ECOLOGICAL FACTORS OF LYNX REPOPULATION IN CROATIA

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I Z J A V A

Ja, Ira Topličanec, potvrđujem da je moj doktorski rad izvorni rezultat mojega rada te da se u njegovoj izradi nisam koristio/-la drugim izvorima do onih navedenih u radu.

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(potpis)

Zagreb, 2023.

This doctoral dissertation was developed at the Department of Veterinary Biology, University of Zagreb, Faculty of Veterinary Medicine, Croatia, under the supervision of Professor Tomislav Gomerčić, PhD and Associate professor Magda Sindičić, PhD. Part of the research was funded by the European Commission under the LIFE Programme: project „Preventing the Extinction of the Dinaric-SE Alpine Lynx Population Through Reinforcement and Long-term Conservation“ (LIFE16 NAT/SI/000634).

INFORMATION ON SUPERVISORS

Associate professor Magda Sindičić has worked at the Departement for Hunting and Wild Animals since 2008. She received her doctorate on research into the genetic diversity of the Eurasian lynx, and her scientific activity led to the conclusion that the lynx population is threatened with extinction due to inbreeding. Today she is leading Croatian activities within the European project for the Dinaric lynx population reinforcement (LIFE16 NAT/SI/000634). Her role as a mentor was primarily focused on the development of a sustainable national population monitoring system and monitoring the health aspect of resident and translocated lynx individuals. She has publised 126 scientific papers and presented her work at 82 scientific conferences.

Professor Tomislav Gomerčić has worked at the Departement for Veterinary Biology since 2001. He recieved his master's degree at the Faculty of Science and Mathematics, University of Zagreb, in the field of biology and ecology. The topic of his master’s research was the craniometrics of the Eurasian lynx, while his scientific activity is focused on the ecology of large carnivores and marine mammals. His role as a mentor, was focused on the ecological aspect of lynx reintroduction research and the developement of ecological methods necessary for the preparation of this dissertation, as well as the field work. He has publised 94 scientific papers and presented his work at 132 scientific conferences.

Zahvaljujem mentorima Magdi Sindičić i Tomislavu Gomerčiću na razvoju ideje za izradu ove disertacije te kontinuiranoj podršci, dostupnosti i vodstvu. Izuzetno je motivirajuće profesionalno se razvijati u prijateljskoj atmosferi uz visoke standarde stručnosti.

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ABSTRACT

The Eurasian lynx (*Lynx lynx*) is a strictly protected species, whose small and isolated population in the Dinaric Mountains is threatened with extinction due to inbreeding. The only possibility for its survival was the inclusion of new individuals from another population. For this reason, a lynx reinforcement project was carried out in Croatia and Slovenia as part of the project LIFE Lynx (LIFE16 NAT/ SI/000634). The aim of this dissertation was to investigate the status of Croatian lynx population prior to the reinforcement and to analyse the early post-release behaviour of the translocated animals using telemetry data. In the 2018 – 2020 period a minimum of 89 adult lynxes were identified using camera traps, representing the first scientifically – based estimate of the lynx population size and distribution in Croatia. Photos of lynx pelts originating from the period 1978–2019, revealed a phenotypic change in the population. Specifically, there was a significant difference ($p < 0.05$) in the occurrence of the four coat patterns between individuals photographed in the period 1978 - 1999 and those photographed in the period 2001 – 2019, with the frequency of big spots pattern increasing over time. After the translocations, an effective method for monitoring the movements of released lynxes was implemented. The animals involved in the study (N=6) settled on average 23 days (SD = 16.5) post-release, with the main direction of movement of the released animals being towards NW-SE, which corresponds to the orientation of the predominant ridgelines of the Dinaric Mountais. The first kill sites of all animals were detected on average 3.4 days (SD = 1.7) after release. Moreover, when comparing the use and availability of the terrain, we concluded that the lynx preferred to move along the mountain range rather than perpendicular to it. The knowledge gained in this study provided valuable insight into the reinforcement process of the Dinaric lynx population and demonstrated that the release of new animals amidst resident territorial individuals had a positive outcome and contributed to a more effective conservation strategy for this and future reinforcement programmes.

Key words: Eurasian lynx, reinforcement, abundance, distribution, movement

PROŠIRENI SAŽETAK

UVOD: Euroazijski ris (*Lynx lynx*, Linnaeus 1758.) po sistematici spada u razred sisavaca (Mammalia), red zvijeri (Carnivora), porodicu mačaka (Felidae) te potporodicu pravih mačaka (Felinae). Autohtone populacije euroazijskog risa u Europi nastanjuju područje Fenoskandinavije, Baltika, Karpata i jugoistok Balkanskog poluotoka, te postoji nekoliko izoliranih, reintroduciranih populacija u zapadnoj i jugozapadnoj Europi kojima pripada i dinarska populacija. Populacija risa u Dinaridima nastala je reintrodukcijom šest jedinki koje su 1973. godine naseljene iz slovačkih Karpata u Sloveniju, odakle se populacija proširila u Hrvatsku te Bosnu i Hercegovinu. Početkom 2000.-ih uočen je pad brojnosti populacije, dok je 2013. godine dokazano da je populacija risa u Dinaridima na rubu opstanka, kao posljedica parenja u srodstvu, budući da su se potomci šest reintroduciranih životinja parili isključivo međusobno. Jedina šansa za opstanak risa u Dinaridima je bilo uključenje novih jedinki u populaciju. U sklopu projekta „Spašavanje dinarske i jugoistočne alpske populacije risa od izumiranja" (LIFE16 NAT/SI/00634), skraćeno LIFE Lynx, se provela repopulacija risa u Hrvatsku i Sloveniju te je u razdoblju 2019. – 2023. ukupno 18 životinja uhvaćenih u Slovačkoj i Rumunjskoj preseljeno u Hrvatsku i Sloveniju. Za uspješnu repopulaciju važno je osigurati da se odluke o provedbi zahvata repopulacije temelje na razumijevanju ekoloških čimbenika bitnih za risa te je repopulacija u sklopu projekta LIFE Lynx na području Dinarida fokus istraživanja ovog doktorskog rada. U Hrvatskoj su brojnost i rasprostranjenost populacije risa prije ove disertacije procjenjivani na temelju mišljenja stručnjaka, bez upotrebe znanstvene metode na cjelokupnom području rasprostranjenosti. Posljednja dva desetljeća, razvoj automatskih fotoaparata, tzv. fotozamki, omogućava neinvazivan pristup istraživanju divljih životinja, te je financijski isplativija od molekularnih i telemetrijskih metoda. Fotozamke su posebno korisne u praćenju životinjskih vrsta s jedinstvenim uzorkom krzna, koje žive povučeno od ljudi i koje nastanjuju teško pristupačna područja, gdje klimatski i reljefni uvjeti otežavaju direktno istraživanje. Naime, risovi pripadaju porodici mačaka te većina populacija ima točkasti uzorak krzna, jedinstven za svaku jedinku. Nadalje, u europskim populacijama prepoznato je i nekoliko različitih tipova krzna kao što su krzna s velikim točkama, malim točkama, rozetama, kombinacijom malih točaka i rozeta te krzna bez točaka. Razlikovanje jedinki na temelju tipa krzna i njegovog jedinstvenog uzorka je preduvjet za procjenu veličine populacije primjenom metode ponovnog bilježenja iste jedinke fotozamkama. U odnosu na fotozamke, telemetrijska istraživanja su skupa, te zahtijevaju puno napora, vremena i iskustva od strane istraživača. Unatoč tome, prilikom reintrodukcija i repopulacija, telemetrijsko praćenje ispuštenih jedinki je neizostavna metoda kako bi se pratilo njihovo preživljavanje te kretanje od ispuštanja do uspostave teritorija. Istraživanje uzorka kretanja te prijeđene udaljenosti ispuštenih životinja, važno je za razumijevanje ponašanja jedinki prilikom ispuštanja na novom području. Kako se ne bi ponovile greške iz prošlosti, prateći IUCN smjernice za reintrodukcije i repopulacije te koristeći znanstveno utemeljenu metodu u skladu s ekološkim potrebama vrste, cilj ove doktorske disertacije je bio uspostaviti i optimizirati metode za prikupljanje i analizu podataka o ključnim pokazateljima stanja domaće populacije risa, kao što su brojnost i rasprostranjenost, te utvrditi prijeđene udaljenosti i trajanje kretanja naseljenih jedinki risova od ispuštanja do uspostave teritorija.

HIPOTEZA: Hipoteza disertacije jest da brojnost autohtone populacije i rasprostranjenost teritorijalnih jedinki utječu na uspješnost repopulacije. Prisutnost teritorijalnih jedinki istog spola će se odraziti produženim vremenom migracije naseljenih jedinki.

MATERIJALI I METODE: Za optimizaciju metode razlikovanja jedinki na temelju tipa krzna i njegovog jedinstvenog uzorka, analizirano je 195 fotografija krzna risa iz Hrvatske, od čega 92 fotografije trofejnih krzna iz razdoblja 1978. – 1999. godine, 20 fotografija krzna živih risova uhvaćenih za telemetrijska istraživanja u razdoblju 2001. – 2019. godine, fotografije krzna jednog mrtvog risa pronađenog 2019. godine te fotografije 82 risa dobivene fotozamkama u razdoblju 2011.-2019. godine. Krzna risova s fotografija su prvo vizualno podijeljena u četiri tipa: velike točke, male točke, rozete i bez točaka. Zatim su se korištenjem programa za obradu fotografija ImageJ kvantificirane osobitosti svakog tipa krzna - izmjerena je površina krzna, broj točaka i njihova veličina, udaljenost između pojedinih točaka te količina crne boje. Na kraju je pomoću statističkog testa chi2 uspoređena zastupljenost pojedinog tipa krzna iz razdoblja 1978 – 1999 te 2001 – 2019. Razlike su smatrane značajnima ako je p vrijednost bila manja od 0.05.

U svrhu određivanja brojnosti i rasprostranjenosti risa, u razdoblju od 1. svibnja 2018. do 30. travnja 2020. godine, provedeno je istraživanje pomoću fotozamki. Istraživanje je provedeno na površini od 10 000 km^2 podjeljenoj u mrežu kvadranata veličine 10 x 10 km te je obuhvatilo područja Gorskog kotara, Like i sjeverne Dalmacije. Fotozamke su postavljene na 182 odgovarajuće lokacije kao što su risja markirališta, šumske ceste i životinjski putevi, uz preduvjet da je u svakom kvadrantu postavljena minimalno jedna fotozamka. Svi podatci s fotozamki su pregledani i pohranjeni u programu Camelot. Istovremeno su prikupljani i svi dostupni podatci o pojavnosti risa kao što su viđenja risa, izmet, urin, dlake, plijen risa, tragovi u snijegu te fotografije risa prikupljene fotozamkama u vlasništvu Javnih ustanova za upravljanje zaštićenih područja i u vlasništvu lovaca, fotografije prikupljene tijekom provođenja studija utjecaja na okoliš ili prikupljene od strane pojedinaca. Svi prikupljeni podatci su pohranjeni u bazu podataka o risu Veterinarskog fakulteta Sveučilišta u Zagrebu, javno dostupnu na dresi http://lynx.vef.hr. Za procjenu brojnosti, sve prikupljene fotografije risova su pregledane i uspoređene s već poznatim jedinkama iz prethodnih istraživanja na temelju jedinstvenog uzorka krzna. U slučaju da jedinka nije bila prethodno poznata, dodijeljena joj je nova identifikacijska oznaka. Budući da svaka jedinka ima različiti uzorak krzna na lijevoj i desnoj strani tijela, identifikacija je bila moguća na temelju samo jedne strane tijela, lijeve ili desne, te obje strane. Time se dobiva raspon veličine populacije unutar kojeg zbroj jedinki identificiranih na temelju obje strane tijela i onih identificiranih na temelju samo jedne strane tijela određuje sigurnu minimalnu veličinu populacije. Za izradu karte rasprostranjenosti svi podaci prikupljeni u navedenom razdoblju su kartirani korištenjem programa QGIS. Rasprostranjenost risa je određena na mreži kvadranata 10 x 10 km, pri čemu je na temelju međunarodno ugovorenih SCALP kriterija rasprostranjenost opisana kao dokazana (na temelju čvrstih dokaza) te moguća prisutnost (bez čvrstih dokaza). Ukupna površina dokazane i moguće rasprostranjenosti risa u Hrvatskoj izračunata je zbrojem površina kvadrata sa zabilježenim opažanjima.

