lynx that match a scat sample collected in 2011.

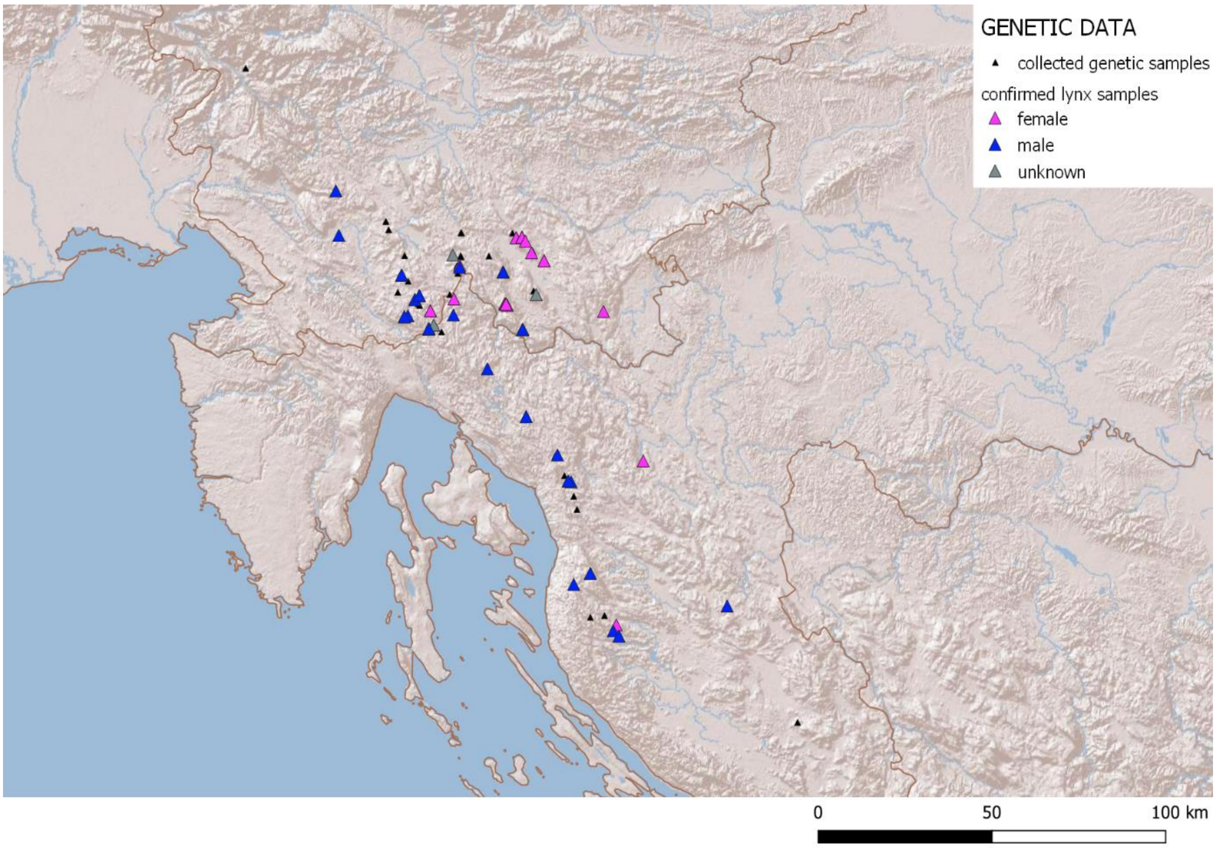

Figure 4. Genetic samples collected from 1.5.2019 to 30.4.2020. Samples which were confirmed to belong to lynx are categorized by those belonging to females (pink triangles) and males (blue triangles) or those for which sex could not be determined (grey triangles). Collected samples that did not yield results are marked with black triangles.

2.4 Population genetics - further genetic erosion and first effects of population augmentation

Our baseline population genetics study (Skrbinšek et al. 2019) showed how the Dinaric lynx population deteriorated genetically since the 1973 reintroduction, with the population approaching severe levels of inbreeding relative to the source population in Slovak Carpathians. New samples of the remnant lynx population in the Dinaric mountains (excluding the animals translocated from Romania and Slovakia in this project, and their offspring) allow us to further track this development. On the other hand, the genotypes of the translocated animals and their offspring allow us to catch a glimpse of how we can expect the population to develop if the translocated animals manage to successfully reproduce and include their genes into the population.

We analysed the additional samples of the remnant Dinaric lynx, which were collected since our last analysis to track the further population deterioration. On the other hand, since the newly translocated lynx and their offspring are now physically a part of the Dinaric population, we re-ran the analysis with samples of these animals and their offspring included, to explore the effect they may have on the population if they successfully breed.

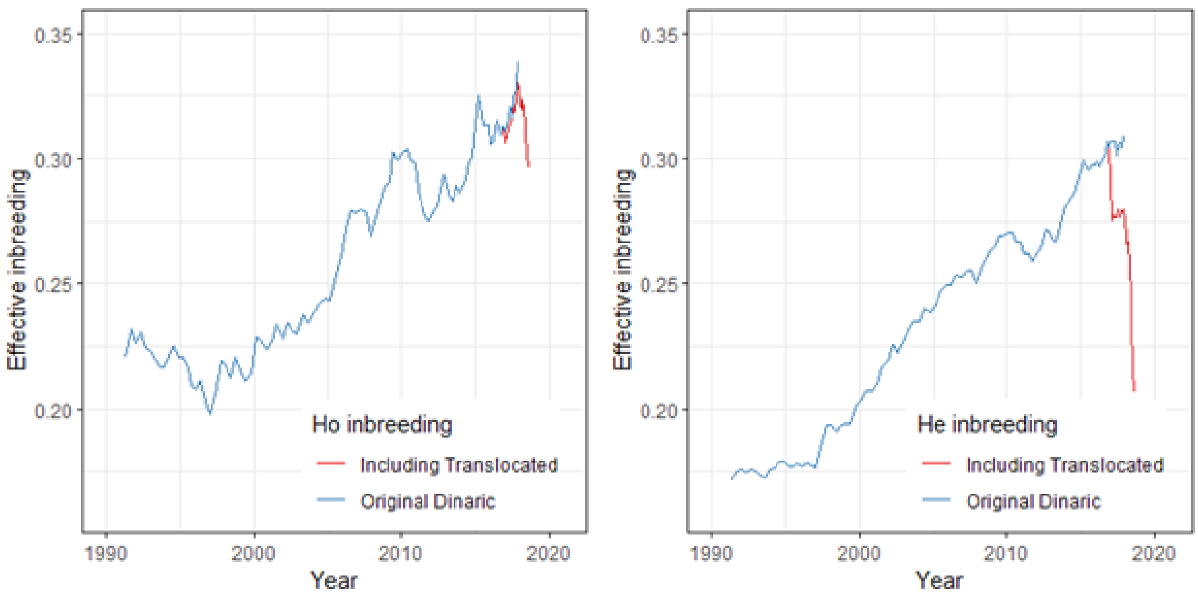

Figure 5. Effective inbreeding of Dinaric lynx relative to the source population in Slovak Carpathians, calculated from observed (left) or expected (right) heterozygosity, with and without including translocated animals and their offspring, calculated with 60-sample travelling window. As expected the effect of the introduced animals is much higher with expected heterozygosity (right) due to the Wahlund effect (direct immigrants in the population), while the actual current effect is better estimated from the observed heterozygosity (left). However, large increase in expected heterozygosity indicates the potential for rapid inbreeding decrease if the introduced animals continue to successfully reproduce.

Since very little time has passed since we last explored the inbreeding status of Dinaric lynx (Skrbinšek et al. 2019), the change in inbreeding in the remnant lynx population is not very different from what we described before (between 0.31 and 0.34), just reaffirmed by more data. However, when including the translocated animals and their offspring, the picture improves significantly. If the introduced animals and offspring would form 15% of the total population (simulated in the graph above), the inbreeding estimated from expected heterozygosity would drop to 0.21. While this is still high, it is closer to the range we observed in the 1980s (around 0.18) when population still seemed viable.

In the next few years, a lot will depend on reproductive performance of the translocated animals. In the Florida panther, *Puma concolor*, population augmentation there seemed to be considerable heterosis (fitness advantage of outbred animals) in the first generation (F1) crosses between indigenous and introduced animals which contributed to rapid expansion of introduced genes in the population (Johnson et al. 2010). There is a good chance that this will happen also in the Dinaric lynx, and we will continue monitoring the situation closely over the following years to keep track of that. The introduced lynx bring many private alleles that were not present in the Dinaric lynx population prior to the translocations (Table 4), which makes the offspring of these animals very easy to detect.

Table 4: Alleles found in translocated lynx that were not previously detected in the Dinaric lynx population. These "private" alleles make offspring of the translocated lynx very easy to detect.

Locus	**F115**	**F115**	**Fca001**	**Fca001**	**Fca001**	**Fca123**	**Fca132**	**Fca132**
Allele	248	252	181	191	187	140	179	175
N observations	6	1	1	1	1	3	5	3
Locus	**Fca161**	**Fca424**	**Fca650**	**Fca650**	**Fca723**	**HDZ700**	**HDZ700**	
Allele	184	180	131	129	179	141	145	
N observations	1	1	1	1	4	4	1	

2.5 Telemetry

Important part of surveying the reinforcement process is GPS-telemetry tracking of all translocated and some of their offspring or remnant lynx. GPS-telemetry can be used for studying lynx behavioral patterns, such as habitat use, dispersal, movements, predation, feeding and reproduction (Schadt et al. 2002, Krofel et al. 2013, Heurich et al. 2014, Hočevar et al. 2020). The main focus of our tracking of the translocated animals is on lynx survival, territory establishment, movement patterns, and reproduction. We also gathered information about prey species, sex and age structure of the prey and calculated the kill rates. Locating fresh kill sites further enabled us to record lynx with the help of camera traps to assess their physical condition. We also recorded the presence of the scavengers at the kill sites and their influence on prey consumption by lynx.

In addition to the translocated lynx, we also collared two remnant lynx and two offspring of translocated and remnant lynx as part of the LIFE Lynx project (Table 5).

Table 5: Overview of GPS-collared lynx tracked within the LIFE Lynx project with basic information.

Lynx name	Origin	Date collared/ released	End of tracking	Current status	Home-range size
Goru	Translocated (Romania)	12.2.2019/14.5.2019	Still tracked	Established territory on Mala gora	144 km^2
Doru	Translocated (Romania)	27.2.2019/4.5.2019	30.1.2020	Signal lost	130 km^2
Katalin	Translocated (Romania)	16.1.2020/ 31.3.2020	Still tracked	Established territory on Menišija/Rakitna	258 km^2
Boris	Translocated (Romania)	25.1.2020/ 28.5.2020	Still tracked	Unclear (possibly established territory)	N/A
Maks	Translocated after rehabilitation (Slovakia)	2.6.2020/23.6.2020	Still tracked	Dispersed into the Alps (without established territory)	N/A
Pino	Translocated (Slovakia)	25.3.2020/ 30.5.2020	30.5.2020	Signal lost	N/A
Alojzije	Translocated (Romania)	20.1.2020/13.3.2020	Still tracked	Established territory in Southern Velebit	167 km^2
Teja	Remnant	12.2.2019	9.2.2020	Collar dropped-off (established territory on Mala gora)	60 km^2
Mihec	Remnant	21.3.2020	Still tracked	Established territory on Racna gora/Snežnik	343 km^2
Mala	Offspring of remnant and translocated lynx	19.1.2020	1.7.2020	Collar battery exhausted (confirmed alive)	78 km^2
Niko	Offspring of remnant and translocated lynx	6.12.2020	Still tracked	Without established territory	N/A
Bojan	Remnant (tracked within 3Lynx Project)	1.12.2019	Still tracked	Established territory in Gorski Kotar	515 km^2

2.5.1 Translocated lynx

So far, we successfully translocated seven lynx to Slovenia (n=4) and Croatia (n=3). Five of them were from Romania and two from Slovakia. Three lynx (Goru, Katalin and Alojzije) have already established their territories, while Boris and Maks, who are younger animals, apparently still did not establish permanent territories. We lost signal from two collars, therefore the status of two individuals (Doru, Pino) is currently unclear. Below we describe history after translocation and current status of each individual.

