LIFE Lynx

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

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Optimal management scenarios for ensuring viability of lynx in the Dinaric mountains and South eastern Alps

Action D.3

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CONTENTS

INTRODUCTION

The Dinaric lynx population was founded by six animals, translocated from Slovak Carpathians in 1973 (Čop & Frković 1998; Kos et al. 2004). The reintroduction campaign was considered one of the most successful reintroductions in Europe (Breitenmoser-Würsten & Breitenmoser 2001) - the population seemed stable until the 2000s, but then a decline started to be observed (Kaczensky et al. 2012). While natural and human-related mortality certainly played their part, the population was becoming considerably inbred (Sindičić et al. 2013). In absence of gene flow from other populations, accumulation of population-level inbreeding has a huge impact on the decrease in the animal's fitness causing lower survival and reproductive success (Allendorf & Luikart 2009). The first genetic study of the Dinaric lynx population showed that this population has the lowest genetic diversity among all studied Eurasian lynx populations (Sindičić et al. 2013). Problematic genetic status of the population was reconfirmed by the baseline genetic study effectuated prior to population reinforcement, where level of inbreeding was estimated to be 0.316 (Skrbinšek et al. 2019), above the expectation for a full-sibling mating which causes an inbreeding coefficient of 0.25. From the genetic perspective, the situation is critical and according to recommendations of the Bonn lynx expert group immediate actions are needed in such cases to restore the genetic variability (Bonn lynx expert group, 2021).

The main goal of the LIFE Lynx project is to rescue the Dinaric - South eastern Alpine lynx population from extinction. During the 2019-2021, 13 lynx were already translocated to Slovenia and Croatia, and some of them were already successfully integrated into the population through successful reproduction. Each new integrated animal increases the chances for survival of the entire population. Previous simulations showed that if the introduced animals and offspring would form 15% of the population, the effective inbreeding would drop to 0.21 (Krofel et al. 2021). This would be a significant improvement, and a step towards what's needed to prevent extinction and establish a genetically and demographically robust population. However, it is clear that in the absence of natural gene flow from other outbred populations the Dinaric - SE Alpine population will need genetic and conservation management in the long run to maintain its viability.

The goal of the D.3 action of the LIFE Lynx project is to track the impact of the project on the long term viability of the Dinaric – SE Alpine lynx population and determine how the population should be managed in the long run. The main tool for the latter is stochastic modelling of population development, aimed to predict the long-term viability of the lynx population in the Dinaric Mountains and the South eastern Alps under different management scenarios, or in complete absence of genetic management. We developed and performed individual-based, forward-time simulations to study dynamics of the key population genetic parameters such as heterozygosity, level of inbreeding and effective population size. However, we need to underline that although modelling is a powerful method of assessing a population's genetic properties, the results should be interpreted with caution. The probability of errors increases for long-term simulations and not all possible factors can be taken into account. Nevertheless, stochastic simulations are a very useful and widely used tool in conservation biology as they are able to provide useful reference points for decision-making that cannot be obtained by any other means. For this modelling, we used the first results of the project as well as literature data. The impact of population reinforcement is being closely monitored (action C.5) through camera-trapping, non-invasive genetics and parentage analyses (Krofel et al. 2021), and the final assessment of the reinforcement (action D.2) will start in 2023. Obtained results will be used to refine the models, improve their predictive power and the long-term strategies for ensuring viability and vitality of the Dinaric-SE Alpine lynx population.

MATERIALS AND METHODS

MODELLING PROCESS

We performed forward-time individual-based simulations using python library SimuPOP v. 1.1.8svn (Peng and Kimmel, 2005). This package allows us to perform simulations of populations with overlapping generations and estimate a wide range of genetic and demographic metrics. The modelling environment integrates the main function for iterative simulation of population dynamics and five secondary functions to set up (1) individual properties, (2) breeding, (3) mortality, (4) reinforcement parameters and (5) genetic statistics estimation. In the simulations we make an emphasis on genetics, which is in a small and inbreeding population the key component influencing the population's survival and fitness. Changes in heterozygosity level, inbreeding and effective population size are used as population genetic statistics to measure the effect of different management strategies on the lynx population. In order to cover various possibilities of population development taking into account stochastic factors, each tested management scenario was run in 50 iterations. We ran each simulation for 100 "years" (a "year" was simulated as one reproductive season) or until complete extinction of the population, i.e. all animals dead. Two populations were simulated – Dinaric-South-eastern Alpine population and a source population for translocations (Figure 1). In these simulations we did not consider gene flow between Dinaric and SE Alpine subpopulations, and modelled the population as a single evolutionary unit. In the simulated population animals were able to mate until they reached the age of 13 (Kos et al., 2004). Lynx males and females reach sexual maturity by 21-33 and 11-21 month of age respectively, as was noted for Scandinavian lynx population (Kos et al., 2004), but for simulation purposes we assumed that both sexes were able to mate when they reached 2 years of age. Ages in the Dinaric-SE Alpine population on initial point of simulation were assigned randomly under negative binomial distribution. The stable lynx populations (Polish and Norvegian) mostly have balanced sex structure (Kos et al., 2004). The simulated source population consisted of adult animals 1-5 years old, and was not exposed to all the processes and factors described below. This fits with the actual translocation process since only healthy, relatively young and genetically unrelated animals should be translocated (Bonn Lynx Expert Group, 2021).