Za utvrđivanje preživljavanja i analizu kretanja naseljenih jedinki risova, korišteni su podatci s telemetrijskih ogrlica šest jedinki ispuštenih u Hrvatskoj (2) i Sloveniji (4) u razdoblju od 2019. do 2020. godine. Korištene su GSM i satelitske ogrlice koje su prikupljale podatke o lokacijama risova u intervalu od 4h do 24h. Kretanja su analiziranja korištenjem programa QGIS. Jednosmjerno kretanje i povećanje udaljenosti od mjesta ispuštanja je definirano kao nomadsko kretanje, dok je poligonalno kretanje uz smanivanje i stabilizaciju udaljenosti definirano kao uspostava teritorija. U obzir se uzelo vrijeme od ispuštanja do prvog pronalaska teritorija, prijeđena udaljenost od lokacije ispuštanja do lokacije uspostave teritorija, zračna udaljenost od lokacije ispuštanja do lokacije uspostave teritorija, ukupna prijeđena udaljenost, prosječna dnevna udaljenost (km/dan), maksimalna dnevna udaljenost (km), minimalna i maksimalna nadmorska visina lokacija s telemetrijskih ogrlica, smjer kretanja, nadmorska visina terena, nagib i šumski pokrov, broj dana do detekcije prvog plijena te prosječni broj jedinki plijena prilikom nomadskog kretanja i prilikom kretanja unutar uspostavljenog teritorija.

REZULTATI I DISKUSIJA: U Dinarskoj populaciji risa identificirana su četiri tipa krzna: velike točke, male točke, rozete i krzno bez točaka. Rezultati analize fenotipa jedinki fotografiranih u razdoblju 1978. – 1999. i onih fotografiranih 2000. – 2019. godine su pokazali značajne razlike u zastupljenosti pojedinih tipova krzna između navedenih razdoblja. Zastupljenost uzorka krzna bez točaka je pala s 14% na 0%, rozete s 23% na 9% te male točke s 16,5% na 11,5%, dok je učestalost velikih točaka porasla s 46% na 80%. Promjena u učestalosti uzoraka krzna može se pripisati maloj veličini populacije s ograničenim protokom gena. Populacija dinarskog risa uspostavljena je reintrodukcijom svega šest jedinki u Sloveniju čiji su se potomci uspješno proširili u Hrvatsku, Italiju, Austriju kao i Bosnu i Hercegovinu, ali je populacija ostala izolirana. Četrdeset godina kasnije, genetskom analizom u populaciji dinarskog risa dokazano je značajno parenje u srodstvu i mala efektivna veličina populacije. Stoga rezultati upućuju da bi se fenotipski profil populacije mogao koristiti kao pokazatelj mogućih posljedica parenja u srodstvu. Prvi znanstveno utemeljeni podatci o veličini i rasprostranjenosti dinarske populacije risa u Hrvatskoj su dobiveni istraživanjem pomoću fotozamki koje se odvilo u razdoblju 2018. – 2020. godine. Na temelju podataka s fotozamki, te drugih podataka o pojavnosti risa prikupljenih u istom razdoblju i pohranjenih u risjoj bazi Veterinarskog fakulteta, rasprostranjenost vrste je potvrđena u Primorsko – goranskoj i Ličko – senjskoj županiji, u južnom dijelu Karlovačke te u sjeveroistočnom području Zadarske županije. Površina područja dokazane rasprostranjenosti risa je 7200 km^2, dok područje potencijalne, nepotvrđene rasprostranjenosti iznosi 1200 km^2. Procijenjeno je da je u Hrvatskoj u istraživanom razdoblju bilo stalno prisutno najmanje 89 odraslih risova te su u 2 sezone fotografirana ukupno 44 mladunca u 23 legla. Sveukupna brojnost je vjerojatnije bliža starijoj procjeni od 130 risova iz 2005. godine nego kasnijoj procjeni od 40 - 60 jedinki iz 2010. godine. Ovako velika varijacija u procjenama ilustrira važnost pravilno osmišljenog i provedenog sustava praćenja populacije risova. Osim istraživanja provedenog na domaćoj populaciji, u sklopu ove disertacije analizirano je i kretanje šest karpatskih jedinki risova ispuštenih u Hrvatskoj i Sloveniji u sklopu zahvata repopulacije. Jedinke su ispuštene na području rasprostranjenosti domaće populacije risova. Pet od šest risova pokazalo je nomadsko ponašanje nakon puštanja. Svih šest risova uspostavilo je teritorij u prosjeku 23 dana (SD=16.5) nakon puštanja i smjestilo se u prosjeku 15,8 km (SD=5,8) od mjesta puštanja. Prve lokacije plijena svih jedinki otkrivene su u prosjeku 3,4 dana nakon puštanja dok je prosječno vrijeme između hvatanja plijenova nakon uspostave teritorija iznosilo 7,76 dana (SD=1,91), što odgovara učestalosti hvatanja plijena domaćih dinarskih jedinki. Rezultati analiza su također pokazali sklonost za kretanjem u smjeru SZ-JI, što odgovara orijentaciji dinarskog planinskog lanca, te da su sve jedinke odabrale kretanje duž planinskog lanca, a ne okomito na planinu, tj. izbjegavali su kretanje uzbrdo i nizbrdo. Analiza kretanja triju risova je potvrdila izrazito lošu propusnost autoceste A1 Ljubljana – Koper koja je identificirana kao značajna barijera za kretanje divljih životinja te onemogućava prirodno širenje populacije risova iz Dinarida u područje Alpa gdje nema zabilježenih teritorijalnih jedinki. Samo je jedan ris (Maks) uspio prijeći autocestu, 4 mjeseca nakon prvog pokušaja. Maks je pod pritiskom domaćih teritorijalnih jedinki koje se nalaze na području južno od autoceste morao produžiti nomadsko kretanje prema sjeveru u potrazi za novim slobodnim teritorijem. Maksova ogrlica je 27. rujna 2021. godine prestala slati podatke te Maks nije naknadno zabilježen na niti jednoj fotozamki što upućuje da je produženo vrijeme nomadskog kretanja negativno utjecalo na njegovu integraciju u populaciju. Najudaljenija točka od mjesta ispuštanja koju su dosegnule neke jedinke je iznosila 50 km, dok su švicarski rezultati pokazali da jedna jedinka može prijeći i do 60 km od mjesta ispuštanja. Stoga se preporučuje da prilikom budućih planiranja ispuštanja jedinki, lokacije ispuštanja budu udaljena barem 60 km od granica područja unutar kojeg je ciljano zadržati jedinke.

ZAKLJUČAK: U sklopu ove doktorske disertacije uspješno je razvijena nova metoda za digitalnu analizu tipova krzna čime je unaprijeđen sustav identifikacije jedinki i pripadajućeg uzorka krzna. Također su dobiveni podatci o mogućem utjecaju parenja u srodstvu na fenotip, odnosno učestalost tipova krzna. Prvi puta u povijesti istraživanja risa u Hrvatskoj je uspostavljen znanstveno utemeljeni sustav praćenja populacije risa pomoću fotozamki koji je rezultirao prvom znanstvenom procjenom minimalne brojnosti risa te uvidom u rasprostranjenost populacije. U sklopu ove studije ispuštene jedinke risova s Karpata uspješno su uspostavile teritorije na području gdje je zabilježena prisutnost teritorijalnih jedinki risova. Međutim, na primjeru risa Maksa je također dokazana hipoteza da prisutnost teritorijalnih jedinki može utjecati na produljeno nomadsko kretanje jedinki i njihovo preživljavanje. Telemetrijskim praćenjem ispuštenih jedinki, utvrdile su se maksimalne udaljenosti koje su jedinke podvrgnute takvim zahvatima sposobne proći, a koje je potrebno uračunati u buduća planiranja sličnih zahvata.

Ključne riječi: Euroazijski ris, repopulacija, brojnost, rasprostranjenost, kretanje

ABBREVIATIONS

ID	identification
GPS	Global Positioning System
GSM	Global System for Mobile communication
VHF	Very high frequency
SLD	straight-line distance from the release site in kilometres
TD	total distance from the release site to initial settlement in kilometres
DD	daily distance in kilometers
SE	south-east
NW	north-west

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1. INTRODUCTION

1.1.The Eurasian lynx (*Lynx lynx*)

1.1.1. Systematics and morphology

Large carnivores are key stone species that reflect the well-being of the entire biodiversity pyramid. They attract the attention of the scientific community and the general public, but at the same time are a very difficult subject of research due to their elusive behaviour, large home ranges, and large body size. Among the large predators in the Dinaric Mountains, the Eurasian lynx (*Lynx lynx*) is the least studied and most endangered animal. The Eurasian lynx taxonomically belongs to the class of mammals (*Mammalia*), the order of beasts (*Carnivora*), the family of cats (*Felidae*), the subfamily of real cats (*Felinae*), and the genus of lynx (*Lynx*). Genus *Lynx* is a monophyletic group comprising four species distributed along the northern hemisphere: the bobcat (*Lynx rufus*), the Canadian lynx (*Lynx canadensis*), the Iberian lynx (*Lynx pardinus*), and the Eurasian lynx (*Lynx lynx*) (KITCHENER et al. 2017). Genetic research confirmed monophyletic ancestry of the four species of genus *Lynx*, as well as their taxonomic position as separate species (JOHNSON et al. 2006). Eurasian and Iberian lynx are the only ones present in Europe (KITCHENER et al. 2017). The Eurasian lynx (hereafter reffered as lynx) is the largest felid of the European continent, twice the weight of the Iberian lynx. Body mass of adults ranges 12-35 kg, with males being bigger than females (BREITENMOSER et al. 2006). Total body length is 70-130 cm, with shoulder height about 65 cm and its characteristic short black tail, round head with ear tufts and flared facial hair, long legs, and large feet. Lynx color coat consists of a combination of a general coloration and spotting, with some exceptions. The coat is greyish with different shades as rusty, yellowish, or reddish at the back and flanks, with a whitish belly (BREITENMOSER et al. 2006). Front feet have five fingers, of which the fifth one does not touch the ground, while the back feet have only four fingers. The claws are retractile, sharp, and strong, especially the claws of the forepaws, which are used for holding the prey. An adult lynx pawprint is round, with diameter between 7-9 cm, forepaws are larger than back paws and their tracks generally follow a straight line (BREITENMOSER et al. 2006).

1.1.2. Ecology and biology

Lynx is a solitary predator with a social organisation based on territoriality. Both males and females occupy individual home ranges, which they mark with gland secretions, urine, and faeces, but they exhibit territorial behavior only towards conspecifics of the same sex. Male home ranges are larger than those of females, therefore there is usually large overlap between male and female territories. Females choose smaller home ranges for nursing kittens according to resources, such as prey and habitat, while the males choose the territory to grant access to females. An adult male monopolizes one or two, and rarely more, females. Thanks to several studies based on telemetry, size of the home ranges of lynx has been estimated in Europe: 180-2780 km^2 for males and 98-759 km^2 for females (BREITENMOSER et al. 2006, HOČEVAR et al. 2020). Distance traveled by lynx within its home range per night depends on several factors, e.g. age, sex, presence of kittens, social status and prey density, but in general it ranges from 1-45 km, while in presence of a fresh kill a lynx can stay in its proximity for several days (BREITENMOSER et al. 2000). Female lynxes are sexually mature at the age of 2 years, whereas males are mature at 3 years old (BREITENMOSER et al. 2006). Data from different regions report that the lynx mating season lasts from February to mid-April, and in late May – first half of June, females usually give birth to 1-4 kittens (LÓPEZ-BAO et al. 2019). The timing of the first dispersal of young lynx ranges from 8.1 to 10.7 months and does not differ between males and females (ZIMMERMANN et al. 2005). After separating from their mother, young lynx stay for a few days in the maternal home range before they disperse (ZIMMERMANN et al. 2005). In Sweden, telemetry studies showed that about one-third of lynx female offspring remained philopatric (SAMELIUS et al. 2011), indicating the potential for geographic clustering of female relatives. Lynx diet varies considerably among different regions (OKARMA 1984, GOSSOW and HONSIG-ERLENBURG 1985, PULLIAINEN et al. 1995, PEDERSEN et al. 1999, BREITENMOSER and BREITENMOSER-WÜRSTEN 2008), while the main prey of Dinaric lynx is roe deer (*Capreolus capreolus*) (KROFEL et al. 2011). Generally, lynx's consumption rate averages 1-2.5 kg of meat per day, but it could be much more after some days of fasting (BREITENMOSER et al. 2006). Eurasian lynx uses two techniques of hunting: ambush and stalking (HOČEVAR et al., 2020). It applies the killing bite to the throat, crushing the windpipe, or with a bite on the back of the neck, severing the spinal cord while holding its prey with its strong forepaws and then starts to eat at a hindquarter (BREITENMOSER et al. 2006, KROFEL et al. 2009). Since felids are mostly solitary hunters, each bite must be made with precision to kill the prey as soon as possible to avoid being hurt during the struggle (KROFEL et al. 2009). Lynx has no natural enemies, apart from some sporadic cases of adult/juvenile individuals killed by wolves, but it also can happen that a large prey animal can fatally injure a lynx during the fight (BREITENMOSER et al. 2000). Mortality among juvenile lynx is high and about 50% of young individuals do not reach the adult age

(BREITENMOSER et al. 2000). However, the main causes of death are influenced by humans, such as hunting, poaching and traffic accidents (BREITENMOSER et al. 2000, SINDIČIĆ et al. 2016). In nature, lynxes can live up to 17 years, while in captivity up to 25 years (BREITENMOSER et al. 2000).