Goru

Figure 6. Lynx Goru photographed with camera-trap on Mala gora, Slovenia.

Lynx Goru was captured in Romania on February 12 in 2019. He is an adult male, currently estimated to be around 6 years old. He was transported to Slovenia on 26 April 2019, where he was released from quarantine in Loški Potok on May 14, 2019. After the release, he first crossed the national border between Slovenia and Croatia, but soon turned north and returned to Slovenia. Seventeen days after the release, he arrived to Mala Gora in Kočevsko, where he established his permanent territory. In this area, a remnant female named Teja was present and collared on April 19, 2019. Her home range overlaps almost completely with Goru's home range, which measures around 144 km^2. They mated in late June in 2019 and in August, Teja gave birth to one kitten, named Mala (see below chapter on Mala for further details). Goru's paternity was confirmed with genetic analysis. In 2020, Goru and Teja mated again, based on the telemetry data from both lynx, as well as later genetic confirmation of paternity of their 2020 offspring. On March 1, 2020, still during the lynx mating season, Goru temporarily left his territory on Mala gora and went towards Ravna Gora area in Croatia, up to 50 km from the edge of his territory. In this area, the presence of three local females was confirmed, therefore there is a chance that he also mated with them. After a month in Ravna Gora area, he returned to his territory on Mala Gora in Slovenia on April 7. On July 14, 2020, he was recaptured and his collar was replaced with a new one that will enable us to monitor him for additional two years. In 2020, female Teja was photographed with two kittens and one of them (male named Niko) was captured on December 6, 2020 and confirmed genetically to be the second litter of Goru's and Teja's offspring. With camera trap surveillance at the kill sites, we recorded Goru on eleven occasions and confirmed that he remains in good physical condition.

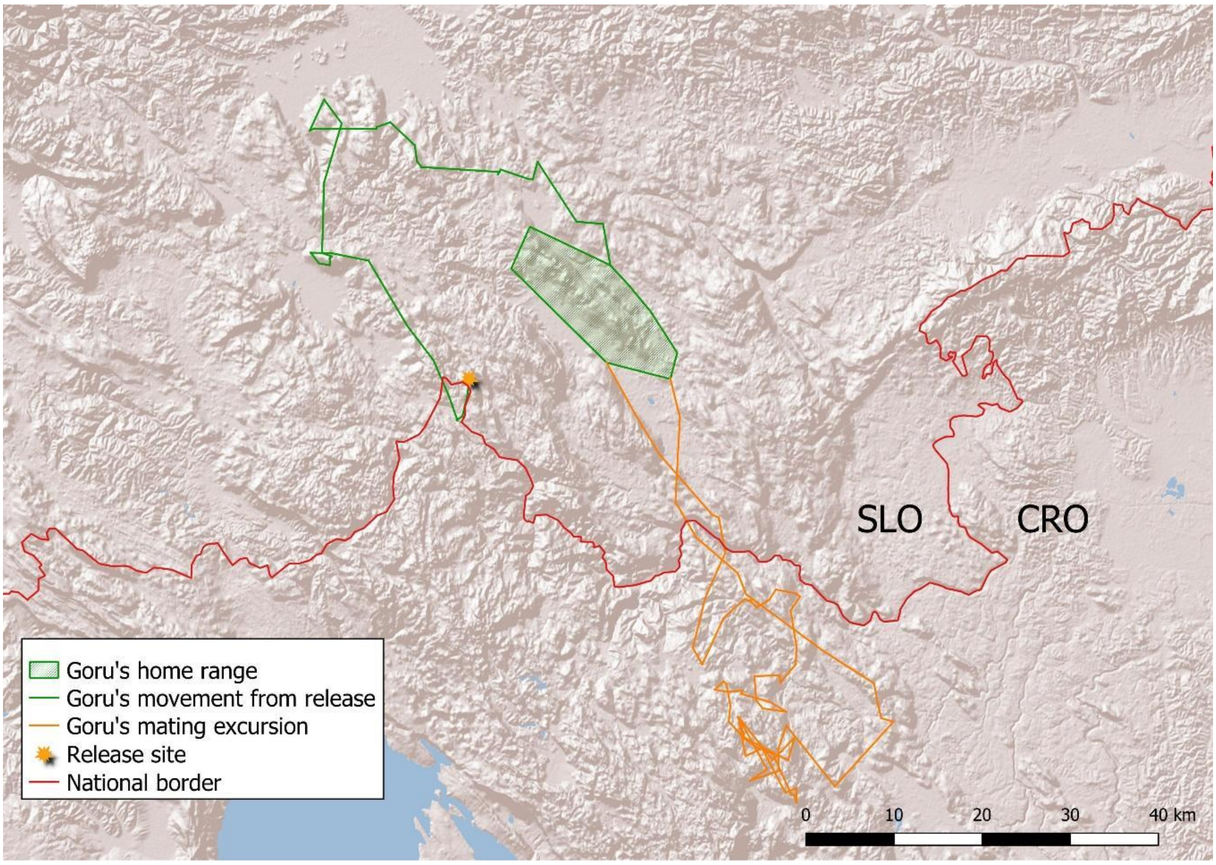

Figure 7. Map of Goru's movement after release (green), his home range (100% MCP) on Mala Gora, and temporary extra-territorial excursion in spring 2020 (orange).

Doru

Figure 8. Lynx Doru when released in Risnjak National Park, Croatia.

Doru was captured in Romania in February 2019 and was released in Croatia, in Risnjak National Park on May 4. Currently he is estimated to be around 5 years old. After the release he spent some days in Risnjak and then moved towards the north, crossing the border to Slovenia. He arrived to Javorniki on June 13, 2019, where he was apparently stopped by the Ljubljana-Koper highway and there established his territory with the home range size of 130 km^2. We recorded him on two kill sites and he appeared in good physical condition. His movements were recorded until the end of January 2020. After that, we lost the signal of his collar and he was also no longer detected on camera traps or from non-invasive genetic samples collected in the area. His status for now remains unknown, but we assume he is no longer alive or at least not present in Dinaric Javorniki area, also because another male was detected in this territory in 2020. If Doru remains unaccounted during the next monitoring season (2020-2021) and if we do not detect his potential offspring, we will declare him lost and following the reinforcement plans (Wilson et al. 2019) consider replacing him with another lynx from the Carpathian population.

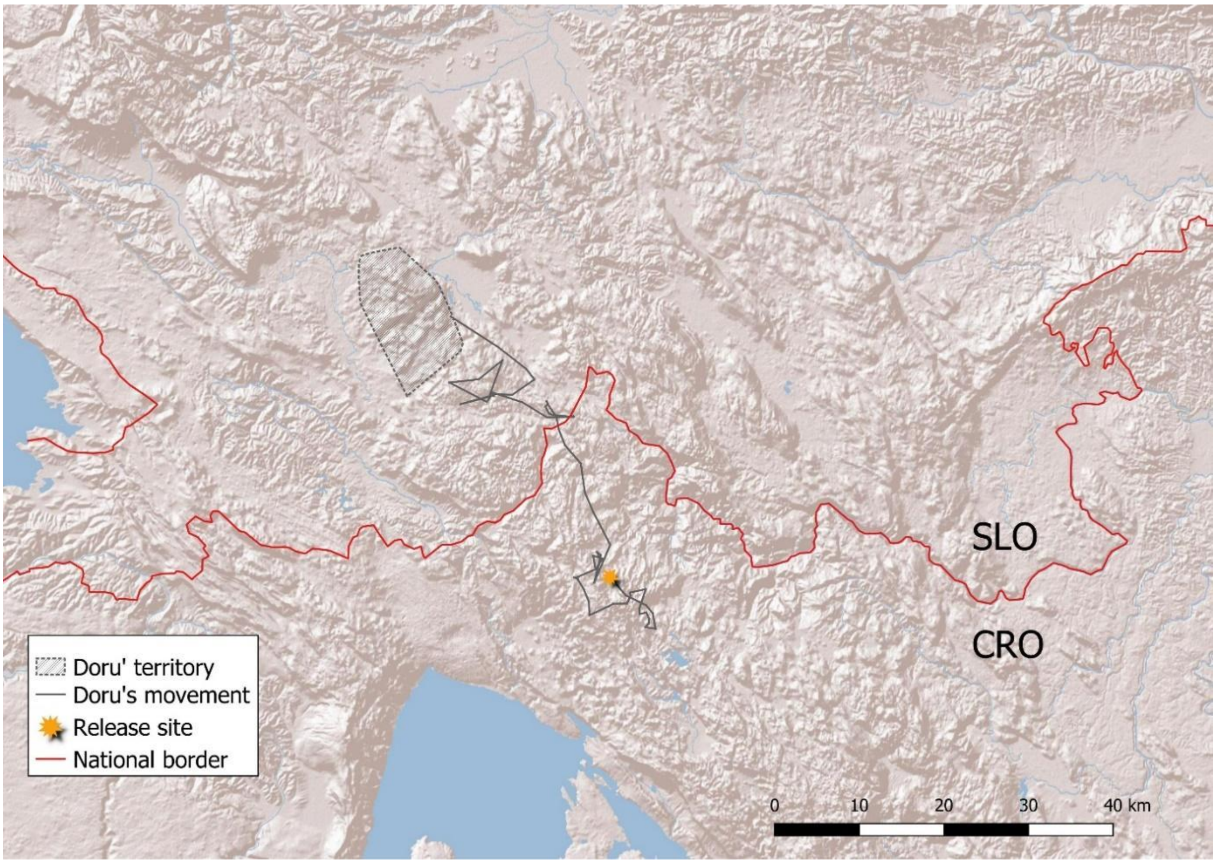

Figure 9. Map of Doru's movement after the release and his home range (100% MCP) established on Javorniki and held at least until end of January 2020.

Katalin

Figure 10. Lynx Katalin in a soft-release enclosure before release on Snežnik plateau, Slovenia.

Katalin was captured in Romania in January 2020 and released to Slovenia on March 31, 2020. He is currently estimated to be 4-5 years old and after the release on Snežnik plateau, he first moved south and crossed the border to Croatia, where he visited the Istra region. Then he turned west and crossed a large part of Gorski Kotar, until crossing the Croatian-Slovenian border again. In Slovenia, he first crossed Kočevsko and arrived to the surrounding of Mt. Krim on 19th April. There he established his territory, which now covers Menišija, Logatec plateau, Rakitna and some parts of Mokrc. On the western side, his home range is limited by the Ljubljana-Koper highway, which he was so far not able to cross. Size of his home range is currently 258 km^2. In summer 2020 he was observed and recorded several times together with another lynx (known as "Menišija1" from the photo-monitoring), which dispersed here as a yearling in 2019 and we assume is probably a female. We recorded Katalin on six occasions with camera traps when feeding on kill sites, among which at least two were shared with this probable female. He appeared in good physical condition. In addition, Katalin was also photographed several times on camera traps within the lynx monitoring program, as well as other camera traps used in the region by local hunters.