Each simulated individual had the following properties: sex, age, genotype, individual id, mother id, father id and IBD (identity-by-decent coefficient, a measure of inbreeding indicating the probability that both alleles in one locus originated from the same recent ancestor), so we assumed an equal sex ratio for the simulated population. In order to make simulated data more compatible with the results of genetic analysis, performed in the frame of the project, we simulated 19 genetic microsatellite loci, the same number as used by Skrbinšek et al. (2019). Allelic diversity – an average number of alleles per locus - in the Dinaric-SE Alpine population was 3.21, in Slovakia – 4.21, in Romania – 3.58 (Skrbinšek et al., 2019). We used the allelic frequencies obtained for Dinaric-SE Alpine, Slovakian and Romanian populations through genetic monitoring to simulate genetic composition of the simulated populations. SimuPOP allows tracking of genealogical relationships between animals, which was used for measurement of inbreeding through identity by descent (IBD). Individual id is an unique number, assigned to each animal, and mother id and father id can be used for tracking relationship between parents and offspring.

Simulation start point was set to be a year before the beginning of the translocations within the LIFE Lynx project, i.e. 2018. During the LIFE Lynx project, the minimum number of adult lynx in the Dinarics - SE Alpine area was estimated as 101 (Krofel et al., 2021). In the simulations, the Dinaric-SE Alpine population size was set as 100 adult animals and 25 kittens. The carrying capacity was set as 240 adult animals (Skrbinšek, Krofel, 2008). Simulated litter size was 2.1± 0.9 kittens as was estimated for the Swiss (Jura mountains) population (Kaczensky, 1991). Simulated baseline mortality of adult and subadult male and female lynx was 0.13, which was the average natural and traffic mortality estimated for Scandinavian lynx in three research areas (Andren et al., 2006). Baseline juvenile mortality was simulated as 0.5 as was estimated for the Swiss (Jura mountains) population (Kaczensky 1991). Probability of survival connected with the inbreeding load was simulated as additive to the baseline mortality and calculated separately. Expected survival probability, defined in Nietlisbach et al., 2018 (equation 4) was calculated for each individual, where F was given as IBD, and B as the number of lethal equivalents:

$$\pi_F = e^{-BF}$$

The intercept A from the original equation (Nietlisbach et al., 2018) corresponds to mortality not related to inbreeding, and it was excluded from the formula since the baseline mortality was applied in the simulations through a separate function. Number of lethal equivalents (B) differs in wildlife populations, and we estimated it as 0.85 (see the Model evaluation section). Calculated expected survival probabilities were used as a success probability to create survival events (0 – dead, 1 – alive), sampled from the Bernoulli distribution. At the time of simulation start, individuals already had a high level of inbreeding, but it is hard to estimate IBD coefficient from the empirical data since a kinship needs to be known for almost the entire population. To calculate the baseline IBD, we have done the simulations from the very beginning - from the reintroduction of 6 animals from Slovakia, as was done in the original 1973 reintroduction. We simulated that some of the animals were related, trying to simulate the situation in the original reintroduction where the founders included a mother and son pair, and another two animals that were siblings (Koubek and Červeny, 1997). For this simulation we performed 500 iterations and 45 years. When we achieved similar inbreeding, allelic frequencies and heterozygosity level to the ones observed in the empirical data, we created a distribution of IBD coefficients, from which we sampled IBDs for further simulations. We used several population genetic metrics to study the dynamics of genetic composition in the simulated population. We estimated the metrics listed below for each "year". Effective population size was calculated using the linkage disequilibrium (LD) method as described in Waples (2006). We estimated the observed heterozygosity (Ho) and the expected heterozygosity (He) (Nei 1978) to explore genetic diversity of the population. We measured inbreeding using IBD (identity by descent) and the total inbreeding coefficient F_{IT} (Wright 1931). The script used for simulations is available on GitHub (https://github.com/PazhenkovaEA/DinLynx).