1.1.3. Geographical distribution and habitat

Eurasian lynx's geographical range includes area from central Eastern Europe to Eastern Asia, comprising a quiet large variety of environments, with different ecological and climatic conditions (SCHMIDT et al. 2011). Three "lynx regions" are distinguished in continental Europe (Bonn Lynx Expert Group, 2021): (1) the Balkan lynx region in the south-east, including the southern part of the Dinaric Range, (2) the Carpathian lynx region between the central Dinaric Range and the southern rim of the Carpathians north to the Harz Mountains, and (3) the Baltic lowland lynx region in the northern part of the continent.

The Eurasian lynx is grouped into 11 populations, based on a range of criteria, such as distribution and other geographical, ecological, political, and social factors (BOITANI et al. 2015). Out of those, five populations are autochthonous (Scandinavian, Karelian, Baltic, Carpathian, and Balkan populations), whereas the other populations, based in central and western Europe, origin from reintroductions in the 1970s and 1980s (Dinaric, Alpine, Jura, Vosges-Palatinian, and Bohemian-Bavarian populations) (BOITANI et al. 2015). In Europe, the lynx inhabits all types of forest such as large deciduous, mixed, and coniferous forests, but it can also survive in more open landscapes (BREITENMOSER et al. 2000; HOČEVAR et al. 2020). Lynx habitat selection is mainly influenced by human presence and prey availability, in fact, FILLA et al. (2017) have shown that Eurasian lynx use more open habitats and areas with high human disturbance during the night time, when they are more active (BREITENMOSER et al. 2000), whereas they prefer dens habitat in undisturbed areas (e.g. forest) for resting during the day, because they have to balance prey hunting with the risk of encounters with humans.

1.2. Phenotipic characteristics and individual identification

In its wide range in Eurasia, a high intra-specific polymorphism has been observed in the coat pattern of lynx (KUBALA et al., 2020; DARUL et al., 2021). This phenotypic polymorphism has been linked to habitat and climatic conditions (ORTOLANI and CARO 1996; ORTOLANI 1999; ALLEN et al., 2011; DARUL et al., 2021), past or current (human-induced) demographic changes that may have disrupted patterns originally established by natural selection (LUCENA-PEREZ et al., 2020), habitat fragmentation, and human-induced mortality (KUBALA et al., 2020). In addition, genetic drift has been found to be responsible for reducing variability in coat type (THULER, 2002; KUBALA et al., 2020). For lynx belonging to the spotted cats (WERDELIN & OLSSON 1997), five types of coat patterns were defined by both THÜLER (2002) and DARUL et al. (2021): large spots, small spots, without spots, rosettes and small spots with rudimentary rosettes, while GREGOROVÁ (1997) and KUBALA (2019) described only three: spotted, rossetted, and uniform. Each individual has a unique coat pattern that can be used to identify individual lynx, which is the basis for estimating population size using camera trapping and capture-recapture methods (WILLIAMS et al. 2002; KARANTH et al. 2006; HIBY et al. 2009; ROVERO and ZIMMERMANN 2016; SARMENTO and CARRAPATO 2019; GIMENEZ et al. 2019). However, it is important to accurately and consistently identify individuals in photographs to avoid estimation errors. Misclassification in photographs can easily occur due to factors such as poor photo quality or limited capacity of trained researchers. Also, some data may be excluded from classification even though they contain sufficient information for reliable identification (JOHANSSON et al. 2020). Therefore, the quantification of phenotypic traits could provide an objective description of individuals and their morphology and reduce the bias caused by human subjectivity, skill, and experience. To date, few methods have been developed for specific taxa (SHERLEY et al. 2010; LE POUL et al. 2014; ALLEN and HIGHAM 2015; LEE et al. 2018), whereas the development of accurate and reliable methods for quantifying coat patterns may be useful for numerous biological studies.

1.3. Population monitoring

Population monitoring is defined as repeated observations of a population of which the results are continuously compared with a specific and desired goal (HELLAWELL 1991). Monitoring the conservation status of a species includes obtaining information on distribution, population size, population dynamics (demography), health, genetic status, threats, and conflicts, such as monitoring of conflicts with human activities (hunting, livestock breeding) (Bonn Lynx Expert Group, 2021). There are many different methods used for lynx monitoring in Europe such as the collection of opportunistic records, questionnaires, camera trapping, mortality records, genetic campling and telemetry, each with its advantages and drawbacks (Hočevar et al. 2020). Some of the methods are an obvious choice for specific areas with specific conditions, but generally, there is no single one-fits-all method that is recommended for all populations and all purposes. In most cases, best results are acquired by combining several methods that would lead to predefined monitoring objectives. Therefore, it is essential that priorities are set and clear monitoring objectives defined before any monitoring scheme is initiated.

Historically, lynx monitoring in Croatia and the Dinaric region relied heavily on mortality records (FRKOVIĆ 2001), which included information about lynx individuals killed, typically through hunting activities, as well as other causes (SKRBINŠEK et al 2012, SINDIČIĆ et al. 2016). Although mortality records provide hard evidence for lynx presence, population monitoring relying solely on mortality records has its limitations. It provides restricted information that is hard to interpret on a population level and sample sizes can be too low to be relevant in small populations since it only accounts for individuals that were killed, omitting crucial data about surviving lynx (SKRBINŠEK et al. 2012). Conversley, camera trapping is one of the most important and commonly used method for lynx monitoring in the last two decades. When designed properly, it is found to be particularly useful for monitoring elusive and individually recognizable wild animals, such as lynx (Hočevar et al. 2020). In Croatia the transition from monitoring only mortality records and hunting bags to comprehensive research and monitoring with camera traps started in the 2011, representing an important improvement in lynx research (KUSAK 2012; KUSAK and MODRIĆ 2012; KUSAK et al. 2013, KUSAK et al. 2014). Nowadays, various studies in Croatia are using camera traps as a research tool. Recent publications present studies on daily migrations of chamois (*Rupicapra rupicapra*) in the Northern Velebit area (ŠPREM et al., 2015), the frequency of wildlife crossings usage, known as "green bridges", by wolves (*Canis lupus*) (ŠVER et al., 2016), for studying the interaction between large carnivores and large herbivores in the Northern Velebit, Gorski Kotar, and Plitvice Lakes National Park areas (ŠPLAJT, 2018) and the temporal overlap between humans and apex predator species (BLAŠKOVIĆ et al. 2022).

1.4.Population reinforcement

Translocations of individuals for reintroduction and population reinforcement have been increasingly used in carnivore conservation and plays a crucial role in lynx recovery in Europe (BREITENMOSER et al. 1998, ČOP and FRKOVIĆ 1998, VANDEL et al. 2006, RUEDA et al. 2021). Reintroduction is the intentional movement and release of an organism within its original range from which it has disappeared, with the goal of restoring a viable population of the target species. Reinforcement is the intentional movement and release of an organism into an existing population of conspecifics. Reinforcement aims to improve the viability of the population, for example, by increasing population size, increasing genetic diversity, or increasing representation of certain demographic groups or stages.

Loss of genetic diversity is considered the greatest threat to the lynx in Croatia, as the entire Dinaric population is descended from six animals that were released into Slovenia from the Slovak Carpathians in 1973, 70 years after the autochthonous population became extinct (SINDIČIĆ et al. 2013). Offspring of these animals successfully spread to Croatia, Italy, Austria, and Bosnia and Herzegovina, but the reintroduced population remained isolated until today. Population growth and range expansion in the first 15 years was followed by a period of stabilization in the late 1980s and early 1990s. However, at the beginning of the 21st century, a decline in the population was recorded. Genetic analyses showed that there were no natural migrations from other lynx populations, so low genetic diversity and inbreeding were the main threats to population survival (SINDIČIĆ et al., 2013). The decline in population size was also exacerbated by high human-caused mortality (SINDIČIĆ et al. 2016), while the lack of proper management indirectly influenced the unfavorable status of the lynx population in Croatia (SINDIČIĆ et al. 2019).

In order to save the population from extinction, a reinforcement process was initiated in 2017 under the LIFE16 NAT /SI/000634 project "Preventing the Extinction of the Dinaric- SE Alpine Lynx Population through Reinforcement and Long-term Conservation" (hereafter referred to as the LIFE Lynx project). The main objective of the LIFE Lynx project was to improve the genetic and demographic prospects of the Dinaric and Southeastern (SE) Alpine population (ČERNE et al., 2019). Prior to the reinforcement, it is important to ensure that decisions regarding the implementation of reinforcement measures are based on scientifically collected data and an understanding of the ecological factors important to lynx (SMOLKO et al. 2019). In addition, monitoring translocated animals is critical to a successful outcome. Telemetry is the main method used for monitoring released individuals during reintroductions and reinforcements (PORT et al., 2020). Although it is expensive in comparison to other monitoring methods, and requires great effort, time, and experience on the part of the researcher, it is an indispensable method for tracking the movements and survival of released individuals (PORT et al., 2020). Movement is the initial behavioral response of translocated animals to “forced dispersal” in a new habitat (STAMPS and SWAISGOOD 2007). Therefore, monitoring movement patterns in the early post-release period is critical for survival and establishment of reintroduced animals (PREATONI et al. 2005, BERGER-TAL and SALTZ

2014). However, available information on early post-release movements of translocated carnivores is limited for most species (VANDEL et al. 2006). This is primarily because there are no published data on telemetry monitoring in the early post-release period, or because it can only be found in the grey literature, such as project reports in local languages (Ryser et al. 2004).

2. AIMS AND HYPOTHESIS

The aim of this dissertation was to optimize the methodology for collecting and analysing data on key indicators of the population status, namely abundance and distribution of territorial individuals according to the sex of the local population. Furthermore, the goal was to determine the distance travelled and the duration of movement of lynx individuals translocated as part of the reinforcement process of the Dinaric lynx population.

The hypothesis of the dissertation was that the status of territorial individuals, as abundance and distribution, would affect the success of the reinforcement. It was hypothesised that the presence of the same-sex individuals would lead to an extended duration of nomadic movement of released individuals.

3. MATERIALS AND METHODS

3.1. Analyzing characteristics and temporality of coat patterns in Croatian lynx population

A total of 195 photographs of *Lynx lynx* individuals from Croatia were used in this study, comprising 92 trophy pelt photographs taken from year 1978 until 1999, 20 photographs of lynx captured in 2001-2019 for telemetry research, 1 of dead lynx found in 2019 and 82 of lynx captured by camera traps from 2011 until 2019. In all statistical analyses described below, differences were considered significant if they were associated with $p < 0.05$.

3.1.1. Definition and quantitation of coat patterns

Lynx coat patterns were visually assigned to one of four types recognized in the Croatian population: no spots, small spots, big spots, and rosettes (Figure 1 – Paper I). To support the visual assignment done by researches and reduce possible bias caused by human subjectivity, a quantitative analysis of four types of coat patterns was also preformed. Quantitative characteristics in each of the patterns were measured using ImageJ software designed for scientific image processing and analysis. The program offers a huge array of functions and can be extended using plugins. Basic features include a wide range of measurement tools such as length measurement, angle measurement and surface calculation were used as described in Paper I. For our analyses, we used only 108 photographs, comprising all 92 trophy pelt images, 1 image of dead lynx and 15 images of live lynx from telemetry and camera trap studies. The quality of the remaining 87 photographs was too low for reliable analysis with ImageJ. Each metric was compared using Student's t-test.

3.1.2. Temporality of coat patterns

All 195 lynx photographs were visually assigned to one of the four coat patterns by two experienced researchers working independently. In 20 (10.26%) cases a third researcher resolved discrepancies when the first two researchers disagreed. These 20 cases involved mismatches between big and small spots (15), rosettes and no spots (2), rosettes and small spots (2), or rosettes and big spots (1). Later, the frequencies of each of the four patterns were compared between images taken in 1978-1999 or 2001-2019 period using chi2 test.

3.2. Abundance and distribution of the local population

In order to estimate abundance and determine the range of lynx distribution in Croatia, a network of camera traps was set up from 1 May 2018 to 30 April 2020 in the areas previously defined as lynx distribution area in Croatia (Sindičić et al. 2010). Simutaneously all available signs of lynx presence were collected from different sources, including photographs, mortality, sightings, lynx prey, footprints, and samples collected for DNA analysis (faeces, urine, hair). All collected data was archived in the database of the University of Zagreb, Faculty of Veterinary Medicine (Gomerčić 2017), which is publicly available at the web address http://lynx.vef.hr. Each sign of lynx presence was registered with details of location and time, as well as the provider of the information, and categorised according to the criteria of SCALP (BREITENMOSER et al. 2006), as presented in Table 1- Paper II.