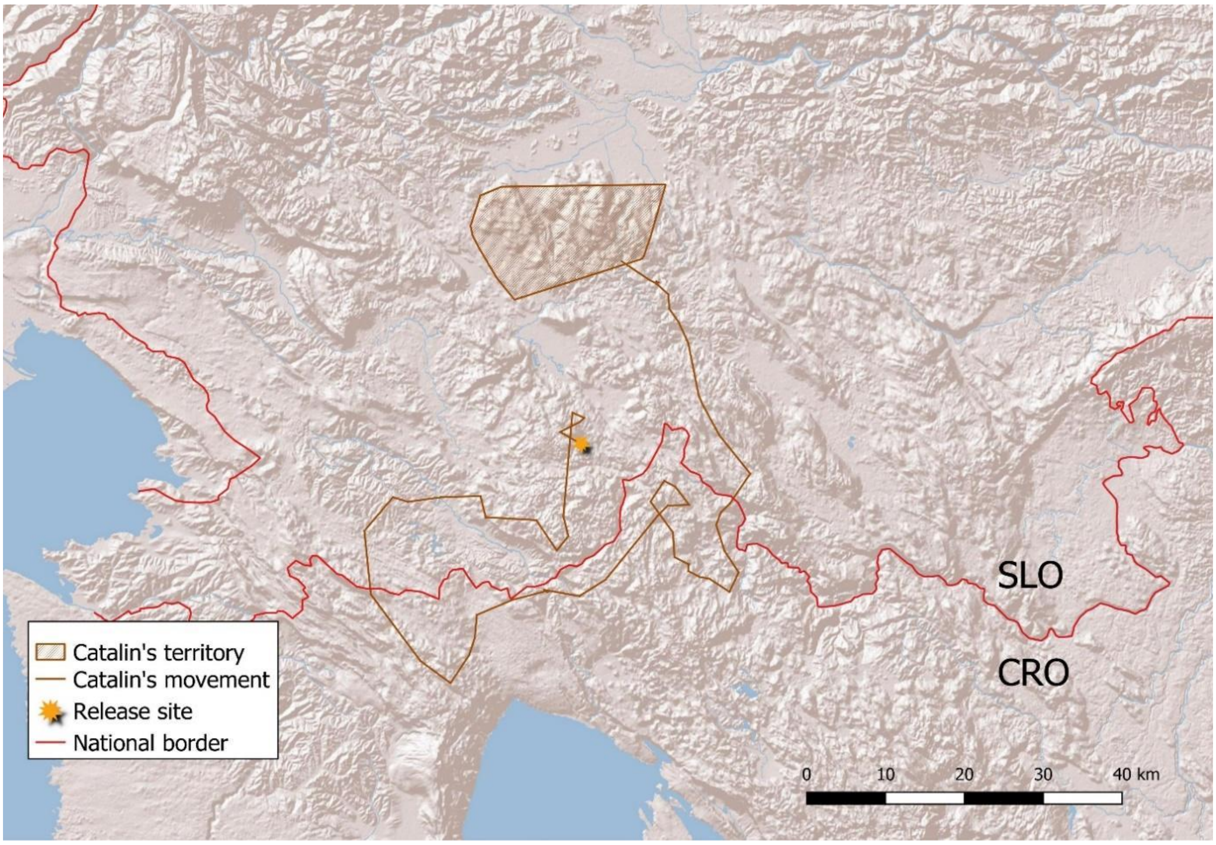

Figure 11. Map of Katalin's movement after the release and his home range (100% MCP) established on Menišija, Logatec plateau, Rakitna and Mokrc.

Boris

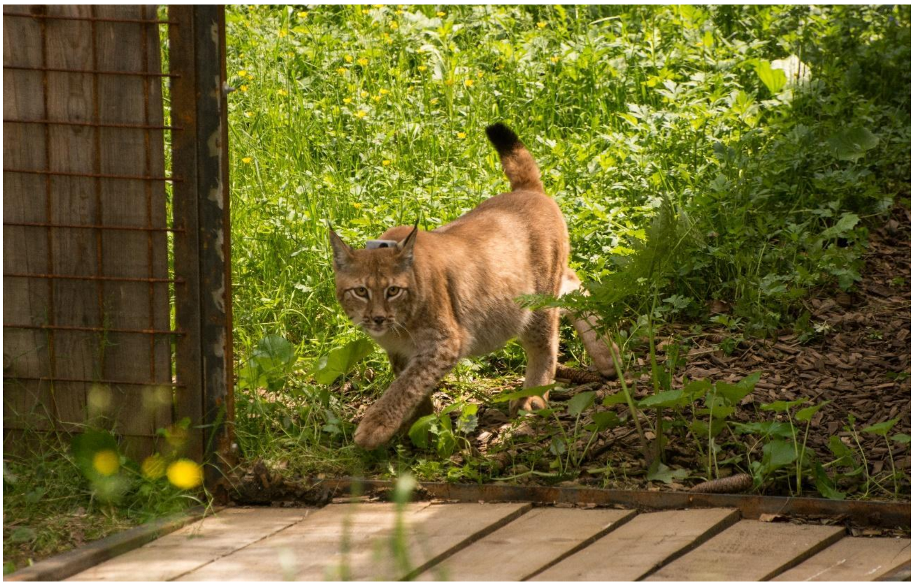

Figure 12. Lynx Boris during release from an enclosure in Loški Potok, Slovenia.

Male Boris is a 2 year old lynx that was captured in Romania in January 2020. He was translocated to Loški Potok enclosure on April 30 and released on May 28, 2020. Soon after the release, he went to Croatia and stayed around Gerovo for about 2 months, apparently establishing a temporary home range. Most likely due to presence of adult male territorial remnant lynx in the surrounding areas, he abandoned this area and moved on south-east to Vrbovsko where he stayed for a month. We recorded a probable encounter with the collared remnant territorial male Bojan (tracked as part of the 3Lynx project), which apparently triggered this movement. Soon afterwards, he again moved further south to Ogulin and Mala Kapela area where he is present since October 14, 2020 and might be establishing his territory, although at the moment this cannot be definitely confirmed. He appears in good condition, mostly feeding on roe deer.

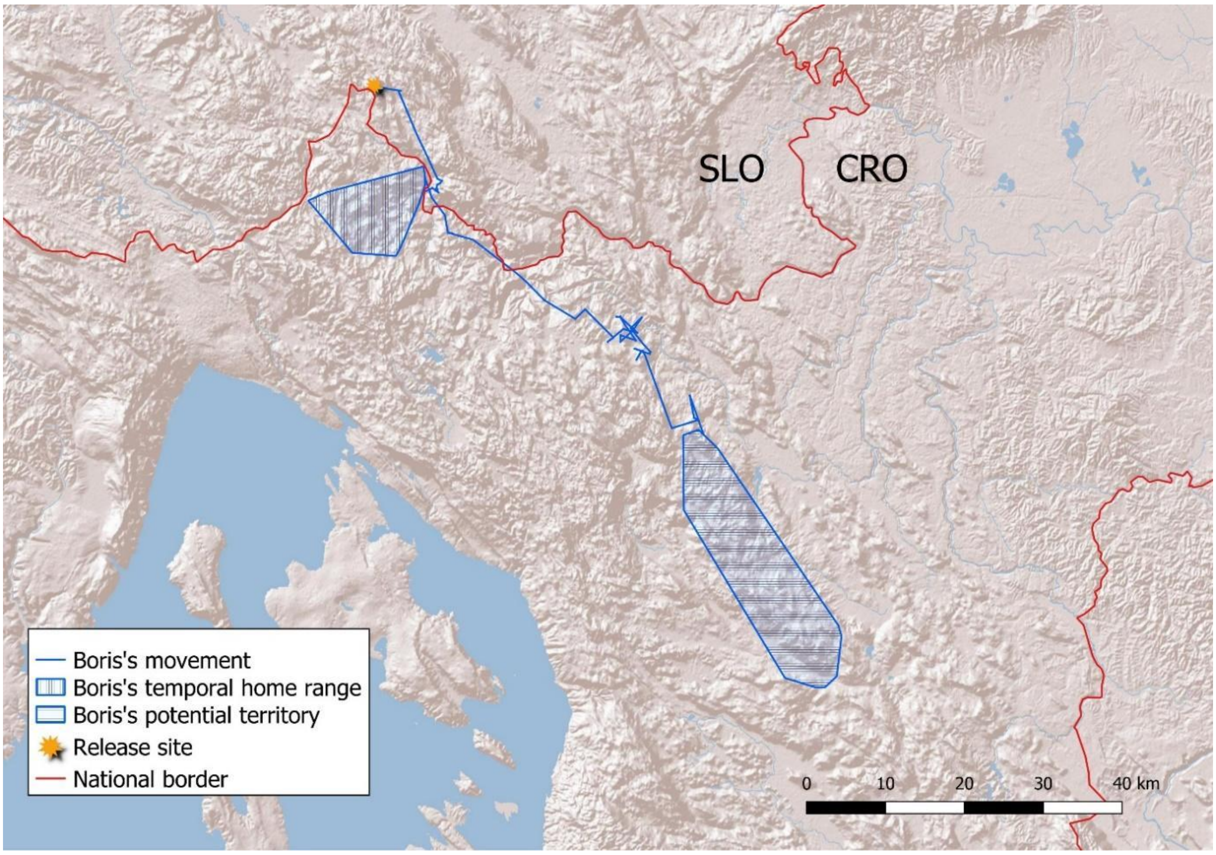

Figure 13. Map of Boris' movement after the release and his (temporary) home ranges (100% MCP).

Maks

Figure 14. Lynx Maks during release from an enclosure on Snežnik plateau, Slovenia.

Lynx Maks was translocated from Slovakia to Slovenia. He was found as an orphan lynx with a broken paw in 2019 in Slovakia. He was rehabilitated and after he was vital again, he was transported to Slovenia in June 2020. He was released from the enclosure in Snežnik plateau on June 23. After the release, he started his journey north towards Javorniki, where he arrived a few days after the release and was apparently stopped by the Ljubljana-Koper highway. He stayed in the area for three months, apparently establishing his territory in the same area occupied by Doru the previous year. But on September 24, he left this area, a reason for which remains unclear and might be connected with a remnant territorial male that moved here from Hrušica this year. Maks moved to Menišija and Logatec plateau, where he stayed for two months, sharing the area with male Katalin, which arrived here a few months earlier, and another lynx ("Menišija1"), which is assumed to be a female. On November 22, 2020, he left the Menišija region, again perhaps due to presence of another territorial male in the area (Katalin) and crossed the Ljubljana-Koper highway. He first stayed a few days in forests around Logatec, from where he moved towards the Alpine area. He first crossed Cerkljansko hribovje, then Jelovica and the last locations received on 20th December 2020 were from Pokljuka, 85 km of aerial distance from the release location. He is currently still moving northward without any indication of establishing a territory. No other lynx are currently confirmed present in this Alpine region, but releases on Pokljuka and Jelovica are planned for 2021. We managed to record Maks on several camera traps, including at his kill sites on Javorniki, Menišija and surroundings of Logatec, when he appeared to be in good physical condition.

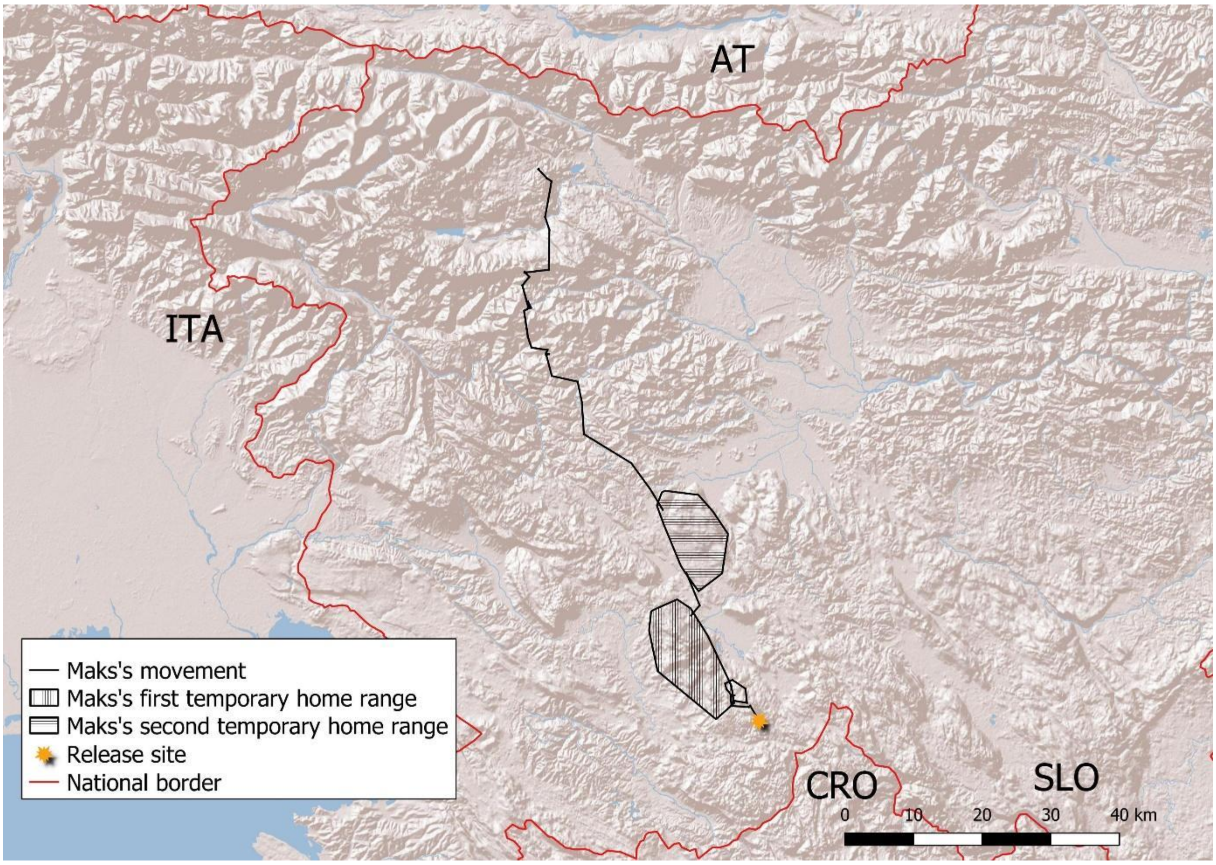

Figure 15. Map of Maks's movement after the release and his two temporary home ranges (100% MCP) on Javorniki and Menišija/Logatec plateau.