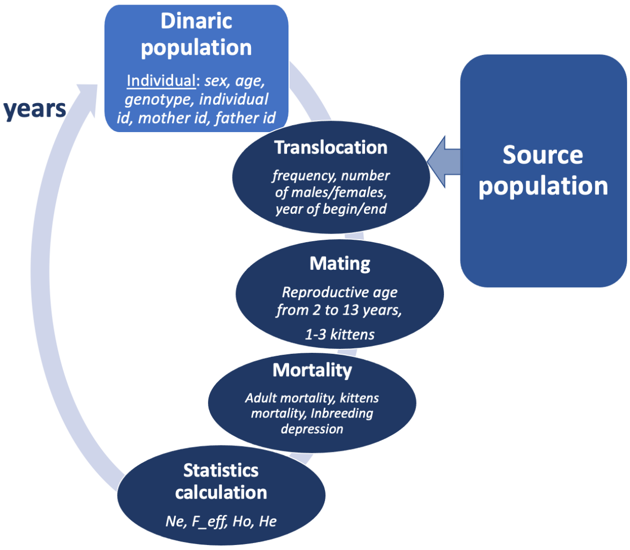

Figure 1. Schematic representation of the modelling cycle.

TRANSLOCATION SCENARIOS

Firstly, we have simulated the possible scenarios of Dinaric-SE Alpine lynx population development (1) if no translocations had been performed at all, (2) if no further actions would be done after the integration of the 14 animals planned in the LIFE Lynx in the population, and (3) if the same project as LIFE Lynx would be carried out again in 43 years (which was two years before the level of inbreeding exceeded the critical level of 0.25 - see below - in the scenario 2). Scenario 2 considers 10 males and 4 females integrated in the Dinaric-SE Alpine population between 2018 and 2021. Since scenarios 2 and 3 resulted in considerable improvement, but the population was still far from the optimal genetic condition, we tried to find the minimum number of additional translocated animals and the corresponding translocation intervals, able to keep the population viable (scenario 4). According to the recommendations for the conservation of the Eurasian lynx, accepted by the Bonn Lynx Expert Group, isolated populations should be managed to keep the F_{IT} below 0.15, and when the inbreeding coefficient increases above 0.25 immediate action is needed (Bonn Lynx Expert Group, 2021). If a simulated translocation scenario succeeded in decreasing the inbreeding coefficient below the 0.15 threshold in the entire period between translocation actions, the scenario was considered successful. We gradually increased the time intervals between translocations (translocations were performed each 3, 5, 10, 15, 20 and 25 years), and for each time interval we estimated the minimum effective number of translocated lynx that would provide a successful outcome. Finally, we estimated for how long the population can keep the level of inbreeding below the critical level if we stop the translocations after 27 years from the beginning, simulating a sudden failure of management. We did the simulations separately for Slovak and Romanian source populations, and modelled a balanced sex ratio of translocated animals.

RESULTS

MODEL EVALUATION

We simulated population development from reintroduction in 1973 until 2017 to evaluate model parameters and to calculate individual IBD coefficients for further simulations (Figure 2). We assumed that the founder animals included a mother and son pair, and that another two animals were siblings, as was reported by (Koubek and Červeny, 1997). We tested different numbers of lethal equivalents (B) in range from 0.1 to 6, and B=0.85 resulted in the effective population size, inbreeding coefficient and heterozygosity close to empiric estimates reported by Skrbinšek et al. (2019).