In addition to lynx photographs from the camera traps, opportunistically collected photos of lynx from other sources (i.e., hunters, institutions for management of protected areas, private individuals, as well as from the environmental assesment study done by Geonatura Ltd. company) were included in the abundance and distribution analysis.

The estimation of minimum population size and distribution did not include lynxes that were released in Croatia within the LIFE Lynx project.

3.2.1. Estimating the abundance

For the estimation of the abundance, i.e. population size, camera traps were set at 182 locations. For optimal camera trap placement, a 10 x 10 km grid cells were used and a lynx sensitivity (presence probability) map produced by KUSAK et al. (2016). At least one non-baited camera trap was placed within each 10 x 10 km grid cell, while cells that were categorized by Kusak et al. (2016) as unsuitable or low suitably for lynx were excluded from the research. To maximize lynx detectability camera traps were set at optimal locations within cells, where landscape and terrain features were likely to channel lynx movements, like lynx marking sites, forest roads and game paths. Those locations were identified based on previously archived observations of lynx presence and with the help of local hunters and rangers. Although camera traps were intended to stay at each location all year round, due to malfunctions, theft and snow coverage some of them were not active during the entire research period on the selected location. Camera traps were checked at least every two months to change memory cards and batteries. Images were processed in program Camelot (HENDRY and MANN 2017) and for each event, the species, number of animals, age category (juvenile or adult) and sex was defined, while empty photos were erased. An event is defined as one visit of animals lasting 10 minutes during which several photos and videos could be taken. Lynx photos were additionally archived in http://lynx.vef.hr database.

Unique coat pattern of lynx in the Dinaric population enabled the identification of individuals by visual comparison as described in the Paper II. Animals' gender was determined from photographs in cases when the genital area was captured or female was recorded with cubs. For some animals, gender and age were determined when they were captured during the radiotelemetry research, or when found dead. Individuals were categorized as adults or kittens when photographed with an adult (mother).

This estimation of minimum population size does not include lynxes that were released in Croatia within LIFE Lynx project.

3.2.2. Mapping the area of lynx distribution

Collected data was mapped using program QGIS (QGIS.org 2020) as shown in Figure 2 – Paper II. Lynx distribution was determined on a 10 x 10 km Pan-European grid (EUROPEAN ENVIRONMENTAL AGENCY 2017), with permanent presence confirmed for quadrants in which lynx was recorded based on at least one C1 observation or two C2 observations (Figure 3 – Paper II and Figure 1 – Paper III). Quadrants with only one C2 observation were defined as areas of sporadic presence, while quadrants with only C3 observations were defined as areas of possible presence but without solid evidence (KUSAK et al. 2016). Total surface of permanent, occasional and areas of possible lynx presence in Croatia were calculated by summing the surface of quadrants with predefined observations.

3.3. Post-release analysis of the movement of translocated lynx

Telemetry data of six translocated lynx individuals was analysed for the purpose of the early post-release study as shown in Table 1 – Paper III. From the six monitored lynx, five of them (all except one lynx as described in Paper III) were captured in box traps during the winter (January-March) in 2019 and 2020. They were kept in quarantine enclosures in the country of capture for at least three weeks before transportation to Croatia and Slovenia. Age estimation was done following MARTI and RYSER-DEGIORGIS'S (2018) classification while handling animals at the capture sites. We defined the early post-release period as three months (91 days) after the release. To analyse the lynx's first behavioural response, we assessed the movement patterns of released animals and their temporal dynamics based on 12 variables defined in

Paper III. For calculating the distances between the daily locations from telemetry collars, we chose 91 locations for each lynx taken on consecutive days at 24 h intervals. We chose 24 h intervals because, on some collars, that was the minimum scheduled interval. There were cases when the collar failed to fix the location, so we interpolated to 24 h intervals.

We defined straight-line movement and increased distance from the release sites as exploratory movement, while polygonal movement with decreased and stabilised distance indicated a settlement process (BUNNEFELD et al. 2011).

Movement analyses were done using QGIS (Geographic information system, Open source geospatial foundation project 2020). Kill sites were detected using three GPS collar locations in a period longer than 30 h within 300 m, following the GPS location cluster analysis described by KROFEL et al. (2013). Forest cover analyses were done using the CORINE Land Cover inventory for 2018 (BÜTTNER et al. 2021). All shapefiles were projected as the HTRS96/UTM zone 33N coordinate system.

4. PUBLISHED PAPERS

4.1. PAPER I: „Big spots in a small population: Analyzing characteristics and temporality of coat patterns in Croatian lynx population“

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Big spots in a small population: Analyzing characteristics and temporality of coat patterns in Croatian lynx population

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ABSTRACT

Quantification of animal phenotypic traits could allow objective description of individuals and their morphology, reducing bias caused by human subjectivity, skill and experience. The goal of our study was to research a quantitative approach to analyzing the four types of coat patterns recognized in endangered Eurasian lynx (*Lynx lynx*) in Croatia and to track frequencies of coat patterns over time. A total of 195 photographs of lynx individuals from Croatia, collected in the 1978–2019 period, were visually assigned to one of four types of coat patterns: no spots, small spots, big spots, and rosettes. Then, using metrics available within the public-domain software ImageJ, we quantified lynx spot traits and compared the characteristics of the four coat patterns to detect objective differences. All metrics that were analyzed differed significantly between "no spots" and other coat patterns ($p < 0.05$), whereas none of the metrics differed significantly between coats of small spots and coats of rosettes. Luckily, researchers visually distinguished easily coats of rosettes from coats of small spots. In contrast, when coats of big spots were numerically compared to those of small spots, they differed significantly in spot size on the whole body, while in some lynx individuals these two patterns proved to be most difficult for researchers to distinguish by eye. When looking at the occurrence of the four coat patterns inside the two temporal categories, they differed significantly between individuals photographed in 1978–1999 and those photographed in 2001–2019. The frequency of coats without spots fell from 14% to zero, the frequency of rosettes dropped from 23% to 9%, while the frequency of big spots increased from 46% to 80%. Significant inbreeding and low population size were genetically proven in Croatian lynx population, but to link inbreeding with the phenotypic change we observed, further research should be conducted.

1. Introduction

Carnivores show a great diversity of coat colors and markings (Ortolani & Caro 1996; Ortolani 1999; Allen et al., 2011) wherefore in felids, six coat patterns have been identified (Werdelin & Olsson 1997): rosettes, small blotches, blotches, vertical stripes, and "uniform with no distinguishable pattern". For lynx, which belong to the spotted cats (Werdelin & Olsson 1997), five types of coat pattern have been defined both by Thüler (2002) and Darul et al. (2021): large spots, small spots, without spots, rosettes and small spots with rudimentary rosettes, while Kubala et al. (2020) depicted only three: spotted, rossetted and uniform. High degree of intra-specific polymorphism in Eurasian lynx (*Lynx*) coat patterns was observed across its broad distribution in Eurasia (Kubala et al., 2020; Darul et al., 2021). This phenotypic polymorphism was linked to habitat and climatic conditions (Ortolani and Caro 1996; Ortolani 1999; Allen et al., 2011; Darul et al., 2021), past or recent (human-induced) demographic changes that may have disrupted patterns that originally evolved by natural selection (Lucena-Perez et al., 2020), habitat fragmentation and human-induced mortality (Kubala et al., 2020), while genetic drift was found to be responsible for reducing coat type variability (Thuler, 2002; Kubala et al., 2020).

Accurate and reliable methods to quantify coat patterns can be useful in numerous biological studies, for example in identifying individuals based on coat patterns, which is the base for estimation of population size in many cat species using camera traps and

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capture - recapture methodology (Williams et al., 2002; Karanth et al., 2006; Hiby et al., 2009; Rovero & Zimmermann 2016; Sarmento & Carrapato 2019; Gimenez et al., 2019; Gomerčić et al., 2021). However, precisely quantifying variations in color patterns is challenging (Schneider et al., 2012a; Van Belleghem et al., 2018) because of differences in photographic conditions, picture quality, animal position and small discrepancies while manually processing photographs. Until now, only few methodologies for specific taxa have been developed (Sherley et al., 2010; Le Poul et al., 2014; Allen & Higham 2015; Lee et al., 2018).

Dinaric lynx population (*Lynx*) was established by a reintroduction of six individuals into Slovenia in 1973 from the Slovakian Carpathian Mountains, 70 years after the extinction of autochthonous population. Offspring of those animals successfully dispersed to Croatia, Italy, Austria as well as Bosnia and Herzegovina, but the reintroduced population remained isolated until today. Population growth and range expansion during the initial 15 years was followed by a period of stabilization in the late 80s and early 90s. However, at the beginning of the 21st century recent studies have indicated potential threats to lynx population in Croatia. Genetic analysis showed there were no natural migrations from any other lynx populations, consequently low genetic diversity and inbreeding are threatening the survival of the population (Sindičić et al., 2013). In order to save the population from extinction, a reinforcement process started in 2017 under the LIFE Lynx project (LIFE16 NAT/SI/000634) with the main goal to improve the genetic and demographic outlook of the Dinaric- SE Alpine population (Černe et al., 2019). Today, the population is estimated to minimum 95 lynx in Slovenia and Croatia (Fležar et al., 2022), and an unknown number in Bosnia and Herzegovina.

The present study aimed to: (a) gain insight into a quantitative approach to analyzing four coat patterns visually recognized in Eurasian lynx in Croatia and (b) track frequencies of Croatian lynx population coat patterns over time. Our goal was to develop a simplified approach based on ImageJ software in the public domain (Schneider et al., 2012b) to determine quantitative differences in coat pattern types recognized in the Croatian lynx population and see whether their occurrence changed over the years.

2. Material and methods

A total of 195 photographs of *Lynx lynx* individuals from Croatia were used in this study, comprising 92 trophy pelt photographs taken from year 1978 until 1999, 20 of lynx captured in 2001–2019 for telemetry research, 1 of dead lynx found in 2019 and 82 of lynx captured by camera traps from 2011 until 2019. In all statistical analyses described below, differences were considered significant if they were associated with $p < 0.05$.

2.1. Definition and quantitation of coat patterns

We visually assigned lynx coat patterns to one of four types recognized in the Croatian population: no spots, small spots, big spots, and rosettes (Fig. 1). Then we aimed to identify and measure quantitative characteristics in each of the patterns using ImageJ software designed for scientific image processing and analysis. The program offers a huge array of functions and can be extended using plugins. Basic features include a wide range of measurement tools such as length measurement, angle measurement, surface calculation and much more. For our analyses, we used only 108 photographs, comprising all 92 trophy pelt images, 1 image of dead lynx and 15 images of live lynx from telemetry and camera trap studies. The quality of the remaining 87 photographs was too low for reliable analysis with ImageJ.

Full-color images were converted to 32-bit grayscale images, then the measurement unit was set to be the distance in pixels from the base of the lynx's tail to the top of the scapula divided by 1000 (Fig. 2). This normalization allowed us to cancel out differences due to animal size. We then selected three regions of interest (ROIs) using the "ROI Manager" and measured their surface using the "Measure" tool. ROI 1 was the surface of the front leg from the chest to the line behind the scapula, ROI 2 was the trunk area between the posterior edge of the front leg and anterior edge of the hind leg, and ROI 3 was the surface of the hind leg (Fig. 2). Spots, treated as "particles" in ImageJ, were defined by selecting dark areas using the "Threshold" command. We excluded dark particles whose area was <50 pixel units in order to eliminate the influence of speckles (Fig. 2).

Then we measured dark areas within the three ROIs using the following metrics within the "Set measurements" command: area of ROI, number of spots, particle area (mean, SD, min and max), area fraction (percentage of black pixels in the thresholded image), and the standard deviation of each of these metrics. Each metric was compared among the three ROIs and the four coat patterns using Student's t-test.

2.2. Temporality of coat patterns

All 195 individuals shown in the photographs were visually assigned to one of the four coat patterns by two experienced researchers working independently. In 20 (10.26%) cases a third researcher resolved discrepancies when the first two researchers disagreed. These 20 cases involved mismatches between big and small spots (15), rosettes and no spots (2), rosettes and small spots (2), or rosettes and big spots (1). Later, the frequencies of each of the four patterns were compared between images taken in 1978–1999 or 2001–2019 using chi2 test. Unfortunately, we were not able to divide photographs in more than two time categories.

3. Results

3.1. Quantification of coat pattern characteristics

All metrics that we analyzed differed significantly between "no spots" and other coat patterns ($p < 0.01$), small spotted coats differed significantly in size compared to big spots, whereas none of the metrics differed significantly between coats of small spots when compared to coats of rosettes.