Pino

Figure 16. Lynx Pino when released in Sjeverni Velebit National Park, Croatia.

Male Pino was captured in Slovakia in March 2020 and is estimated to be currently 5 years old. He was released in Sjeverni Velebit National Park in Croatia on May 30, 2020. Unfortunately, his collar stopped working after the release and no telemetry data were received. He was not captured on camera traps, but in August 2020 a local hunter reported a sighting of a collared lynx on central Velebit. No other collared lynx were present there at that time so this indicates potential presence of Pino in this area. At the moment his status is uncertain and we will wait for potential records from camera traps or through non-invasive genetic material, before making any assumptions about his fate.

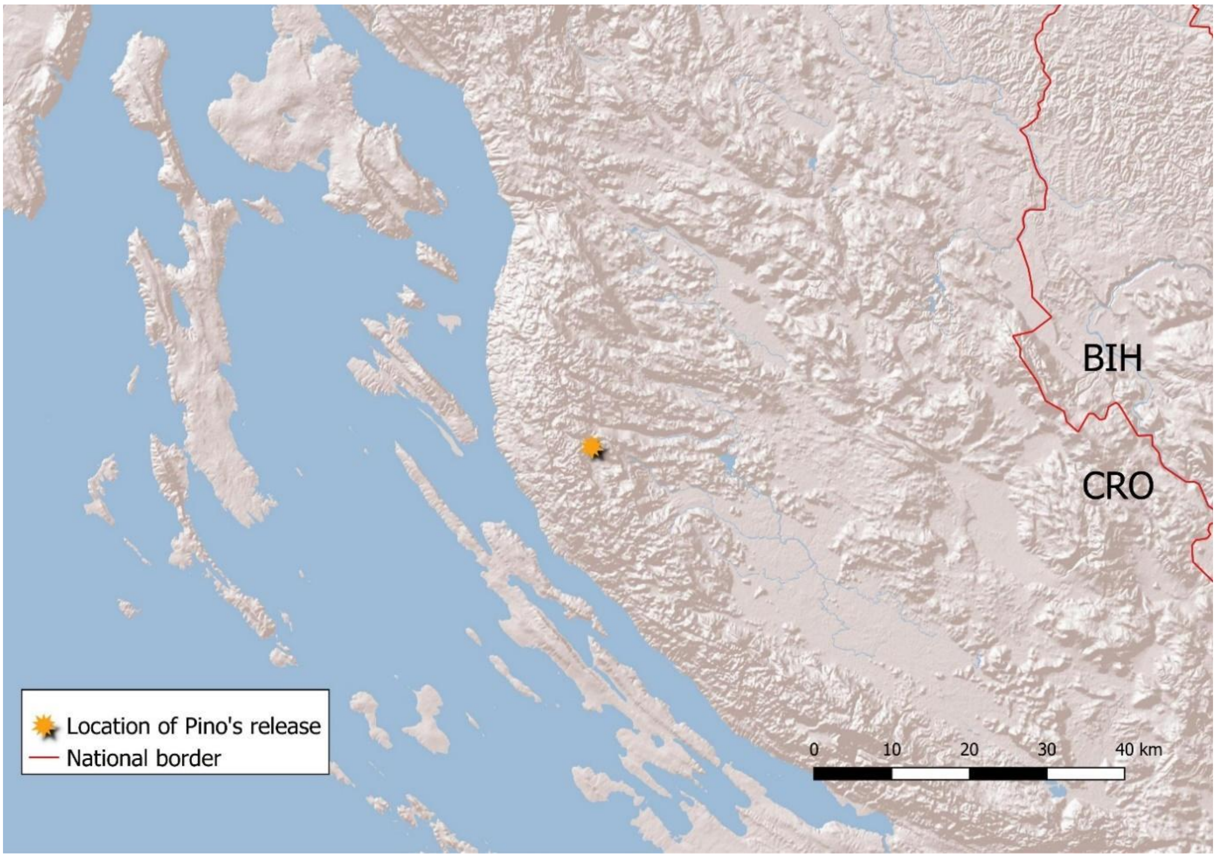

Figure 17. Map of Pino's release site. No telemetry data was received after his release.

Alojzije

Figure 18. Lynx Alojzije when released in Paklenica National Park, Croatia.

Male Alojzije was captured in Romania on January 20, 2020 and is currently estimated to be 3-4 years old. Alojzije was released in Paklenica National Park on March 13, 2020. After the release, he first moved towards north-west on Velebit Mountains until Baške Oštarije, where he turned back towards the south-east shortly and came back to the vicinity of the release site on April 24, where he established his territory around Sveti Rok. His current home range measures 166 km^2 and is located on the very south of Velebit Mountains. Alojzije is frequently crossing over the highway Bosiljevo-Split above the Sveti Rok tunnel. In 2019, before this translocation, we recorded one female in the area, but in 2020 we only documented two males in the surrounding area. We managed to locate two of his kills since the release – red deer fawn and a roe deer buck. We have now increased our camera trapping effort within his home range in order to document females in the area and his potential offspring in 2021 and the following years.

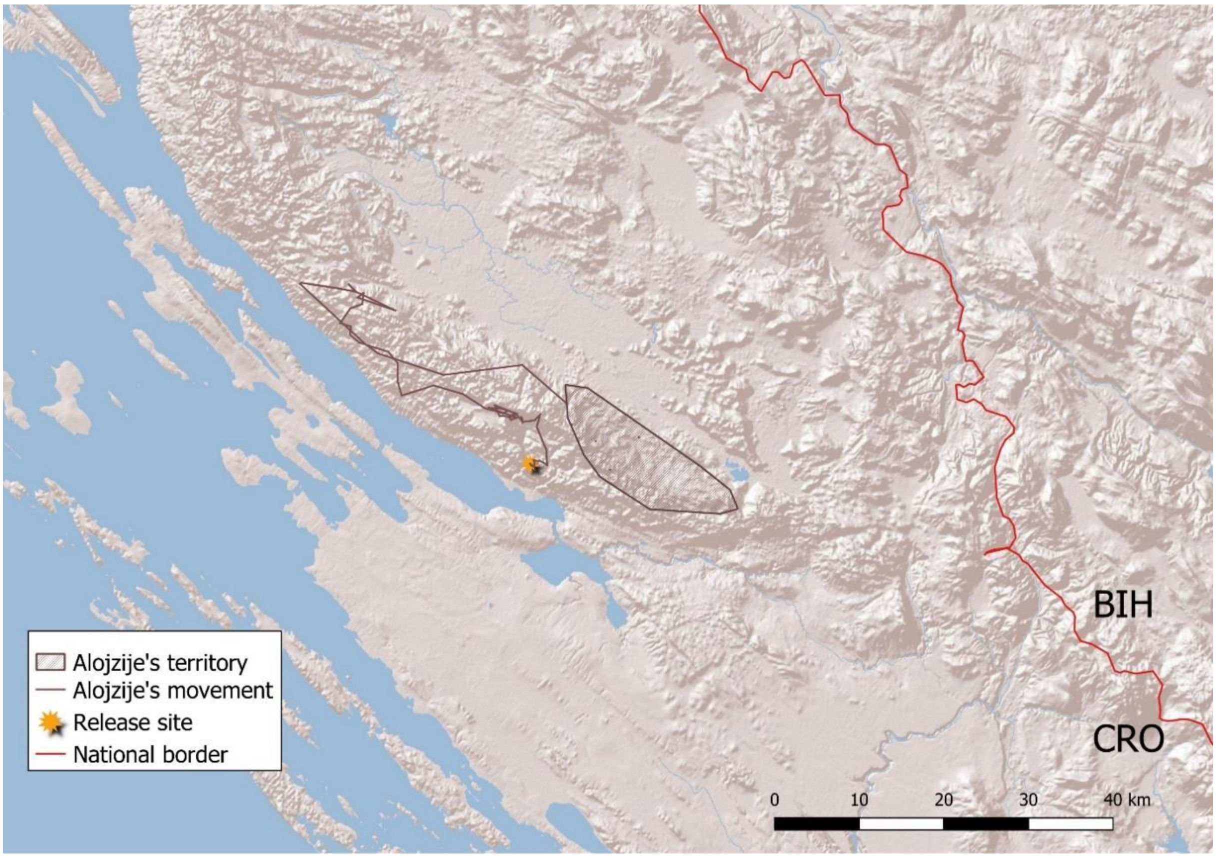

Figure 19. Map of Alojzije's movement after the release and his home range (100% MCP) established in the southern part of the Velebit Mountains.

2.5.2 Remnant lynx and offspring of translocated lynx monitored with telemetry

Within the LIFE Lynx project, so far two remnant lynx were captured and collared in Slovenia, as well as two offspring of a remnant female and a translocated male. This helps us to gain additional understanding of the reinforcement processes, such as mating with translocated lynx and dispersal of their offspring, as well as to better understand the territorial distribution of lynx in current population, which can importantly affect movements of translocated animals and establishing of their territories. So far we captured one adult remnant female (Teja), one adult remnant male (Mihec) and two juvenile lynx (female named Mala and male named Niko), who are offspring of translocated lynx Goru and remnant lynx Teja. Below we provide details on the movement of three individuals collared during the 2019-2020 lynx-monitoring year, while the second juvenile, Niko, was captured a few days before completion of this report (December 6, 2020), therefore his telemetry data will be presented in the next annual report. We also provide information on a remnant male Bojan tracked within another project (3Lynx), but note that also other remnant lynx are tracked with telemetry in Croatia. Since we were not able to obtain permission to show their data, this is not presented in this report, but we took this information in account when writing synthesis of the current situation (chapter 3).

Teja

Figure 20. Lynx Teja photographed with camera-trap on Mala gora, Slovenia.

Female lynx Teja was first recorded with a camera-trap on September 7, 2018 on Mala Gora near Ribnica. According to her vocalizations recorded with a video in March 2019, it looked like she was looking for a mate. Apparently there was no male lynx present in her territory at that time. Teja was captured and collared on April 19, 2019 on Mala Gora. On June 1 translocated lynx Goru reached Teja's territory and met her on the first day of his arrival. In the next weeks they often met and moved together, as well as shared kills. Movement analysis showed that Teja gave birth on August 15, 2019 which is 2.5 months later compared to the expected delivery period for Eurasian lynx. On November 19, 2019 we recorded Teja's kitten (named Mala) with a camera-trap and obtained a non-invasive genetic sample which showed that Goru is the kitten's father. Despite a late birth date, the kitten survived to independence. In the beginning of February Teja's collar dropped off but we monitored her movement until the beginning of May through Mala's collar. According to the telemetry data, we assume that Goru and Teja mated for the second time between February 21 and 24, 2020. Teja and Mala were moving together till May 1, 2020, when Mala moved out of mother's territory to become independent, and Teja gave birth to another litter, this time with two kittens. We first recorded them with camera-traps in September and confirmed their good physical condition. We managed to capture and collar the male kitten (Niko) and sampled it for genetic analysis that confirmed Goru is the father of this year's litter.