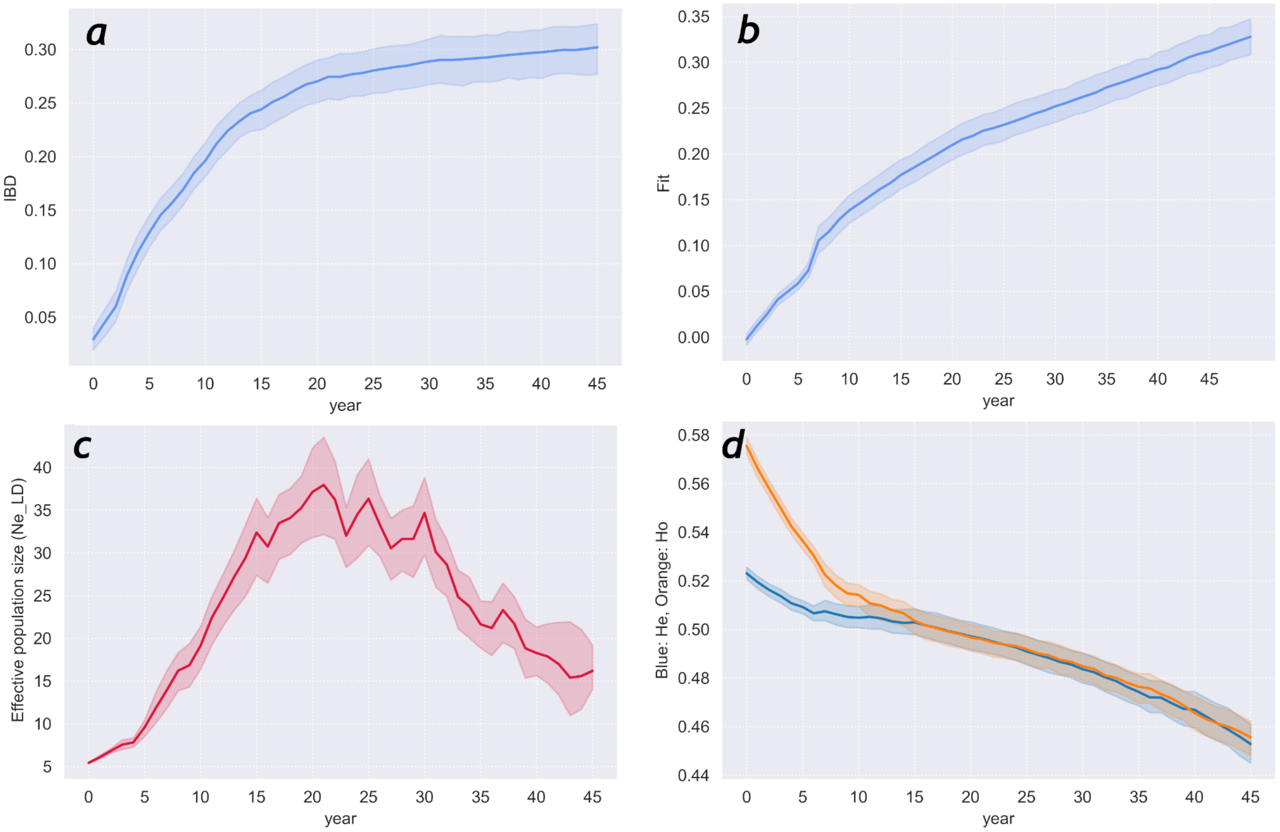

Figure 2. Dynamics of inbreeding level, measured as identity-by-descent (IBD) (a) and F_{IT} (b), effective population size (c) and heterozygosity (d; expected in blue, observed in orange) of the Dinaric lynx population assuming no translocations had been performed. Year 0 corresponds to the year 1973, when 6 lynx from Slovakia established the Dinaric lynx population.

EFFECT OF THE PERFORMED TRANSLOCATIONS ON THE POPULATION SURVIVAL

Scenario 1, no conservation action. We modelled the scenario of the future population development if no conservation actions had been performed. Assuming no translocations had been done within the project, the Dinaric population would be completely extinct after 28 years from the year when genetic monitoring was done (2017). Fatal loss of genetic diversity leading to the decrease of the effective population size and heterozygosity as well as to dramatically increasing the total inbreeding was observed (Figures 3,4).

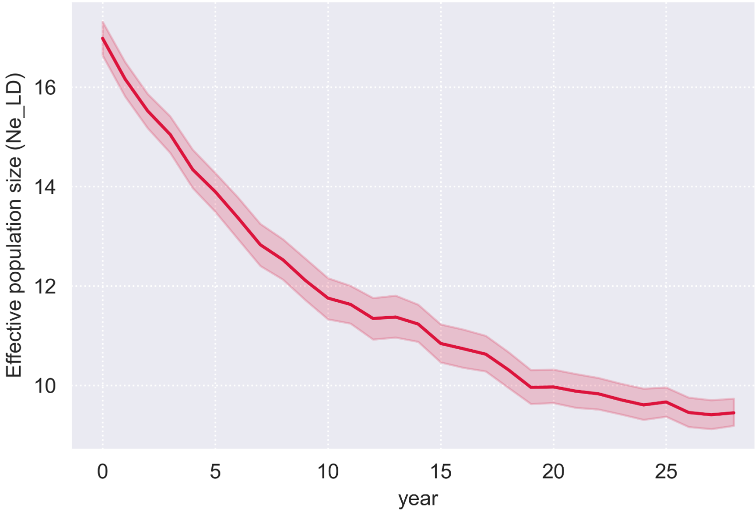

Figure 3. Dynamics of effective population size of the Dinaric lynx population assuming no translocations had been performed. Year 0 corresponds to the year 2017. The population size went to 0 on average in 29 year (not shown on the plot).