Spot size, quantified as "particle area", increased in the expected trend from no spots < rosettes < small spots < big spots (Fig. 3). Spot size differed significantly when coats of big spots were compared to other three types of coats ($p < 0.05$) and in all cases when coats of no spots were compared with other coat types ($p < 0.01$). Only coats of small spots showed no significant difference when compared to coats of rosettes.

The fraction of coat area covered by spots, quantified as "area fraction" or the percentage of black pixels in the thresholded image, did not differ significantly among coats of rosettes, small spots and big spots ($p > 0.05$). When the three coat patterns were compared with coats of "no spots" they differed significantly in all three cases ($p < 0.01$) (Fig. 4).

Spot density differed significantly in all three cases when each of the coat pattern type was compared to coats of "no spots" ($p < 0.01$). Spot density differed significantly on the trunk (ROI 2) ($p < 0{,}05$) and the hind leg (ROI 3) ($p < 0.01$) between coats of small spots and coats of big spots, while between coats of big spots and rosettes it differed significantly only on the hind leg (ROI 3) ($p < 0.01$). Spot density between coats of small spots and rosettes showed no significant difference. On each coat type, spot density

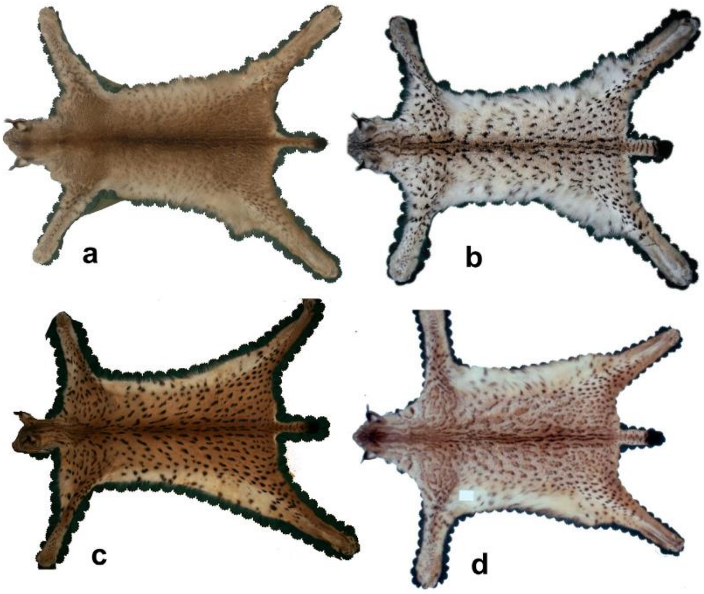

Fig. 1. Lynx coat pattern types, shown in representative photographs of trophy pelts as **a** no spots; **b** small spots; **c** big spots; **d** rosettes.

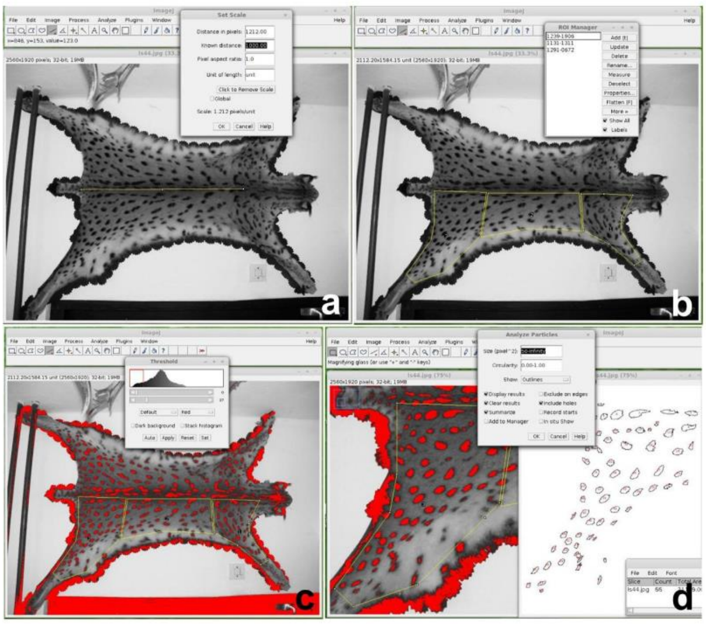

Fig. 2. a Normalization of pixel length to 1/1000th of the distance from the base of the tail to the shoulder line; **b** selected ROIs for quantitative analysis of coat patterns using ImageJ; **c** dark areas on the lynx coat after selection and extraction using the "Threshold" command; **d** counting and measuring spots ("particles") in each thresholded ROI using the "Analyze particles" command.

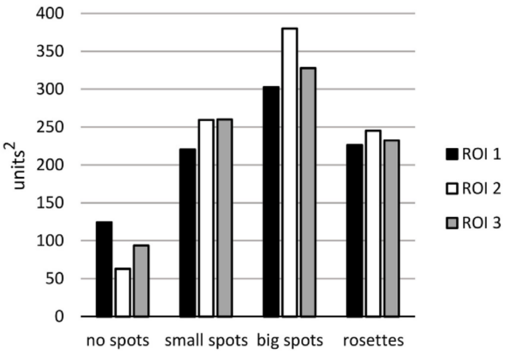

Fig. 3. Spot size in the four coat patterns in three preselected ROIs: ROI 1 – front leg, ROI 2 – trunk, ROI 3 – hind leg.

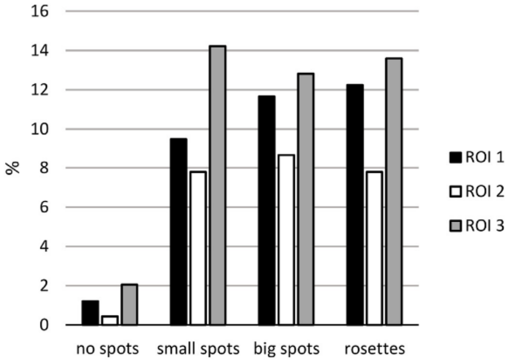

Fig. 4. Fraction of coat area covered by spots in the four coat patterns, based on three preselected ROIs: ROI 1 – front leg, ROI 2 – trunk, ROI 3 – hind leg.

was greatest on the hind leg (ROI 3), followed by the front leg (ROI 1) and then the trunk (ROI 2) (Fig. 5). In coats of no spots and coats

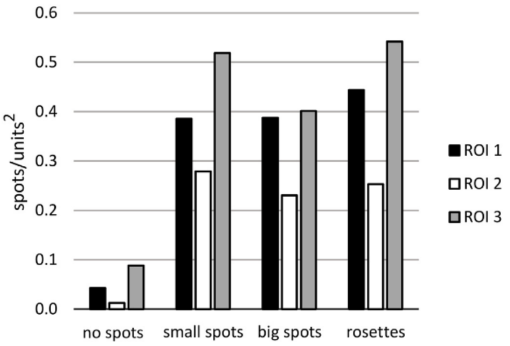

Fig. 5. Spot density in the four coat patterns, based on three preselected ROIs: ROI 1 – front leg, ROI 2 – trunk, ROI 3 – hind leg.

of big spots, our analysis showed that the number of spots, their size and area fraction differed significantly between limbs (ROIs 1 and 3) and trunk (ROI 2). Conversely, in coats of small spots and rosettes, number of spots, their size and area fraction did not differ significantly between limbs and trunk.

3.2. *Temporality of coat patterns*

The frequencies of the four coat patterns differed significantly between individuals photographed in 1978–1999 and those photographed in 2001–2019 (Table 1). The frequency of coats without spots fell from 14% to zero, the frequency of rosettes dropped from 23% to 9%, while the frequency of big spots increased from 46% to 80%.

4. Discussion

Quantification of animal phenotypic traits could allow objective description of individuals and their morphology, reducing bias caused by human subjectivity, skill and experience (Kühl & Burghardt 2013). Using ImageJ program, we were able to measure the patterns and quantify four different lynx coat traits, although clear numerical difference was not shown between all of the coat patterns. We easily distinguished coats of small spots from coats of big spots, which differed significantly in spot size and density (Figs. 3 and 5), even though these two patterns proved as the most difficult for researchers involved in this study to distinguish by eye. On the other hand, it was the easiest for researchers to distinguish coats of small spots from coats of rosettes, while these two patterns showed no significant differences on any of the metrics that we examined. This likely reflects the inability of individual metrics to capture the characteristic visual appearance of rosettes. In the context of this study, it also highlights the fact that automated software currently cannot rival the "human eye" for integrating multiple types of visual information for defining and differentiating patterns. Furthermore, it was interesting that among the coat patterns of spots and rosettes, the amount of black color ("area fraction"), did not differ significantly. In other words, these coats contained the same overall extent of black cover, regardless of spot size or density.

Our findings in the quantitative analysis of coat patterns supported the visual assignment of four types of coat patterns in the temporal analysis of the frequencies of coat patterns in Eurasian lynx population from Croatia. Through the end of the 20th century a similar distribution of the coat patterns was found in Carpathian, Dinaric and Jura Mountain populations of lynx - spotted pattern as predominating and less frequent rosettes and pattern without spots (Štollmann 1963; Thüler 2002). In Swiss Alpine population, frequencies of different coat pattern types changed from coat without spots in the historical population, to large spots in the early-reintroduced population. Later, the occurrence of small spots increased, to finally end up with a higher frequency of rossetes in

Table 1
Changes in the frequencies of coat patterns in Eurasian lynx population from Croatia, 1978–2019. The frequencies of all four coat patterns differed significantly between individuals photographed in 1978–1999 and those photographed in 2001–2019.

Coat pattern	Total		1978–1999		2001–2019	
	N	%	N	%	N	%
Big spots[a]	125	64.1	42	46.2	83	79.8
Small spots[a]	27	13.8	15	16.5	12	11.5
Rosettes[a]	30	15.4	21	23.1	9	8.7
No spots[a]	13	6.7	13	14.3	0	0.0

[a] All types of coat patterns differed significantly between the two periods.

recent (late-reintroduced) populations (Thüler 2002). In a more recent study, Darul et al. (2021) presented that majority (41.5%) of lynx, that they sampled across the entire distribution in Eurasia, had coats with the large spot pattern. The second most common pattern was uniform (36.2%), while rosettes, small spots, and pseudo-rosettes were represented in frequencies below 11%.

The overall frequencies of the four coat patterns in our Croatian lynx population showed the trend big spots > rosettes > small spots >no spots, and this trend was the same when we examined data from 1978 to 1999. However, the trend and the absolute frequencies of some patterns did change substantially between 1978-1999 and 2001–2019. Coats without spots vanished completely from the population, and coats with rosettes became much less frequent. This recent data on the lynx with rosettes, which account for fewer than 9% of Croatian lynx, also showed that they are confined to the area south of the Zagreb - Rijeka highway (Fig. 6). Intensive camera-trapping in 2018–2019 failed to detect any lynx with rosettes in Croatia in area northwest of the Zagreb - Rijeka highway (Gomerčić 2017; Gomerčić et al., 2021). Also, lynx with rosettes were not detected in Slovenia either in that period (Fležar et al., 2019). Conversely, over a similar period (2000–2017), Slovakian lynx population have shown a significant decrease in spotted coats and concomitant increase in coats with rosettes (Kubala et al., 2020). Kubala et al. (2020) found that lynx offspring of a parent with spotted phenotype coupled with rosetted one, would have either of the two parental phenotypes. If both parents were spotted or both without spots, the offspring had the same phenotype, and never rosettes. So, Kubala et al. (2020) hypothesized that phenotypic shift in the marginal parts of the Slovak lynx population was the result of a genetic drift, indicating small population size with limited gene flow. Interestingly, study from Darul et al. (2021) showed a greater differentiation in coat patterns among the populations with lower genetic diversity, and proposed that the observed phenotypic distribution was shaped by the post-glacial expansion from refugia, rather than adaptations to specific climates and environments. Unfortunately, coat patterns of the six animals that founded the Dinaric lynx population in 1973 are unknown but now we will have new opportunity to observe the changes in frequency of coat patterns after new individuals entered the closed population. To save the Dinaric population from extinction, from 2019 to 2021 thirteen lynx from Romania and Slovakia were released into Croatia and Slovenia (Černe et al., 2019). Among the thirteen released animals, seven of them have coats without spots, four with big spots and two are with rosettes. It will be interesting to evaluate their influence on the frequency of coat patterns in the Dinaric population and possibly use quantitative genetics methods (parent–offspring regression) to quantify the proportion of observed phenotypic variation of a trait that is shared between mother and offspring.