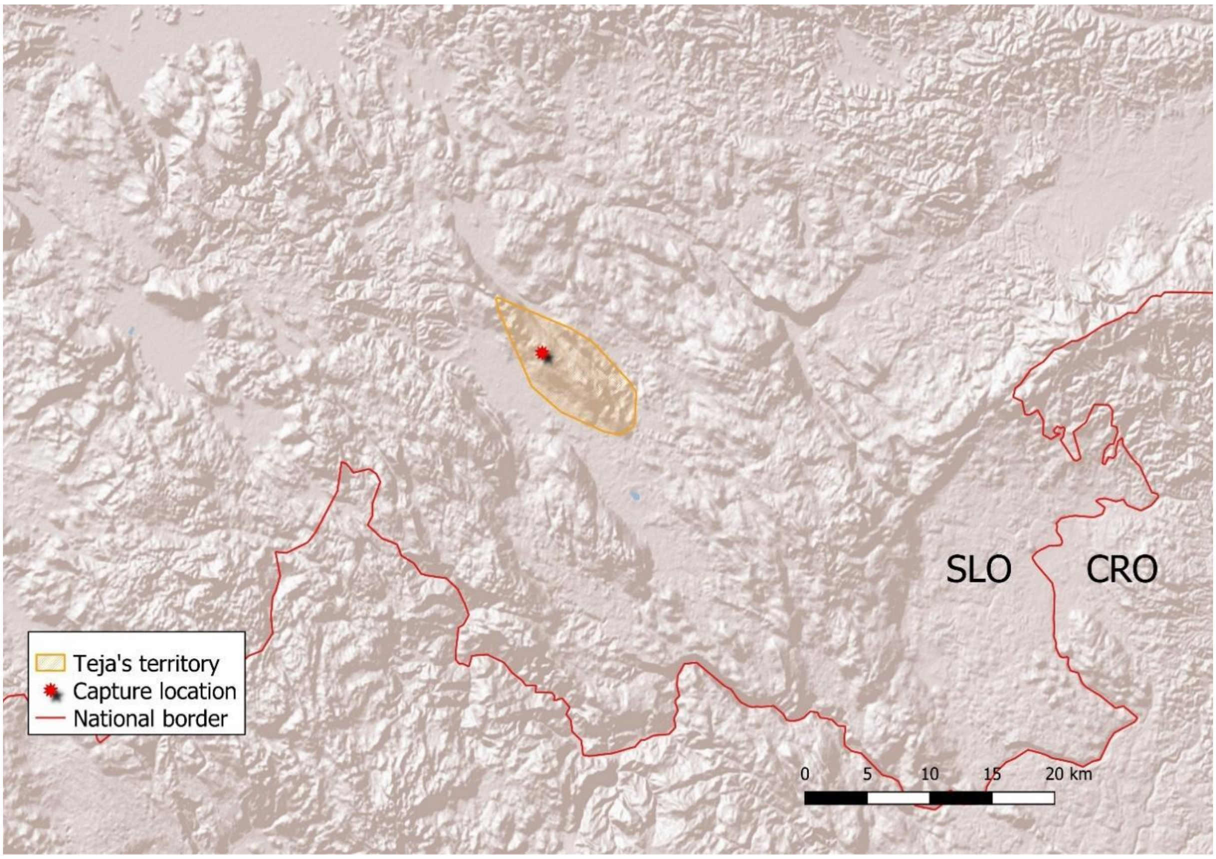

Figure 21. Map of Teja's home range (100% MCP) and capture location.

Mihec

Figure 22. Lynx Mihec photographed before capture with camera-trap near Draga, Slovenia.

Remnant male lynx Mihec was first captured on December 23, 2010 as a 12 kg yearling in the Snežnik area. He was a kitten of GPS-tracked female Snežka. In April 2011 he dispersed from mother's territory to the north-west and established his own territory on Javorniki, where we tracked him until October 2011, when his collar stopped working. The male was spotted several times until 2013, when his collar was found (passively dropped-off). Since then we didn't get any new data about his presence, probably because he became more difficult to identify without a collar. His survival was confirmed in the 2019-20 lynx monitoring season, when he was photographed several times and identified based on a coat pattern. On March 21, 2020 we captured him and genetic analysis confirmed his identity. Telemetry data revealed that sometime during the previous eight years, he shifted his territory from Javorniki to a new territory stretching from north-eastern part of Snežnik plateau, over Racna Gora to the western part of Goteniška gora region. His home range covers around 340 km^2 (MCP 100%). He is sharing his territory with at least two female lynx. In 2020 we photographed one of these females with her offspring and there is a good chance that Mihec is the father, although this needs to be confirmed with genetic analysis.

We regularly obtain records of Mihec through camera-trapping, with over 20 photos from him, especially from hunting clubs Iga vas and Babno Polje. Despite being one of the oldest known lynx in the Dinarics, Mihec appears to be in good shape and is successful in hunting, mainly roe deer, but we also found prey remains of other species, like red deer, dormice, and red fox. Additionally, we found one case of killing and consuming a large male European wildcat..

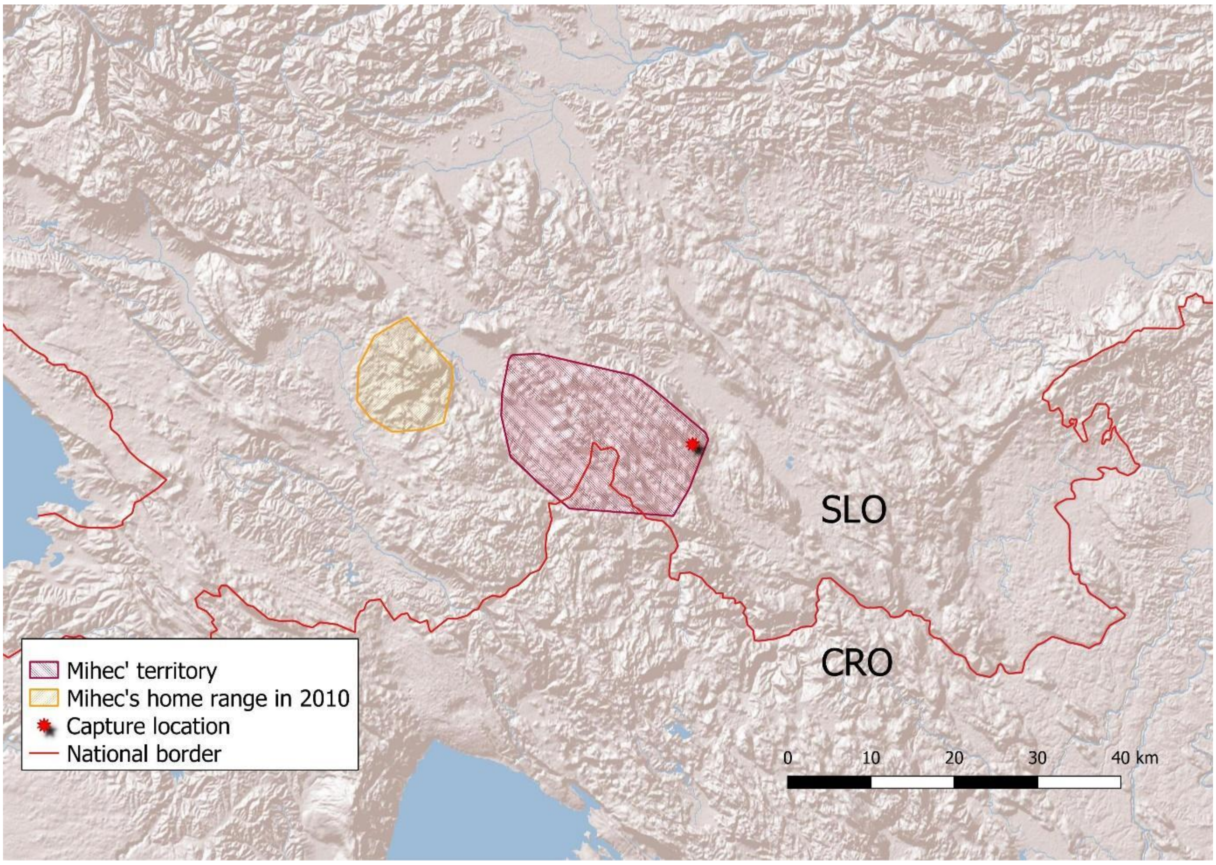

Figure 23. Map of Mihec's home range from 2010 and 2020 (100% MCP) and capture location in 2020.

Mala

Figure 24. Lynx Mala photographed with camera-trap on Mala gora, Slovenia.

Female lynx Mala is a descendant of the remnant lynx Teja and translocated lynx male Goru, born in mid-August 2020, which is a very late date for Eurasian lynx in Europe and was a consequence of absence of male lynx in her mother's territory, before the arrival of a translocated male Goru in the beginning of June. Mala was first photographed at her mother's kill site on November 21, when she was estimated to weigh around 3-4 kg. On January 18, 2020 we captured Mala while trying to recapture her mother and collared her with an ultralight GPS collar. Mala stayed with her mother until the beginning of May when Teja gave birth to the next litter. Mala dispersed north to Suha krajina area but stayed close and occasionally visited her mother's territory. Mala was successful at hunting prey on her own and shortly after independence managed to kill her first roe deer. At the end of June, the batteries in her collar started draining out so we triggered drop-off but unfortunately due to a collar failure it did not activate. Soon thereafter the signal was lost, but we continued to detect her on camera traps and she continues to appear in good physical condition (Figure 23). We continue with efforts to recapture her and replace the failed collar.

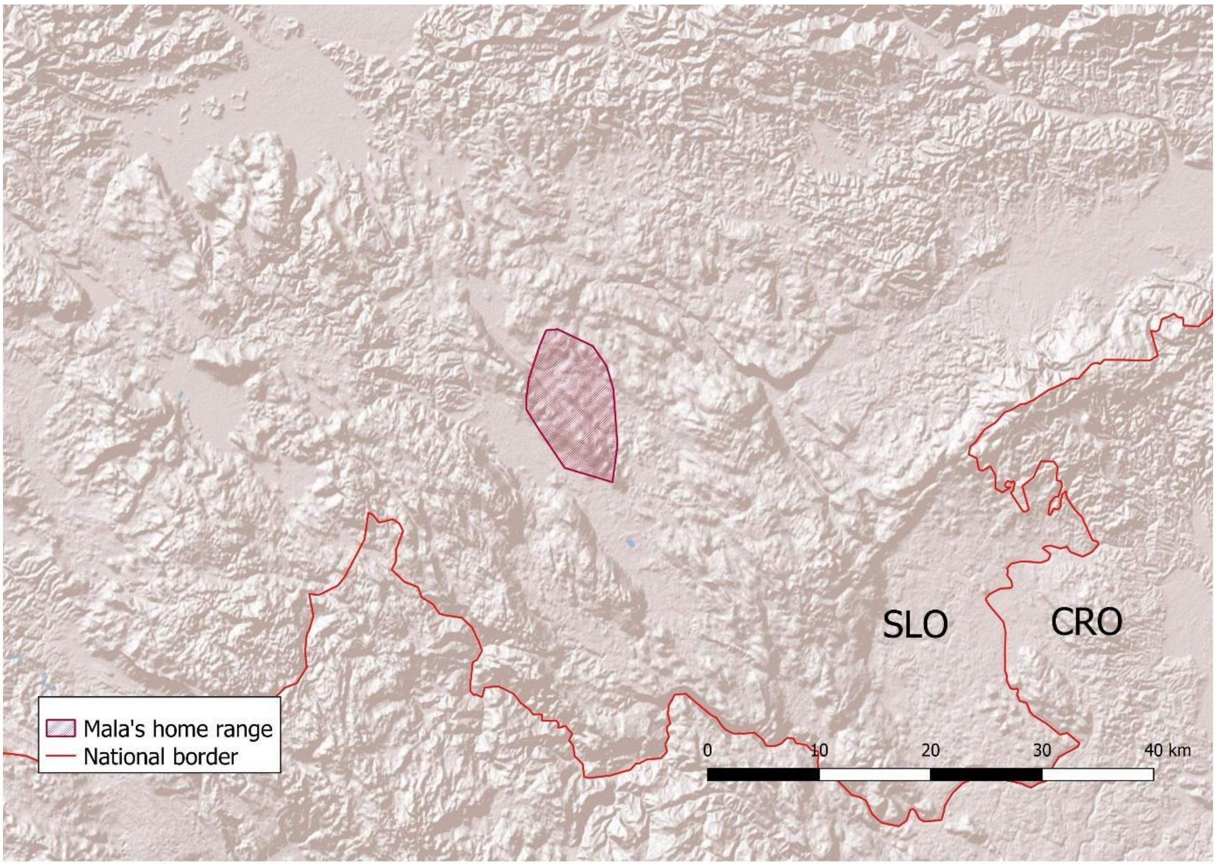

Figure 25. Map of Mala's home range (100% MCP) after independence.