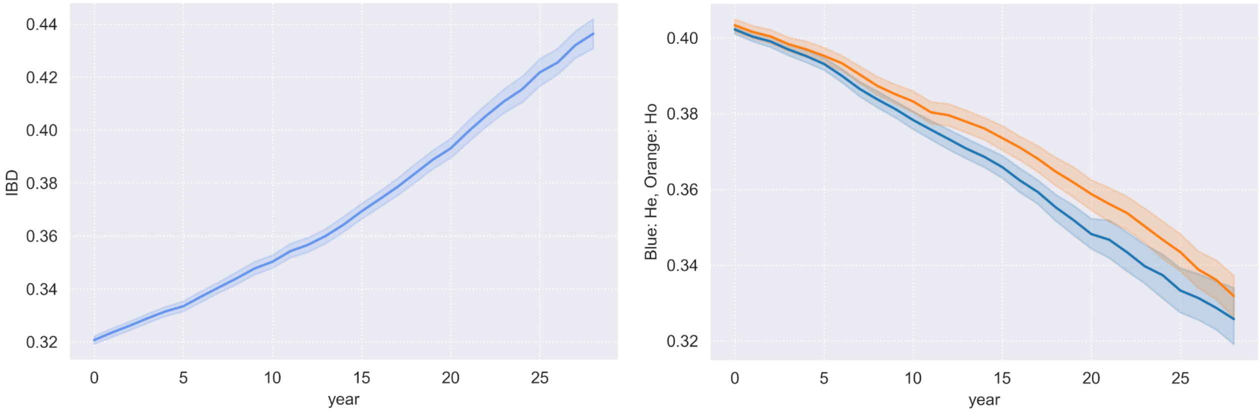

Figure 4. The inbreeding, measured as IBD (left) and heterozygosity (right) of the Dinaric lynx population assuming no translocations had been performed. Year 0 corresponds to the year 2018.

Scenario 2, translocations within the LIFE Lynx project were carried out, but no further actions for population conservation were done. The start of the graph shows a sharp short-term decrease of the effective population size (Figure 5), which is a property of the LD-method of Ne measurement. The method should have a significant downward bias of the effective population size when two gene pools are present in the same population (which happens when animals from another population are translocated). This increases linkage disequilibrium (Nei and Li, 1973), the main signal for estimating effective population size with the LD method, biasing the result. After the subpopulations mixed and formed the common gene pool, the effective population size steadily increased in the next 15 years (Figure 5). The effective population size is not a demographic metric and it does not always reflect the actual number of animals in the population, it defines the rate of genetic drift - how fast the population is going to lose genetic diversity and become inbred. Translocations significantly delayed the fatal decrease of population genetic diversity and inbreeding - for 28 years after translocations the inbreeding level stayed below the 0.15 threshold. However, after 45 years the inbreeding level exceeded 0.25 threshold, which is equal to the full-sib mating (Figure 6) and considered a critical threshold for immediate action (Bonn Lynx Expert Group, 2021). After 53 years from the start of the simulations, the effective population size dropped to the level endangering the population's survival, close to the situation we have had in 2017 before the LIFE LYNX project.

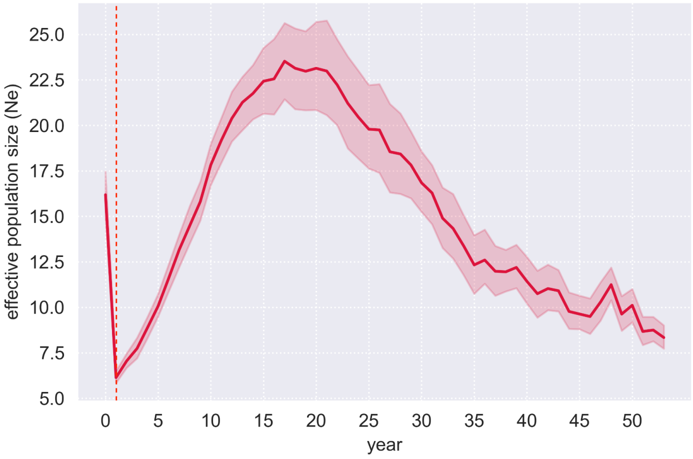

Figure 5. Dynamics of effective population size of the Dinaric lynx population assuming translocations done within the LIFE Lynx project were followed by no further translocations and the population remained isolated. Year 0 corresponds to the year 2018. Dashed red line shows the translocations start.