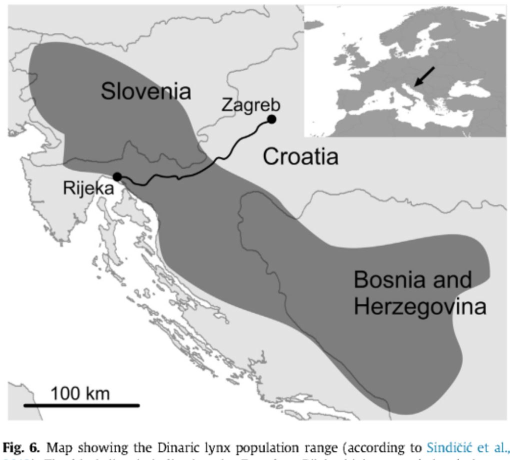

Fig. 6. Map showing the Dinaric lynx population range (according to Sindičić et al., 2013). The black line is indicating the Zagreb – Rijeka highway and the dark grey area is the distribution of the Dinaric lynx population.

In conclusion, our results can give rise to ideas for further research on coat pattern quantification and the use of freely available image analysis software could facilitate widespread implementation of our method not only for lynx but potentially also for other species.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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4.2. PAPER II: „Distribution and minimum population size of Eurasian lynx (*Lynx lynx*) in Croatia in the period 2018–2020“

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Izvorni znanstveni članci – Original scientific papers
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DISTRIBUTION AND MINIMUM POPULATION SIZE OF EURASIAN LYNX (*Lynx lynx*) IN CROATIA IN THE PERIOD 2018–2020

RASPROSTRANJENOST I NAJMANJA VELIČINA POPULACIJE EUROAZIJSKOG RISA (*Lynx lynx*) U HRVATSKOJ U RAZDOBLJU 2018.–2020.

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SUMMARY

Scientific data on distribution and abundance of endangered species are the foundation for their effective conservation and management. In this paper, we present results of the first scientifically – based estimation of lynx population size in Croatia. The goal of the study was to determine the area of lynx distribution and to estimate the minimum size of lynx population in Croatia in the period 2018 - 2020. To determine lynx distribution, 902 signs of lynx presence were collected in the period from the beginning of May 2018 until the end of April 2020. Out of those, 92.8% of lynx observations were categorized as C1, 2.8% as C2 and 4.4% as C3. Permanent lynx presence was confirmed in Primorsko – Goranska and Ličko – Senjska county, in southern part of Karlovac county and north-eastern part of Zadar county on the total surface of 7200 km2. For the minimum population size estimation, 804 camera trap photographs led to identification of 89 – 108 adult lynxes. Among 108 identified individuals there were 29 females, 22 males, while for 7 animals the sex was not determined. During the two reproductive seasons, we photographed 44 cubs in 25 litters. Future important steps in lynx population monitoring are correcting the deficiencies identified in this study and implementation of methodology that will allow us to use spatial capture recapture models for estimation of lynx abundance in Croatia.

KEY WORDS: distribution, abundance, *Lynx lynx*, minimum population size, Croatia

INTRODUCTION
UVOD

Population monitoring implies repeated, standardized assessment of indicators that reveal ecologic processes, and is carried out within a defined area over a specified period of time (Thompson et al. 1998). The term itself is used in very different contexts - from collecting data for assessing population status to planning of interventions (e.g. highway construction or species reintroduction, hunting quotas). Population monitoring is an indispensable activity in the management of a certain population and has a key role in

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the management of protected wild species, where the goal of monitoring is to determine the efficacy of conservation actions (Breitenmoser et al. 2006). Monitoring objectives must be clearly defined to decide which indicators should be monitored and which methods should be used. Basic monitoring involves collecting data on the distribution, abundance and density of the population and their changes over time. This serves as a foundation for efficient population management (Thompson et al. 1998).

The Eurasian lynx (*Lynx lynx*) is protected in Croatia by the Ordinance on declaring protected and strictly protected wildlife species (Official Gazette No 144/13, 73/16) and it is listed as critically endangered (CR) on the IUCN Red List of Threatened Species. Habitats Directive (92/43/EEC) lists Eurasian lynx on Annexes II and IV, requiring strict protection and population monitoring. For the Habitat Directive reporting period 2013-2017, the conservation status of the lynx population in the Alpine region in Croatia was evaluated as unfavorable - bad (U2), while the situation in the Continental and Mediterranean region was assessed as unfavorable - inadequate (U1) (Anonymous 2019). The loss of genetic diversity is considered as the most important threat to lynx in Croatia, as the entire Dinaric population originated from six reintroduced animals (Sindičić et al. 2013). Decrease in population size was also invigorated by high human – induced mortality (Sindičić et al. 2016), while lack of appropriate management indirectly influenced the unfavorable status of lynx population in Croatia (Sindičić et al. 2019).

With the development of technology, photo traps became the most effective and cost-efficient methodology for monitoring lynx population (Rovero and Zimmerman 2016). In Croatia first lynx monitoring activities using automatic cameras (camera traps) were conducted in Gorski Kotar in the 2011 – 2014 period (Kusak 2012; Kusak and Modrić 2012; Kusak et al. 2013, Kusak et al. 2014),while wide – scale population monitoring with camera traps is in place since 2018 within the project LIFE16 NAT/SI/000634 "Preventing the Extinction of the Dinaric-SE Alpine Lynx Population through Reinforcement and Long-term Conservation" (acronym LIFE Lynx) (Sindičić et al. 2018).

In this paper, we present results of the lynx population monitoring in Croatia for the period 2018 - 2020. The goal of the monitoring was to determine the lynx distribution area and to estimate the minimum size of the lynx population in Croatia.

MATERIALS AND METHODS
MATERIJALI I METODE

Signs of lynx presence were collected for two years, from 1st of May 2018 until 30th of April 2020. This period overlaps with two "lynx years" defined as period from the beginning of May to end of April, since kittens are mostly born in May and leave the mother in April of the following year (Zimmerman et al. 2005). All available observations from all possible sources, including photos, mortality, sightings, lynx prey, footprints and samples collected for DNA analysis (feces, urine, hair) were archived in Faculty of Veterinary Medicine University of Zagreb database (Gomerčić 2017), which is publicly available on the internet address http://lynx.vef.hr. Each sign of lynx presence was registered with information about location and time, provider of the information and was categorized according to SCALP criteria (Breitenmoser et al. 2006):

Collected data was mapped using program QGIS (QGIS.org 2020). Lynx distribution was determined on a 10 x 10 km Pan-European grid (European Environmental Agency 2017), with permanent presence confirmed for quadrants in which lynx was recorded based on at least one C1 observation or two C2 observations. Quadrants with only one C2 observation were defined as areas of sporadic presence, while quadrants with only C3 observations were defined as areas of possible presence but without solid evidence (Kusak et al. 2016). Total surface of permanent, occasional and areas of possible lynx presence in Croatia were calculated by summing the surface of quadrants with predefined observations.

For the estimation of minimum population size a network of camera traps was set in Gorski kotar, Lika and northern Dalmatia - areas previously defined as lynx distribution area in Croatia (Sindičić et al. 2010). Three additional camera traps were placed on Pelješac, as we wanted to check several undocumented reports of lynx sightings on the peninsula. For optimal camera trap placement, we used 10 x 10 km grid cells and a lynx sensitivity (presence probability) map produced by Kusak et al. (2016). At least one non-baited camera trap was placed within each 10 x 10 km grid cell, while cells that were categorized by Kusak et al. (2016) as unsuitable or low suitably for lynx were excluded from the research. To maximize lynx detectability camera traps were set at optimal locations within cells, where landscape and terrain features were likely to channel lynx movements, like lynx marking sites, forest roads and game paths. Those locations were identified based on previously archived observations of lynx presence and with the help of local hunters and rangers. Different brands and models of camera traps with active infrared sensor and infrared flash were used, set to capture one photo and 30 seconds of video or three photos without the video. During the period May 2018 - April 2020, camera traps were set at 182 locations. Although camera traps were intended to stay at each location all year round, due to malfunctions, theft and snow coverage some of them were not active during the entire research period on the selected location. We checked camera traps at least

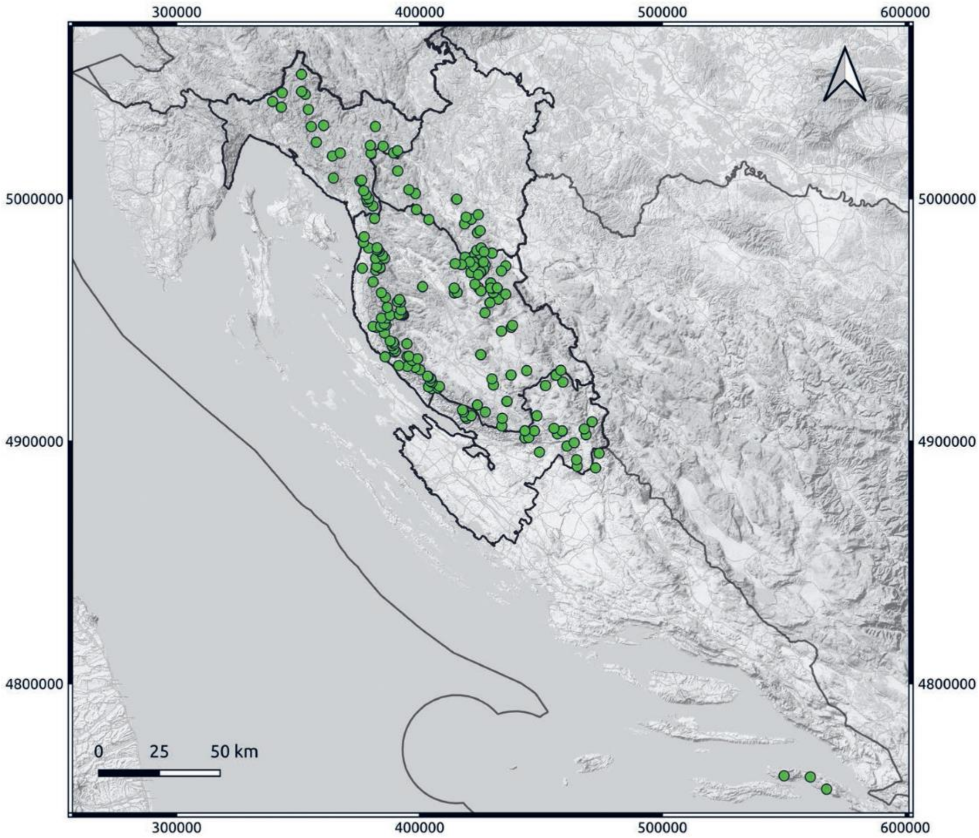

Figure 1. Locations of camera traps used for lynx monitoring in Croatia in the period 1st of May – 30th of April.
Slika 1. Lokacije fotozamki korištene za monitoring risa u Hrvatskoj u razdoblju 01. svibnja 2018. – 30. travnja 2020.

every two months to change memory cards and batteries. Images were processed in program Camelot (Hendry and Mann 2017) and for each event, the species, number of animals, age category (juvenile or adult) and sex was defined, while empty photos were erased. An event is defined as one visit of animals lasting 10 minutes during which several photos and videos could be taken. Lynx photos were additionally archived in http://lynx.vef.hr database.

Opportunistically collected photos of lynx from other sources (i.e. from hunters, institutions for management of protected areas, private persons, as well as from company Geonatura Ltd. comprising results of preconstruction monitoring for windfarm "Lički medvjed" financed by Green Trust Energy Ltd., Split) were also included in the analysis.

Lynx from the Dinaric population have coats with rosettes, large and small spots, while coats without spots are not present (Topličanec et al. in press). Unique coat pattern of lynx in Dinaric population enabled the identification of individuals by visual comparation. Each newly photographed individual was compared with reference photographs of individuals belonging to the same coat pattern type until all photographs of the individuals within the database have been checked. When both flanks of the same individual are known lynx gets a unique identifier (Rovero and Zimmerman 2016). If for a new animal we have photos of one flank only then we cannot connect which right and left flank belong to the same individual. For example, if we have five lynxes with left flank photos only and five lynxes with right photos only, this could be the same five animals or ten different animals. That is why our estimation of the minimum population size has a span. Animals' gender was determined from photographs in cases when the genital area was captured or female was recorded with cubs. For some animals, gender and age were determined when they were captured during the radiotelemetry re-

Table 1. Observations of Eurasian lynx presence in Croatia in the period 1st of May 2018 - 30th of April 2020.
Tablica 1. Pregled znakova prisutnosti Euroazijskog risa u Hrvatskoj u razdoblju 1. svibnja 2018. – 30. travnja 2020.