Bojan

Figure 26. Remnant lynx Bojan after collaring near Kolpa river, Slovenia.

Lynx Bojan is a remnant lynx that was collared within the Interreg 3Lynx project on December 1, 2019. He weighed 24 kg and was estimated to be four years old. He was captured in Slovenia, close to the border with Croatia in the hunting ground Osilnica. He stayed in Slovenia for a few days after the capture, but soon crossed the border with Croatia and since then mostly stayed in Gorski Kotar area. His home range size measures around 515 km^2 (MCP 100 %), which is larger than most other lynx home ranges in Northern Dinarics. It is mostly located in Gorski Kotar in Croatia, partly within Risnjak National Park, with occasional visits to Slovenia, mainly around Osilnica and a single excursion (possibly extra-territorial) to Velika gora. Telemetry data revealed a likely encounter with young translocated lynx Boris, who left the area after this interaction.

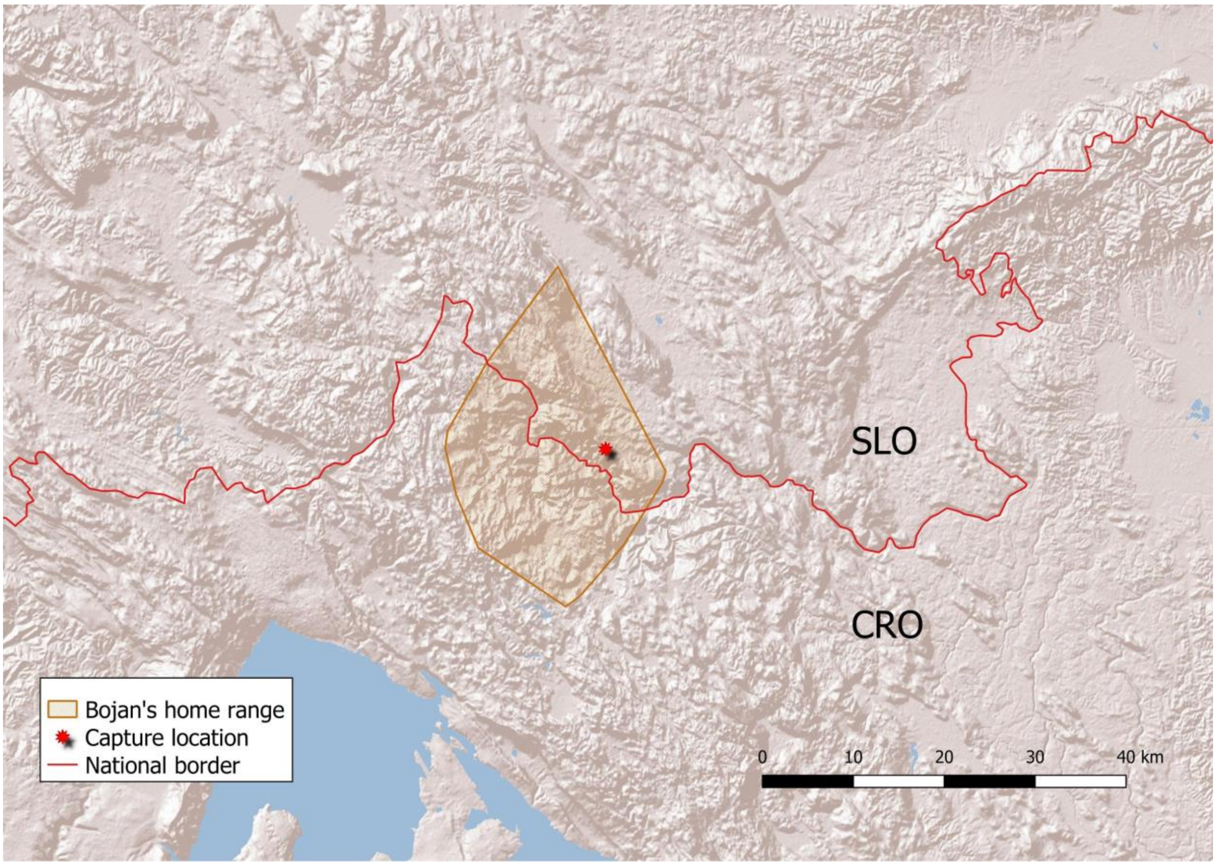

Figure 27. Map of Bojan's home range

2.5.3 Monitoring lynx predation on ungulates

Thanks to lynx behaviour of returning to their ungulate kills for several days (Krofel et al. 2013), telemetry data obtained from GPS tracking can be a useful tool for studying lynx predation and thus enable us to find the kill sites in the field. Collection of data at the kill sites can provide us with insight about lynx diet, which can be later implemented into ungulate management plans and evaluation of the lynx ecological impact. Besides, installing camera traps on fresh kills provides us with an opportunity to obtain footage and assess lynx' physical condition.

In total, we found 148 lynx kill sites in Slovenia and Croatia, often with the help of local hunters. The main prey species found at the kill sites was European roe deer with more than 95% of all detected kills. We also detected red deer (2%), red fox (1%), chamois (<1%), European wildcat (<1%), and fat dormouse (<1%) at the lynx kill sites. Scavenger species that were recorded at the lynx kill sites include red fox, brown bear, Eurasian badger, golden jackal, beech marten, wild boar, common buzzard, northern goshawk, common raven, Eurasian jay, and coal tit.

In Table 6 we present sex and age structure of animals killed by the collared translocated and remnant lynx for the most important prey species, the roe deer. Lynx mostly killed adults and somewhat more females than males. In general, these results suggest similar predation patterns to those observed in previous research on remnant lynx from the Dinaric population (Krofel et al. 2014).

Table 6: Age and sex structure of roe deer killed by collared lynx.

	roe deer	sex			Total (%)
		male (%)	**female (%)**	**unknown (%)**	
age	adult	32(22.5)	40 (28.2)	10 (7)	82 (57.7)
	juvenile	6 (4.2)	20 (14.1)	12 (8.5)	38 (26.8)
	unknown	0 (0)	0 (0)	22 (15.5)	22 (15.5)
	Total (%)	38 (26.8)	60 (42.3)	30 (31)	142 (100)

With the telemetry data and GPS cluster analysis (see Krofel et al. 2013 for details), we could also calculate the kill rates for each of the collared lynx, except for Doru, Alojzije and Pino, where this was not possible due to too low GPS fix rate for this purpose or collar malfunction. The average interval between consecutive kills was 5.8 days for the translocated lynx. The highest kill rate was observed for lynx Boris and Katalin, with an average interval between the kills of 5.6 days. The lowest kill rate was observed in lynx Goru, with an average kill made every 6.3 days. Compared to the previous research on remnant Dinaric lynx (average kill interval of 7.2 days; Krofel 2012) this kill rate appears higher. We assume that this could be connected with higher densities of wild ungulates in some of the areas occupied by monitored lynx, as well as potentially naïve prey in areas recently colonized by the translocated lynx, similar to observations reported from elsewhere (Duľa & Krofel 2020). Additional reason for the observed difference could be due to the male-biased sample of lynx monitored during the first period of this project compared to the previous study in this area.

2.6 Lynx mortality

Only one lynx was found dead in the Dinaric SE Alpine region in the lynx-monitoring year 2019-2020. It was killed on Slovenian A1 highway Ljubljana-Koper near Postojna on December 27, 2019. The remains were much destroyed and only a sample of tissue was collected for genetic analysis while necropsy was not possible. The skin was mostly preserved and was taken to a taxidermist so it can be used for educational purposes.

Additional mortality was recorded after the end of the lynx-monitoring year 2019-2020, in August 2020. It was a remnant orphan female named Martina, which was found as a kitten in Croatia in 2019 and was released in 2020 from Risnjak National Park after successful rehabilitation. Later it dispersed to Slovenia and died from an unknown cause after the collar had already failed. Only bone remains were found in Pivka valley, so necropsy was again not possible. Further details about this lynx will be presented in the next annual report on 2020-2021 lynx-monitoring year.

There were two other potential lynx tissue samples sent to the Faculty of Veterinary Medicine University of Zagreb in Croatia anonymously, believed to belong to poached animals, however, genetic analysis showed that the samples did not belong to lynx. Therefore they are not included in Table 3.

3. REGIONAL SYNTHESES

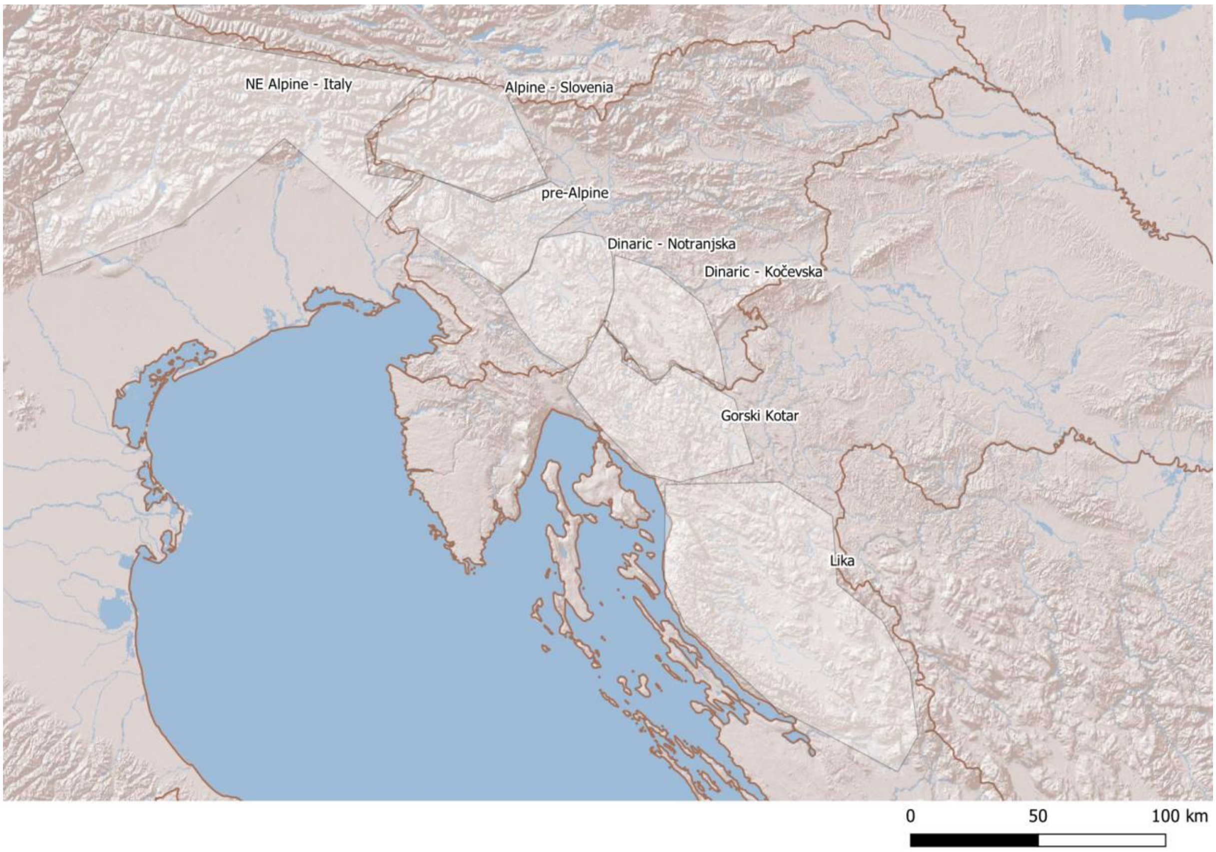

Figure 28. The division of Dinaric-SE Alpine project area in distinctive regions for which we present the syntheses in chapters 3.1 – 3.5. The NE Alpine – Italy region is omitted due to almost no data collected there.