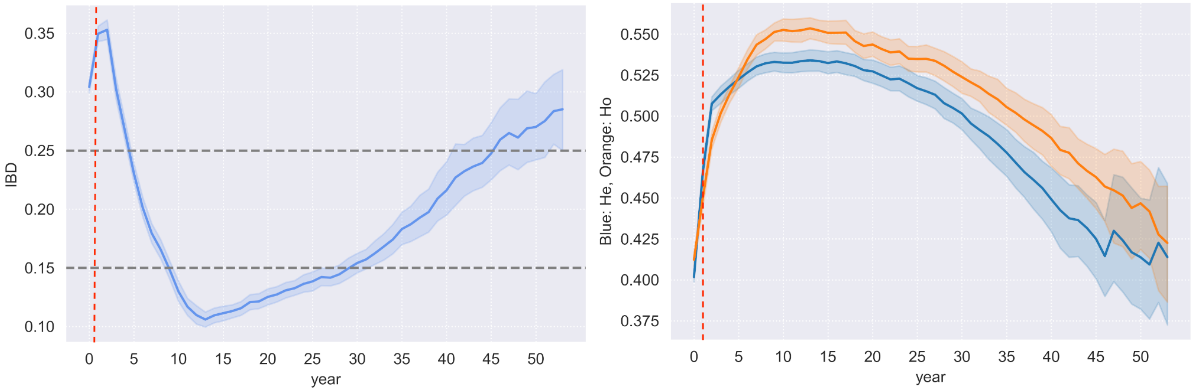

Figure 6 The inbreeding, measured as IBD (left) and heterozygosity (right) of the Dinaric lynx population assuming translocations done within the LIFE Lynx project were followed by no further conservation actions. Year 0 corresponds to the year 2018. Dashed red line shows the translocations start. Grey dashed lines indicate upper and lower inbreeding thresholds.

The Dinaric population, established by 6 animals, survived for almost 50 years, and would (according to scenario 1) probably have non-zero population size for another 25+ years before going completely extinct. It is somewhat counterintuitive that a translocation of 14 animals would delay the extinction just for another 25 years. First, the founder population seems to have a low number of lethal equivalents and got lucky to survive the first time around, and there is no reason to expect this will happen again. The number of lethal equivalents we estimated in this study by fitting the simulations of the development of the population after the 1973 reintroduction to the empirical data is considerably lower than what we expected from literature - 0.85, recent study report lethal equivalents in vertebrates between 0.4 and 7.5 with an average of 3.5 (Nietlisbach et al, 2019). Previously the average number of reported lethal equivalents was 6 (O'Grady et al., 2006). There is no guarantee that the deleterious alleles brought by the newly translocated animals won't create a more severe effect when the population starts to inbreed again, but we decided to use the 0.85 lethal equivalents estimate since this is the best we have for the particular species. In the simulations where we used the lethal equivalents reported in literature with no translocations the populations collapsed considerably faster - in 20 years and in 17 years with the number of lethal equivalents of 3.5 and 6, respectively. Second, when including new animals into the population that already has a high level of inbreeding, such an "augmented" population can behave differently than a newly established population. As a result of a long period of absence of gene flow and small effective population size, the inbreeding reached the critical level. Since the majority of animals are highly inbred, introduction of new alleles to the population can be difficult. But on the other hand, outbred individuals often demonstrate increased fitness (the phenomenon called "heterosis"), and we implemented it in the simulations only as a relatively lower mortality of outbred individuals compared to inbred individuals. In practice, this effect may be more significant and may be observed during the monitoring of the population.

Scenario 3, translocations within the LIFE Lynx project were carried out and repeated only before inbreeding reached the upper threshold. If a similar population augmentation as in the LIFE Lynx project was repeated every 43 years (2 years before inbreeding level reaches the critical level of 0.25, see Figure 6), the population would survive, but every subsequent translocation would be less efficient - after the next cycle of translocation the effective population size would not increase that much, making the population vulnerable to demographic stochasticity and inbreeding faster. This makes optimization of the translocation scenarios a must if we wish to maintain continuous viability of the population.