Type of observation *Vrsta znaka prisutnosti*	Season (May 1 – April 30) Sezona (01.05. – 30.04.)		Total	SCALP category *SCALP kategorija*		
	2018-2019	2019-2020		1	2	3
Photography – *Fotografija*	345	459	804	802		2
Captured animal – *Uhvaćena životinja*	1	5	6	6		
Mortality – *Smrtnost*	2	2	4	4		
Footprint – *Otisak šape*	12	9	21		20	1
Hair – *Dlaka*	11	14	25	14		11
Prey – *Plijen*	1	5	6		5	1
Scat – *Izmet*	5	10	15	10		5
Urine – *Urin*	4	1	5			5
Sighting – *Viđenje*	10	6	16			16
Total – *Ukupno*	391	511	902	836	25	41

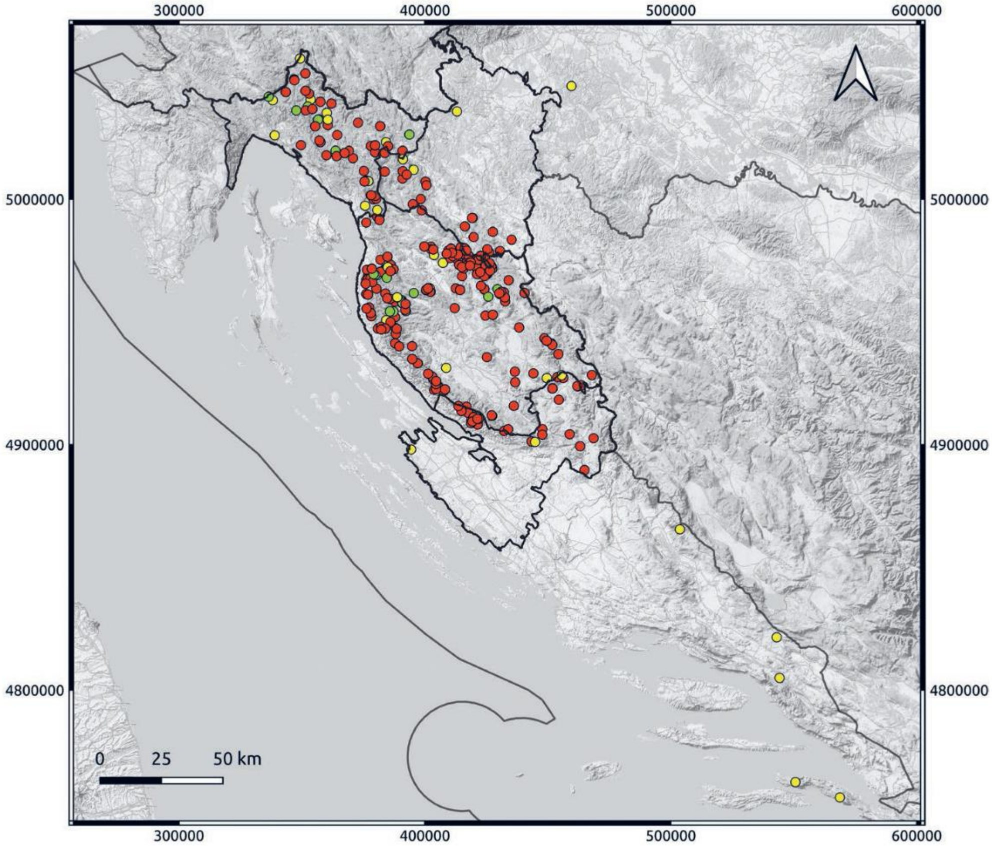

Figure 2. Signs of lynx presence in Croatia collected in the period 1st of May – 30th of April. C1 observations are presented with red dots, C2 – green dots, C3 observations – yellow dots. Black lines define borders of Croatian counties – Primorsko - Goranska, Ličko – Senjska, Karlovačka and Zadar county.

Slika 2. Znakovi prisutnosti risa u Hrvatskoj prikupljeni u razdoblju 1. svibnja 2018. – 30. travnja 2020. C1 prisutnost je predstavljena crvenim točkama, C2 – zelene točke, C3 – žute točke. Crne linije obilježavaju granice hrvatskih županija – Primorsko-goranska, Ličko-senjska, Karlovačka i Zadarska županija

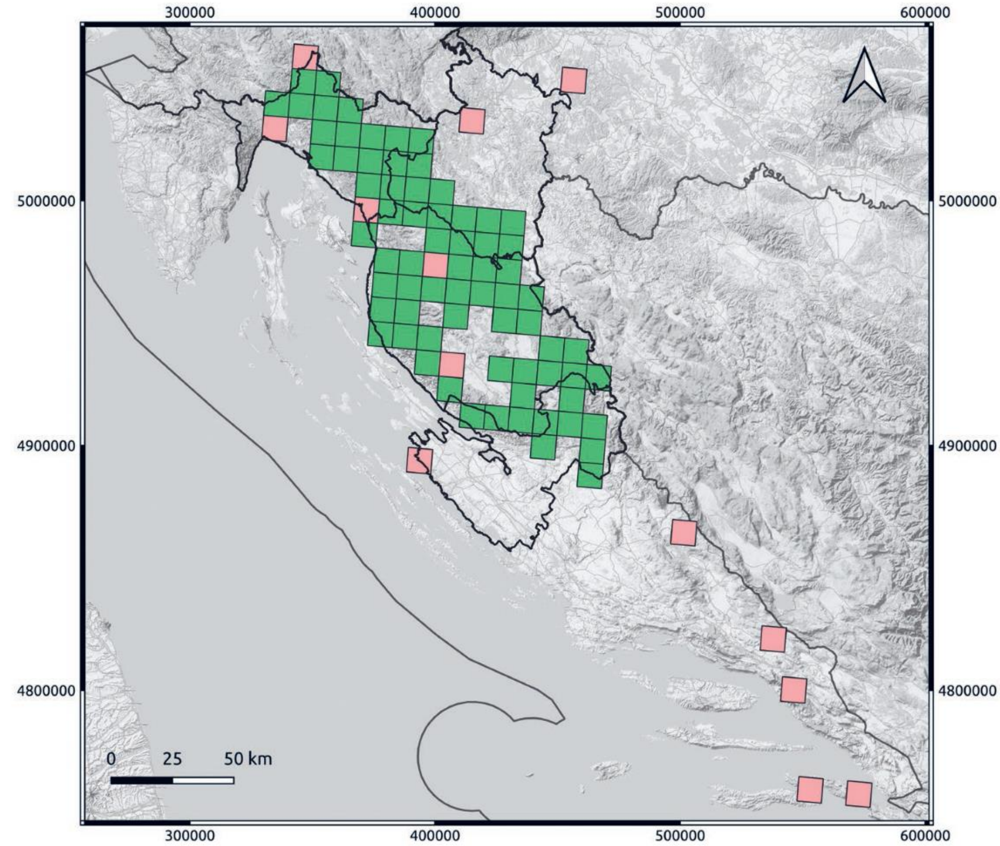

Figure 3. Lynx distribution in Croatia for the period 1st of May – 30th of April. Squares marked in green represent the area of permanent distribution, while squares colored in red represent the area of possible, unconfirmed distribution. Black lines define borders of Croatian counties – Primorsko - Goranska, Ličko – Senjska, Karlovačka and Zadar county.

Slika 3. Rasprostranjenost risa u Hrvatskoj u razdoblju 1. svibnja 2018. to 30. travnja 2020. Kvadrati označeni zeleno predstavljaju područje stalne prisutnosti, dok crveni kvadrati predstavljaju područja moguće, nepotvrđene rasprostranjenosti. Crne linije obilježavaju granice hrvatskih županija – Primorsko-goranska, Ličko-senjska, Karlovačka i Zadarska županija

search, or when found dead. Individuals were categorized as adults or kittens, when photographed with an adult (mother).

This estimation of minimum population size does not include lynxes that were released in Croatia within LIFE Lynx project. Out of seven lynxes released in Croatia and Slovenia in 2019 and 2020, two males – Alojzije and Boris, established their territories in Croatia.

RESULTS
REZULTATI

A total of 902 records of lynx presence were collected in Croatia in the period from 1st of May 2018 until 30th of April 2020 (Table 1). Out of those, 92.7% of observations were categorized as C1, 2.8% as C2 and 4.5% as C3.

Permanent lynx presence was confirmed in Primorsko – Goranska and Ličko – Senjska county, in south part of Karlovac county and north-eastern part of Zadar county on the total surface of 7100 km². Areas of occasional presence were not registered according to the used methodology, while on the surface of 1300 km² lynx signs of presence were recorded as C3 observations, i.e. those which could not be verified. Those include Pelješac peninsula, then mountains Biokovo and Dinara, which are apart from the core of the permanent distribution range in Croatia but are bordering to lynx distribution area in Bosnia and Herzegovina (Anonymous 2018).

Table 2. Number, sex and age of lynxes identified in Croatia in the period 1st of May 2018 – 30th of April 2020
Tablica 2. Broj, spol i starost risova identificiranih u Hrvatskoj u razdoblju 1. svibnja 2018. – 30. travnja 2020.

Number of identified lynx *Broj identificiranih risova*		Season *Sezona* 2018-2019	2019-2020
Adults *Odrasli*		52-62	69-82
Kittens *Mladunčad*		21	23
Sex (adults) *Spol (odrasli)*	Male *Mužjak*	14	19
	Female *Ženka*	21	24
	Unknown *Nepoznato*	27	39
Photographed body side *Fotografirana strana tijela*	Both *Obije*	39	50
	Left *Lijeva*	10	13
	Right *Desna*	13	19

The total effort of lynx recording by camera traps at 182 locations was 31710 camera-trap days or on average, camera traps were active at each location for 163.13 days. Those camera traps recorded 687 lynx events, while 117 records were obtained from other sources.

During the 2018 - 2019 season we identified 39 adult lynxes based on both sides of the body, while additional ten individuals were identified based only on the left body side and 13 based only on the right body side. We identified 21 females and 14 males, while sex could not be identified for 27 animals. If we assume that none of the animals photographed only from the right side matches the one photographed from the left side, then the maximum number of adult animals identified in the season 2018 – 2019 was 62. However, if all 13 lynxes photographed only from the right side match the animals photographed only from the left side, then the minimal number of identified lynxes was 52.

During the 2019 – 2020 season, we identified a minimum of 69 and a maximum of 82 adult animals; 50 lynxes were identified based on both sides, while additional 19 individuals were identified based only on the right and 13 more based only on the left body size. We could identify 24 females and 19 males, while for 39 individuals sex could not be determined. Out of 82 individuals identified in 2019-2020 season, 36 (43.9%, 35 adults and one kitten) of them were already known for the season 2018-2019.

We identified a total of 89 – 108 different adult animals during both seasons. Out of those, 61 were identified from both sides, 28 from the right and 19 only from the left flank. Among 108 adult individuals there were 29 females, 22 males and 57 animals of unknown sex. A total of 30 animals (27.8% out of 108) were observed only once, while three lynxes with the highest number of observations were observed 46, 21 and 20 times.

We compared the identified animals with data from Slovenia (Fležar et al. 2019), and found that seven animals were recorded both in Croatia and Slovenia.

During the two seasons, we photographed 44 kittens belonging to 25 different litters. There were two cases of females with three kittens, 15 cases of females with two, and we recorded a single kitten in eight cases. Seven offspring from the 2018-2019 season could be identified based on their coat pattern (five based on both sides, two by the right side only). Only one kitten from the first study season was recorded in the second season as an adult individual.

DISCUSSION
RASPRAVA

Scientific data on distribution and abundance are the foundation for effective population management (Breitenmoser et al. 2006). Since the reintroduction of lynx to Slovenia in 1973 lynx monitoring in Croatia was mainly limited to the mortality records (Frković 2001). Only in the early 2000s research and monitoring of various aspects of lynx biology and ecology started (Gomerčić et al. 2009; Gomerčić et al. 2010, Kusak 2012). Even though, one of the goals of Croatian lynx management plan for the period 2010 – 2015 was to establish a national monitoring system (Sindičić et al. 2010), this was achieved only in 2018 as combined effort of LIFE Lynx project implementation (Sindičić et al. 2018), lynx monitoring in protected areas (especially National park Plitvice lakes and Nature park Velebit), cooperation with numerous hunting grounds and wildlife monitoring contracts of company Geonatura Ltd. Since at the beginning of this study almost 10,000 km^2 was considered as potential lynx distribution area in Croatia (Sindičić et al. 2010), the first challenge of our research was to establish monitoring of an elusive species over such a large area. Weingarth et al. (2015) advise that when establishing monitoring in a new area, a survey should be carried out for as long as possible and then optimize the methodology for future monitoring based on the collected data. Therefore, we established our monitoring system over the entire assumed area of lynx distribution in Croatia with photo traps active throughout the year, to record as many different individuals as possible and get a basic insight into the population demography. Afterwards, based on this data, we can plan the optimal methodology for future lynx monitoring and perform more accurate estimate of population size (e.g. using the spatial capture-recapture model).