3.1 Slovenian Dinaric - Notranjska and Kočevska

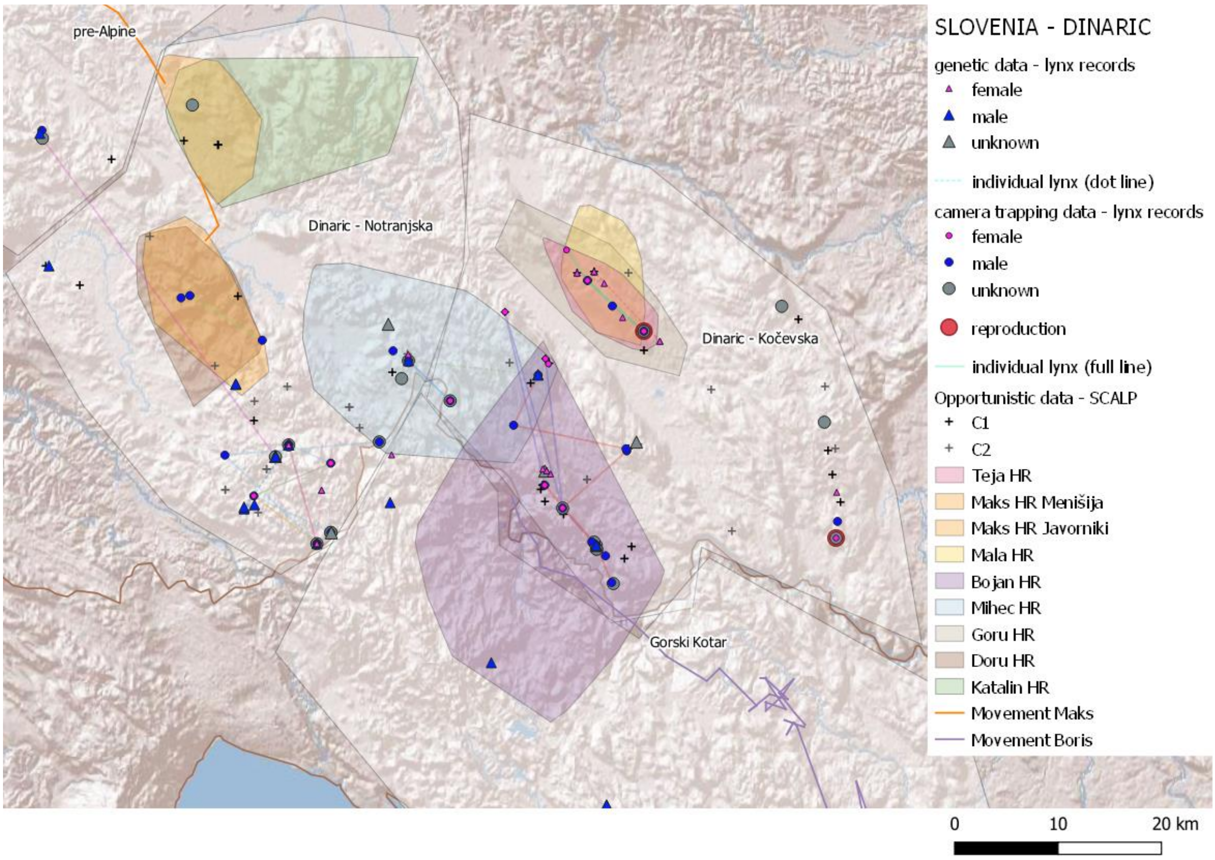

Figure 29. Overview of all confirmed records of Eurasian lynx collected during the 2019-2020 monitoring year in Kočevska and Notranjska regions in Dinaric Mountains of Slovenia. Shown are records from the systematic camera-trapping and non-invasive genetic monitoring (with information about the sex of detected lynx, when available), home-ranges (HR) and movements of translocated lynx tracked with GPS telemetry and confirmed opportunistically-collected records (C1 and C2 category). Straight lines connect genetic samples and camera-trap records of the same individual.

In the Slovenian part of the Dinaric Mountains south of the Ljubljana-Koper highway (i.e. Notranjska and Kočevska) most of the region is currently occupied by lynx, including several territories occupied by male and female lynx and a total of minimum 18 different adult lynx were detected in this region in 2019-2020 lynx-monitoring year, including two translocated males. However, in this monitoring year we recorded a surprisingly small number (2) of successful lynx reproductions (we detected another kitten near Postojna, but it died in a vehicle collision on the highway). The good news is that one of these reproductions was the first successful mating between the translocated male from the Carpathian population (Goru) and remnant female from Dinaric population (Teja).

Lynx pairs sharing a territory were confirmed in Kočevski rog/Poljanska gora (with reproduction), Mala gora (with reproduction), Goteniška gora, Velika gora, Racna gora, Snežnik plateau (probably 2 pairs), Javorniki/Vremščica (with unsuccessful reproduction) and most likely also on Menišija/Rakitna (we are still waiting for confirmation that the lynx observed together with Katalin is a female). Areas where apparently only single lynx are present and which could therefore be the most suitable areas for future translocations include Stojna (probably only male present) and northern part of Kočevski rog (one lynx of unknown sex). Another potentially suitable area is Mokrc and surrounding forests, from where no confirmed lynx records were available during the 2019-2020 season, but where at least one lynx was detected through opportunistic monitoring in the second half of 2020, although its sex remains unknown for now (genetic samples were collected, but were not yet analysed; however, according to the scent-marking frequency rate observed during snow-tracking, it is likely a female). We recommend this area to be included in the future systematic monitoring, same as for Vremščica and forests north of it, where unsuccessful reproduction was confirmed (kitten killed on highway), but it is not clear who were the parents and whether they are the same lynx that were detected in the nearby Javorniki area.

3.2 Pre-Alpine, Slovenia

In the pre-Alpine region (i.e. Polhograjsko hribovje, Hrušica, Nanos, and Trnovski gozd) we were able to confirm lynx presence on Hrušica and Trnovski gozd. During both of the last two monitoring seasons two different males were detected genetically and with camera-traps on Hrušica. Interestingly, both of them were also detected genetically on the other side of the Ljubljana-Koper highway on Javorniki (one in 2018-2019 season and the other in 2019-2020). From Trnovski gozd we obtained only one confirmed opportunistic record, a photo of a lynx of unknown sex. At the end of 2020, also one of the collared translocated lynx (male Maks) crossed this area, but did not settle here. No confirmed records were obtained from Nanos, although lynx presence there is suggested by the roadkill of a kitten on a nearby highway. Further intensification of monitoring efforts is recommended, especially for Trnovski gozd and Nanos.

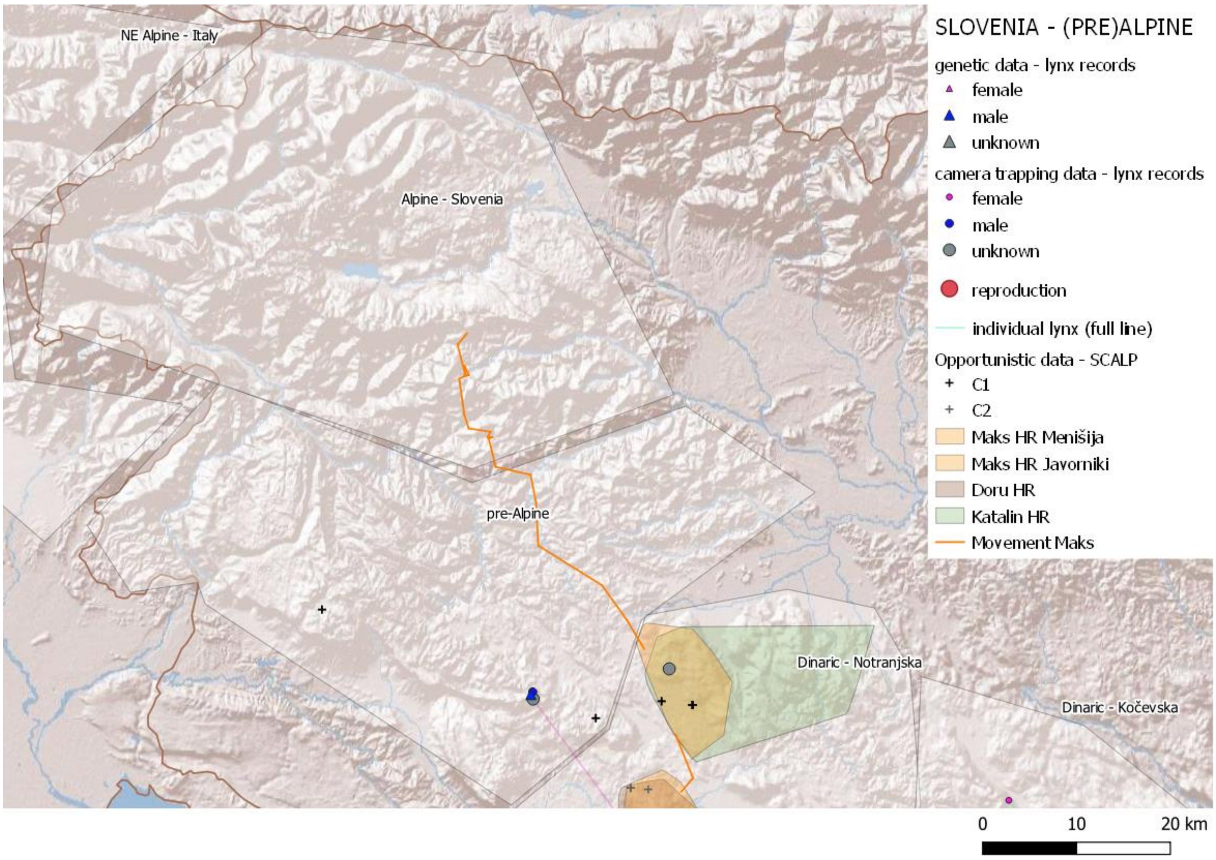

Figure 30. Overview of all confirmed records of Eurasian lynx collected during the 2019-2020 monitoring year in pre-Alpine and Alpine regions of Slovenia. Shown are records from the systematic camera-trapping and non-invasive genetic monitoring (with information about the sex of detected lynx, when available), movement of translocated lynx tracked with GPS telemetry and confirmed opportunistically-collected records (C1 and C2 category). Telemetry data of lynx from other regions is not shown.

3.3 Julian Alps, Slovenia

No confirmed records were obtained from this region in 2019-2020. Occasional unconfirmed information was reported, but until more reliable evidence is obtained, the presence of remnant lynx in this region remains questionable. At the end of 2020, one of the collared translocated lynx (male Maks) arrived in this area, but so far there is no indication that he would be establishing his permanent territory here.

3.4 Gorski Kotar, Croatia

In Gorski Kotar we identified 25 adult lynx, 21 from both sides of the body, 3 from only right side and one from the left side only, among which were 12 females, 9 males and 4 animals of unknown sex. Lynx Boris is mostly present on Kapela Mountain, an area which is still suspected to be covered with land mines, so we could not put camera-traps here and therefore we do not have a good insight into lynx presence in this area.

All of the lynx that were released in Gorski Kotar in the past years (one translocated Romanian lynx and two rehabilitated orphans from Croatia) left this region and crossed the border to Slovenia. Also, other translocated or remnant lynx that dispersed through Gorski kotar did not establish their territories here. This and our monitoring data indicate that most of the territories in this region are already occupied. This presents limited options for translocated lynx to establish new territory in Gorski kotar, so for the next season we do not plan to release any new animals in this region.