OPTIMIZATION OF THE MANAGEMENT SCENARIOS

According to our simulations, the translocations within the LIFE Lynx project are having a great impact on genetics of the Dinaric lynx population. Following successful implementation of the project, the simulations indicate that the total inbreeding would remain under the optimal threshold of 0.15 for 28 years and under the critical threshold of 0.25 for 45 years after the translocations even if no additional actions were taken (Figure 6). This means that the critical steps of saving the population from extinction caused by genetic erosion are already being done. However, to ensure long-term population viability it is critical for conservation measures to continue. We compared the effect of the integration of animals, originated from the Romanian and Slovak populations, when translocations were performed once per 3, 5, 10, 15 and 25 years (scenario 4). For each translocation frequency we calculated the minimum number of animals, integrated to the population, that would be enough to keep the population below the inbreeding threshold of 0.15 in the periods between translocations, and to maintain an acceptable level of genetic variability in the Dinaric lynx (Table 1). We further estimated the total number of lynx that would be translocated in 50 years to be able to compare scenarios with different time intervals. In order to test the robustness of the suggested strategies, we additionally estimated for how long the population can keep the inbreeding coefficient below 0.25 if the conservation program is discontinued ("years after stop"). We calculated the standard deviation of IBD in the time interval between translocations, and then calculated an average between these values to quantify how inbreeding fluctuates through time. Any crash of population to high inbreeding carries a risk of extinction, particularly if the number of lethal equivalents increases through translocations, which makes the scenarios where inbreeding never gets really high inherently more robust.

Table 1. Effect of the translocations on the Dinaric-SE Alpine lynx population. Minimum number of animals integrated to the population per action (N min), number of animals per 50 years, for how many years after reaching inbreeding level below 0.15 the population can keep inbreeding level below 0.25 without additional actions (Years after the last action), mean of standard deviations, calculated for IBD coefficients between each two translocations (Variation IBD).

	Slovak source population				*Romanian source population*			
Year interval	N min per action	N of animals per 50 years	Years after the last action	Variation IBD	N min per action	N of animals per 50 years	Years after the last action	Variation IBD
3	2	33	20	0.033	1	17	25	0.034
5	3	30	25	0.033	3	30	28	0.032
10	5	25	29	0.035	5	25	30	0.035
15	7	23	34	0.036	7	23	34	0.036
20	10	25	30	0.0375	9	23	31	0.039
25	12	24	27	0.038	12	24	28	0.04

In general, translocations from the Romanian population were more efficient since they had a longer post-translocation effect, which is expected considering that the Dinaric and Romanian populations are genetically less similar (SkrbinŠek et al., 2019). However, translocations from the Slovakian population also have a significant impact on genetics of Dinaric-SE Alpine lynx population. Interestingly, relatively frequent translocation of a few animals (once per every 3 years) seemed to have a less significant long-term effect and higher inbreeding level fluctuations than translocations of a larger number of animals, which are performed in longer time intervals and resulted in a lower total number of translocated animals over the long term. We should underline that Table 1 contains the minimal number of animals required to keep inbreeding below the critical level in the short term. It does not take into account possible complications during translocations, and we assumed in the simulations that all of the translocated animals participate in reproduction. The percentage of translocated lynx successfully included in the population is being estimated during the project, and the conclusions or decisions made on the basis of these simulations should be adjusted accordingly.

According to the modelling results, all the suggested strategies modelled under scenario 4 are able to maintain the population viable in the long term. We suggest the following criteria to improve the effectiveness of the reinforcement actions:

1. Genetics: minimise fluctuations in inbreeding coefficient, which makes the population robust to the sudden demographic collapse (variation of IBD).
2. Management: too long intervals between actions may increase the probability of changes in the management plans on the administrative level. Too short may cause additional difficulties in organisation of the actions, but may encourage development of routine genetic management strategies with translocations every couple of years.
3. Economy: lower total number of translocated animals reduces total costs of translocations, but establishment of routine strategies with shorter intervals between translocations and smaller-scale translocations may prove more economically feasible (lower costs per action) and, if optimized, may end up being more cost-effective.
4. Robustness: in case of management failure, it becomes important for how many years the population can survive after the translocations stop.

It seems that translocation of 7 animals every 15 years demonstrated the longest post-translocation effect in both cases of the Slovak and the Romanian source population. Also, this scenario has the lowest number of animals per 50 years for the Slovak source population and one of the lowest for the Romanian source population. Variance in inbreeding is about the average. On the other hand, this period may be too long for routine management. In any case, all scenarios shown here are viable from the biological perspective, and the final decision on which one to use will depend on practical management, implementation and funding circumstances.

CONCLUSION

The establishment of the Dinaric lynx population in 1973 is a case of an extremely successful reintroduction of a large carnivore. However, the small number of animals translocated and lack of any post-reintroduction gene flow (natural or otherwise) resulted in reduction of fitness through inbreeding depression. The translocations done within LIFE Lynx are having a great impact on genetic parameters, and seem to be increasing the probability of the population's survival for at least 55 more years. Still, even after this critical action is completed, the population might remain small and isolated, and might as such continue to inbreed. In this case, if we wish to ensure long-term viability of the population, we need further genetic management. Any management actions should be accompanied by continuous monitoring of genetic and demographic status, which helps to bring models closer to reality. In the frame of D.3 action we have developed and tested a flexible tool for prediction of population development mostly from the genetic perspective, but also taking into account demographic parameters. Simulations showed that translocations from a more genetically distant Romanian population have stronger and longer effects on genetic diversity of the recipient population, and require translocation of fewer animals to achieve the same effect. On the other hand, bringing animals from the Slovak population or from the both source populations also significantly improved genetic characteristics of the Dinaric population, and also seems a completely viable option. We provide several possible management strategies, which have different pros and cons from the ecological, genetic, managerial and economical points of view. In general, 10-20 year intervals between translocations of 5-10 animals per action demonstrate longer after-translocation effect, then shorter or longer intervals, and such a timing also allows to detect a response of the recipient population to the reintroductions via monitoring and to keep the genetic parameters at acceptable level. However, short (3-5 year) intervals allow to minimize fluctuations in the inbreeding coefficient.

One of the goals of the LIFE Lynx project is to improve the connectivity of the Dinaric lynx population with other populations via creating a population "stepping-stone", making natural gene flow possible. This is a key measure in population reinforcement, which will help to slow down genetic erosion. However, at the moment the conservation of the Dinaric-SE Alpine population requires active steps, such as translocations from other populations, due to its small size and isolation. In our simulations we assumed no natural gene flow between the Dinaric population and other populations as in this condition the population is the most threatened. If stable connections between populations do get established, the model needs to be revised.

REFERENCES

Bonn Lynx Expert Group 2021. Recommendations for the conservation of the Eurasian lynx in Western and Central Europe. Cat News 14, 78-86

Breitenmoser-Würsten C. & Breitenmoser U. 2001. The Balkan Lynx Population-History, Recent Knowledge on its Status and Conservation Needs. KORA Bericht Nr. 7e, 38 pp.

Cop J, Frković A. 1998. The re-introduction of the lynx in Slovenia and its present status in Slovenia and Croatia. Hystrix 10:65–76. Available from Cop & Frkovic 1998 Status of the lynx in Slovenia and Croatia.pdf.

Kaczensky P. 1991. Untersuchungen zur Raumnutzung weiblicher Luchse (Lynx lynx), sowie zur Abwanderung und Mortalität ihrer Jungen im Schweizer Jura 14–63. (In German). - Diploma thesis, Lehrstuhl für Wildbiologie und Jagdkunde, Forstwissenschaftlichen Fakultät an der Universität München, Germany

Kos I, Potočnik H, Skrbinšek T, Skrbinšek Majić A, Jonozovič M, Krofel M. 2004. Ris v Sloveniji: strokovna izhodišča za varstvo in upravljanje. Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia.

Koubek, P. and Cerveny, J. 1997. Lynx in the Czech and Slovak republics (Appendix). Acta sc. nat. Brno 30(3): 2-60.

Krofel M., Fležar U., Hočevar L., Sindičić M., Gomerčić T., Konec M., et al. 2021. Surveillance of the reinforcement process of the Dinaric-SE Alpine lynx population in the lynx-monitoring year 2019–2020. Ljubljana. 45 pp

Nei M., Wen-Hsiung L. 1973. Linkage disequilibrium in subdivided populations Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 75(1): 213–219.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Nietlisbach P., Muff S., Reid J. M., Whitlock M. C., Keller L.F. 2018. Nonequivalent lethal equivalents: Models and inbreeding metrics for unbiased estimation of inbreeding load. Evolutionary Applications 12(2), 266-279.

O'Grady J. J., Brook B.W., Reed D.H., Ballou J.D., Tonkyn D.W., Frankham R. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biological Conservation 133:42–51.

Peng B. & Kimmel M. 2005. simuPOP: a forward-time population genetics simulation environment. Bioinformatics 21 (18), 3686–3687

Sindičić M., Polanc P., Gomerčić T., Jelenčič M., Huber Đ., Trontelj P. & Skrbinšek T. 2013. Genetic data confirm critical status of the reintroduced Dinaric population of Eurasian lynx. Conservation Genetics 14, 1009–1018

Skrbinšek T. & Krofel M., 2008. DinaRis report. Analiza kvalitete habitata, hrana in kompeticija. Ljubljana. 56 p (in Slovene).

Skrbinšek T., Boljte B., Jelenčič M., Sindičić M., Paule L., Promberger B., et al. 2019. Baseline (pre-reinforcement) genetic status of SE Alpine and Dinaric Lynx population. Ljubljana. 24 pp.

Waples R. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conservation Genetics 7:167–184. Springer Science+Business Media B.V., Formerly Kluwer Academic Publishers B.V.