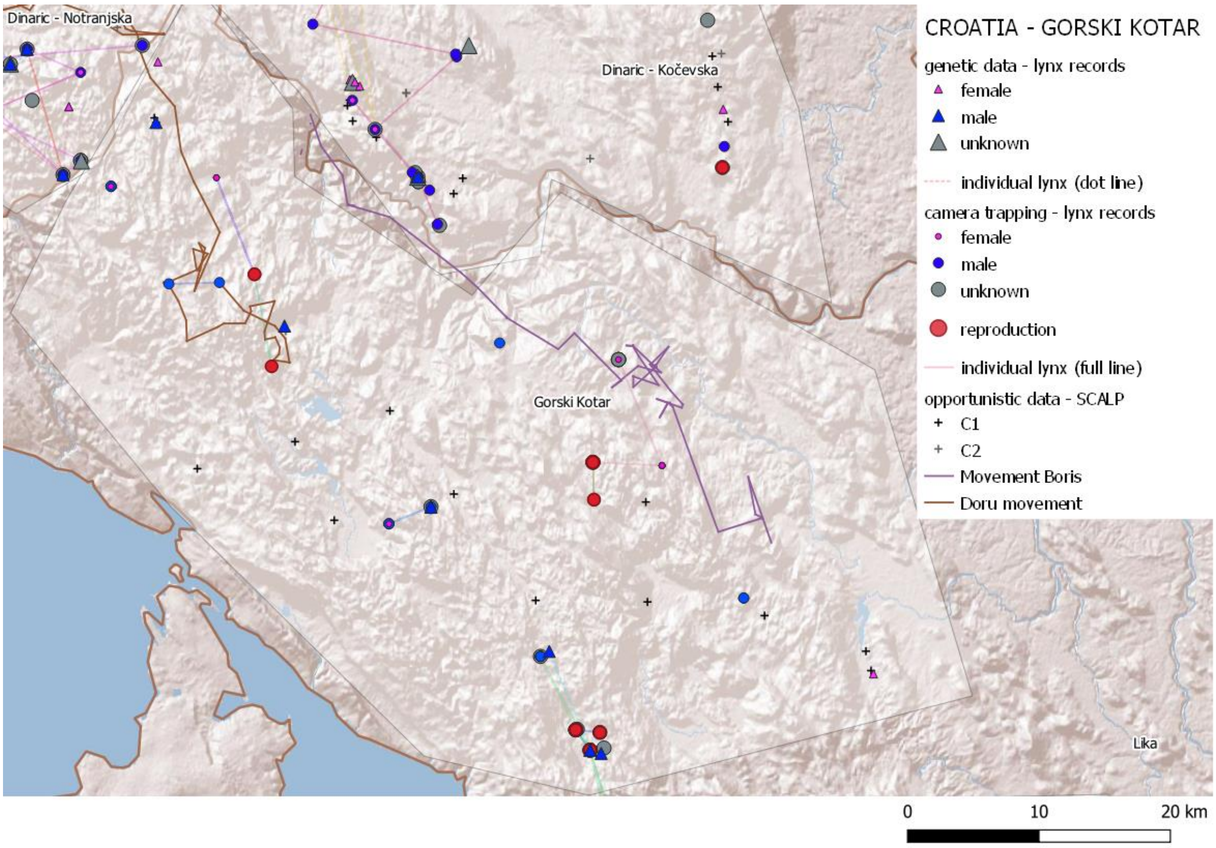

Figure 31. Overview of all confirmed records of Eurasian lynx collected during the 2019-2020 monitoring year in Gorski Kotar, Croatia. Shown are records from the systematic camera-trapping and non-invasive genetic monitoring (with information about the sex of detected lynx, when available), movements of

translocated lynx tracked with GPS telemetry and confirmed opportunistically-collected records (C1 and C2 category). Straight lines connect genetic samples and camera-trap records of the same individual.

3.5 Lika, Croatia

A total of 58 adult animals were identified in the wider Lika region (including several animals in Karlovac and Zadar county), 30 from both sides of the body, 16 only from the right, 12 only from the left. Among them were 12 females, 11 males, 35 animals of unknown sex. Compared to the Gorski Kotar region, we have poorer understanding of the current situation in Lika, where only 52% of the animals were photographed from both sides of the body, sex was not determined for 60% of the individuals and a total of 14 individuals were photographed only once. This is a consequence of two main factors: in Gorski Kotar region most of the camera traps are located on marking locations where it is easier to photograph animals from both sides, while in Lika region we were able to find only a small number of marking locations. Also, marking locations are important for collection of samples for DNA isolation, which reflects the overall number of collected samples and identified sexes of individuals. In 2019-20, snow cover was present for a very short period in Lika region, and mostly only in northern Velebit, which also resulted in a low number of samples collected in southern parts.

In Lika region, translocated lynx Alojzije established a territory in southern Velebit, where we also recorded a female lynx in this and previous year. In central Lika we do not have signs of lynx presence, but this area is not suitable for lynx presence (karst fields and settlements). Based on our data, the southeastern area of lynx distribution in Croatia (Zadar county) has lower lynx density, so we recommend central Velebit as the next release location in Croatia, where animals will also have opportunity to disperse towards southeast.

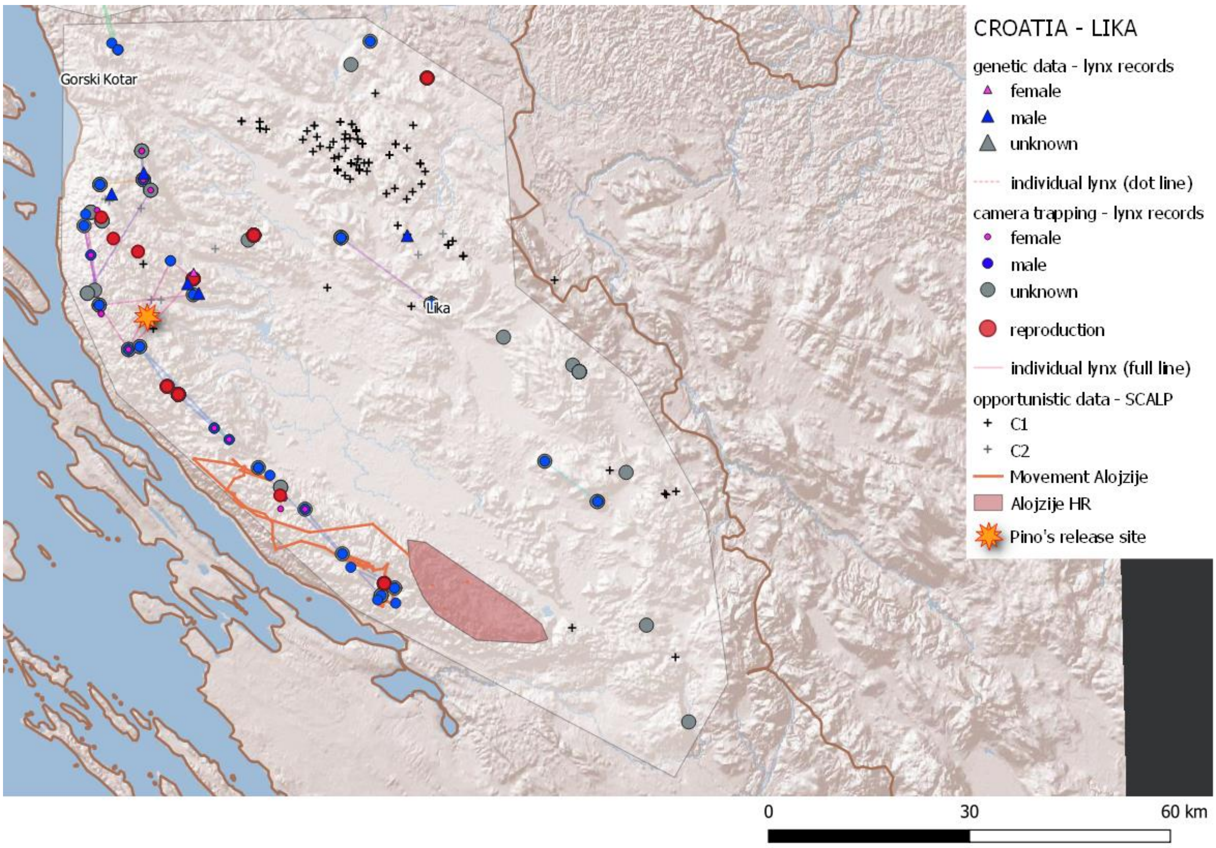

Figure 32. Overview of all confirmed records of Eurasian lynx collected during the 2019-2020 monitoring year in Lika, Croatia. Shown are records from the systematic camera-trapping and non-invasive genetic

monitoring (with information about the sex of detected lynx, when available), movement and home range (HR) of translocated lynx tracked with GPS telemetry and confirmed opportunistically-collected records (C1 and C2 category). No telemetry data was received from one of the released lynx, therefore only his release site is indicated.

4. CONCLUSIONS WITH RECOMMENDATIONS FOR FURTHER RELEASES

According to available data, in 2019-2020 the Dinaric-SE Alpine lynx population generally appears similar to the previous year, with several territories across the Dinaric Mountains of Slovenia and Croatia occupied by lynx of both sex, small number of individual lynx in the pre-Alpine region in Slovenia and without confirmed records of presence of remnant lynx in the Alpine region of Slovenia or north-eastern Italy. The most important changes observed in this lynx-monitoring year include the translocation of the first animal from the Carpathian population in the frame of the reinforcement project and the first births of the offspring of the translocated and remnant lynx. Another notable difference compared to the previous year is a small number of successful reproductions detected in Slovenia. Whether this is a consequence of imperfect detection or genuine decline in reproduction cannot be assured at the moment. Given small population size, stochastic events like this would not be unexpected, but we stress the need to pay close attention to trends of this parameter in the next monitoring seasons.

Similar to the previous year, camera trapping provided the most informative insight into demographic status of the population, especially in Slovenia where camera-trap density was high and monitoring activities intensively coordinated. This could only be achieved by close cooperation with hunters, which should be further nourished in order to yield good results. In addition to camera-trapping, also genetic and opportunistic data importantly complemented the picture. Genetic monitoring is crucial for assessing genetic status and influence of ongoing translocations. But due to difficulties of collecting enough samples over the entire area, genetic monitoring could so far only be used as supplementary data to camera trapping for understanding the demographic status and population parameters. However, genetic samples gave us important insight into the level of effective inbreeding, which is the most important threat to this population and which could not be monitored with other methods. While inbreeding is still high, we can already see important improvements due to population reinforcement efforts and large increase in expected heterozygosity indicates the potential for rapid decrease of inbreeding if the translocated animals continue to successfully reproduce.

In 2021, translocations and releases of Carpathian lynx will focus on the Alpine region. Since no confirmed presence of remnant lynx was recorded here, simultaneous releases of males and females will be essential for the successful formation of a stepping-stone population nucleus. Two areas (Pokljuka and Jelovica) have been selected as the most suitable for these releases according to the habitat requirements, connectivity to the population core area in Dinaric Mountains and local public support for translocations.

In case additional releases will be conducted also in Dinaric Mountains, the most suitable areas in Slovenia include territories where only single lynx are currently present (Stojna, northern part of Kočevski rog, potentially also Mokrc), although information of their sex would require additional confirmation. In Croatia, next releases are recommended to take place in Lika, especially in the central Velebit area. Based on this information, the most appropriate location for potential releases in Slovenia 2021 is the enclosure in Loški Potok. We will continue the population surveillance through the lynx-monitoring year 2020/21 and make the final decision based on the last available information, if needed. More releases in the Dinaric region of Slovenia and Croatia are planned for 2022, which should be based on updated results of the monitoring in the next (2020-2021) season.

At the moment, destiny of two translocated lynx, whose signal from GPS collars was lost (Doru and Pino), remains unclear. If their survival or presence of their offspring could not be established in the next monitoring seasons, we recommend to consider replacing them with two new lynx from the Carpathian population, according to the reinforcement plan (Wilson et al. 2019). Two additional lynx released in 2020 (Maks and Boris) have so far not yet established permanent territories and we will continue to monitor whether they will be successfully included in the population.

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