To determine the number of lynxes in Central European populations, it is recommended to use a 2.7 x 2.7 km cell grid (Zimmermann et al. 2013), while in the Slovenian part of the Dinaric population, a 3 x 3 km grid was used (Fležar et al. 2019). In this study, it was not possible to cover the entire study area with recommended density of camera traps, due to the financial limitations. Moreover, areas of Kapela and Velebit mountains were partly not accessible due to the danger of mine fields. Therefore, our results present the minimum and not the actual number of lynx individuals present in Croatia in the studied period. Until now, results of population census using camera traps were published for several Eurasian lynx populations. The largest dataset comes from Switzerland, where monitoring with camera traps started already in 1999 (Pesenti and Zimmermann 2013). Weingarth et al. (2012) used camera traps for the estimation of lynx population size in German National park Bavarian forest, Blanc et al. (2013) we considered 4 scenarios comparing low versus high detection probability and small versus large populations and contrasted abundance estimates obtained from both approaches. Standard CR and SECR models both provided minimally biased abundance estimates, but precision was improved when using SECR models. The associated confidence intervals also provided better coverage than their non-spatial counterpart. We concluded SECR models exhibit better statistical performance than standard closed CR models and allow for sound management strategies based on density maps of activity centers. To illustrate the comparison, we considered the Eurasian lynx (Lynx lynx) implemented their research on French Jura Mountains, while Gimenez et al. (2019) estimated lynx population size in French part of Jura and Voges in the period from 2011 until 2016. In these studies, camera traps were active between two and four months per year, mostly during the winter months (January – April), but area of survey were much smaller than the area covered in this study. One of the challenges of population monitoring on such a large area arises from the different terrain configuration and the differences in previously available data for certain areas. For instance, we noticed differences in the quality of results between the two geographical areas - Gorski kotar (i.e. Primorsko - Goranska county) and Lika (i.e. Ličko-Senjska county). In Gorski kotar, a significant number of marking sites were known from previous research period (Kusak 2012; Kusak and Modrić 2012; Kusak et al. 2013; Kusak et al. 2014), while in Lika (except northern Velebit and Plitvice Lakes National Park) photo-traps have never been used before to monitor lynx. As a result, in Gorski kotar (where most of camera traps are placed on marking sites) we have a higher percentage of animals identified based on both flanks, and a lower proportion of animals of unknown sex and those recorded only once. To reach this level of data reliability in Lika, it is necessary to enhance our camera trap network, what was not been possible in all locations within this study.

During the two seasons, we identified a total of 89 - 108 adult lynxes. During the second season (2019-2020) we determined that a minimum of 69 - 82 adult lynxes were present in Croatia. The fact that we conducted monitoring throughout the year and had a high percentage of animals recorded only once (27.8%), indicate that we recorded a certain number of individuals in dispersion. Probably those individuals did not establish a territory in Croatia but were recorded in their search for territory or during the mating season. Another explanation for the low rate of repeated records could be insufficient detectability of lynxes caused by low density of camera traps in some areas, then also partly by eventually high turnover of lynx individuals in Dinaric population. Although 43.5% of individuals identified in 2018 - 2019 were not photographed during the 2019 - 2020 season, we cannot claim that all of those individuals perished from the population but probably some of them were not captured due to the low density of camera traps. Also, results of long-term monitoring in certain areas (Gorski kotar, northern Velebit) suggest that some animals are re-recorded (recaptured) after more than a year of absence from photo-traps (unpublished data).

The actual lynx number is more likely to be closer to older estimation of 130 lynxes (Firšt et. al. 2005), then to later estimation of 40 – 60 individuals (Sindičić et al. 2010). This wide variation in estimates illustrates the importance of properly designed and performed monitoring system. This research presents the first published scientifically – based estimation of lynx population size in Croatia. Thus, we cannot state that there was an increase in lynx population size in Croatia when we compare this study with past results. Future important steps in lynx population monitoring are correcting the deficiencies identified in this study and implementation of methodology that will allow us to use spatial capture recapture models.

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SAŽETAK

Znanstveni podaci o rasprostranjenosti i brojnosti temelj su za učinkovito upravljanje i zaštitu ugroženih populacija. U ovom radu predstavljamo rezultate prve znanstveno utemeljene procjene veličine populacije risa u Hrvatskoj. Cilj praćenja bio je utvrditi područje rasprostranjenosti risa i procijeniti najmanju veličinu populacije risa u Hrvatskoj u razdoblju 2018. - 2020. godine. U svrhu utvrđivanja rasprostranjenosti populacije, prikupljena su 902 znaka prisutnosti risa u razdoblju od 1. svibnja 2018. do 30. travnja 2020. Od toga je 92,8% podataka kategorizirano kao C1, 2,8% kao C2 i 4,4% C3. Trajna prisutnost risa potvrđena je u Primorsko-goranskoj i Ličko-senjskoj županiji, u južnom dijelu Karlovačke županije i sjeveroistočnom dijelu Zadarske županije, na ukupnoj površini od 7200 km^2. Za procjenu minimalne veličine populacije, prikupljene su 804 fotografije s fotozamki tijekom obje sezone te je identificirano 89 do 108 odraslih životinja. Među 108 identificiranih jedinki, bilo je 29 ženki, 22 mužjaka i 57 životinja nepoznatog spola. Tijekom dvije sezone fotografirali smo 44 mladunca u 25 legla. Budući važni koraci u praćenju populacije risa su ispravljanje nedostataka utvrđenih u ovoj studiji, kako bi se omogućila procjena brojnosti korištenjem modela prostornog hvatanja i ponovnog hvatanja jedinki.

KLJUČNE RIJEČI: rasprostranjenost, *Lynx lynx*, najmanja veličina populacije, Hrvatska

4.3. PAPER III: „Early post-release behaviour of Eurasian lynx translocated to the transboundary region of the Dinaric Mountains“

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RESEARCH PAPER

Early post-release behaviour of Eurasian lynx translocated to the transboundary region of the Dinaric Mountains

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Abstract. Translocations of individuals for re-introductions and population reinforcements have been increasingly used in carnivore conservation. Movement is the first behavioural response of reintroduced animals to "forced dispersal" in a new habitat. Our study investigated space use and movement patterns of six male Eurasian lynx (*Lynx lynx*) translocated from the Carpathian to the Dinaric Mountains and released at four different sites in Croatia and Slovenia. Data were collected during their early post-release period (i.e. three months after the release) to investigate the first behavioural response following the translocation. Released lynx were monitored with GPS-GSM-VHF telemetry collars set to collect GPS locations in intervals between 4 and 24 h. All animals settled during the study period, on average 23 days (SD = 16.5) after the release. Although outside of the monitoring period that was the focus of this study, two lynx left their first territory 102 and 92 days after their release and went on a second exploratory movement. The main movement direction of the released animals was to the NW-SE, corresponding to the orientation of the predominant ridgelines of the Dinaric Mountain range. Furthermore, by comparing the use and availability of the terrain aspect, we concluded that the lynx chose to move along the mountain range and not perpendicular to the mountain, i.e. they avoided moving uphill and downhill. First kill sites of all animals were detected on average 3.4 days (SD = 1.7) after the release. This study brought valuable theoretical and practical knowledge on the early movement behaviour of translocated lynx that should be considered when planning translocations.

Key words: *Lynx lynx*, reinforcement, exploratory movement, post-release monitoring

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Introduction

Translocations of individuals for re-introductions and population reinforcement have been increasingly used in carnivore conservation (Breitenmoser et al. 1998, Cop & Frkovic 1998, Vandel et al. 2006, Rueda et al. 2021). However, such efforts can be extremely costly, risky, and fraught with political and social challenges (Breitenmoser et al. 1998, Fischer & Lindenmeyer 2000, Devineau et al. 2010). Nevertheless, several key publications that conducted extensive surveys and reviews of re-introduction efforts present important findings regarding the factors that affect success rates and offer relevant guidance for future translocation efforts (Miller et al. 1999, Fischer & Lindenmayer 2000, Linnell et al. 2009, Pérez et al. 2012, Rueda et al. 2021). Others demonstrate that little information exists about past translocation efforts, nor have these cases been well studied, while unsuccessful translocations are generally not reported or underreported (Miller et al. 1999, Linnell et al. 2009).

Movement is the first behavioural response of translocated animals to "forced dispersal" in a new habitat (Stamps & Swaisgood 2007). Thus, movement patterns in the early post-release period are critical for the survival and establishment of reintroduced animals (Preatoni et al. 2005, Berger-Tal & Saltz 2014). However, information available on the early post-release movement of translocated carnivores remains limited for most species primarily due to the lack of published data on telemetry monitoring early after the translocations (Vandel et al. 2006, Yiu et al. 2015)

The Eurasian lynx (*Lynx lynx*) is a solitary predator with a social organisation based on territoriality. The species has one of the most widespread distributions of the currently living felids (Breitenmoser et al. 2015), but in many parts of Europe, their populations are highly fragmented and at risk of extinction (Kaczensky et al. 2013). The Dinaric-SE Alpine lynx population went extinct at the beginning of the 20th century due to hunting and persecution, habitat loss and lack of prey species. It was successfully reintroduced in 1973 by translocating six animals from a Carpathian source to Slovenia. The animals then spread towards the southeast to Croatia and Bosnia and Herzegovina, as well as to Italy in the west and Austria in the north (Sindičić et al. 2013). However, at the beginning of the 21st century, genetic analysis showed that a founder effect and absence of natural migrations from any other lynx population caused low effective population size and considerable inbreeding (Sindičić et al. 2013). Therefore, the conclusion was that genetic factors, additive to threats like human–induced mortality (Sindičić et al. 2016) and prey base depletion, can quite possibly lead to another extinction of this species from the Dinaric Mountains (Sindičić et al. 2013). So today, the Eurasian lynx is regarded as the most endangered mammal in the region, with minimum population estimates of 95 adult lynx distributed along 12,500 km^2 in Slovenia and Croatia (Gomerčić et al. 2021, Fležar et al. 2022) and an unknown population status in Bosnia and Herzegovina. To save the population from extinction, urgent measures were needed to improve the genetic status and connectivity with other populations. For that reason, 11 partners from five countries came together under the LIFE Lynx project (LIFE16 NAT/SI/000634) with the primary goal of improving the genetic and demographic outlook of the Dinaric-SE Alpine population through reinforcement (Černe et al. 2019).

Data from different regions report that the Eurasian lynx mating season lasts from February to mid-April, and in late May, females usually give birth to an average of 1-4 kittens (López-Bao et al. 2019). The timing of the first dispersal of young lynx ranges from 8.1 to 10.7 months and does not differ between males and females (Zimmermann et al. 2005). After separating from their mother, young lynx stay for a few days in the maternal home range before they disperse (Zimmermann et al. 2005). In Sweden, telemetry studies showed that about one-third of Eurasian lynx female offspring remained philopatric (Samelius et al. 2011), indicating the potential for geographic clustering of female relatives. Lynx diet varies considerably among different regions (Okarma 1984, Gossow & Honsig-Erlenburg 1985, Pulliainen et al. 1995, Pedersen et al. 1999, Breitenmoser & Breitenmoser-Würsten 2008), while the main prey of Dinaric lynx is roe deer (*Capreolus capreolus*) (Krofel et al. 2011).

Our study investigated space use and exploratory movement patterns of six Eurasian lynx subjected to "forced dispersal" as part of a reinforcement process (Černe et al. 2019). Data used in this study were acquired during the first three months post-release to provide an in-depth analysis of the early behaviour of translocated lynx. No previous research investigated the early behavioural response of Eurasian lynx individuals engaged in a population reinforcement process, so we decided to limit the data to only three months post-release to understand this critical period thoroughly. Directions of movements and straight-line distances were calculated and compared over time, as well as total distance (TD) from the release sites and time to the initial settlement, defined as a polygonal movement with the decrease and stabilisation in distance from the release site. We predicted that the calculation of the TD would show a clear difference between the exploratory movement TD and home-ranging TD in translocated animals. The movement patterns of translocated animals and their temporal dynamics should indicate the establishment process of individual animals (Berger-Tal & Saltz 2014). We expect an initial increase in distances from the release site during exploration and stabilisation or reduction when the animal has settled. Lastly, we compared the orientation of lynx movement in relationship to terrain aspect, slope and elevation as we expected the lynx to use ridges for their movement in mountainous landscapes (Zimmermann et al. 2007).

Study Area

The study area includes four release sites within the lynx distribution area (Gomerčić et al. 2021, Fležar et al. 2022) in the Dinaric Mountains in Croatia and Slovenia (Fig. 1): 1) Kočevsko-Belokranjska region (Slovenia): hunting ground "Loški Potok"; 2) Notranjska region (Slovenia): hunting ground "Jelen", Snežnik; 3) Primorsko-Goranska county (Croatia): National Park "Risnjak"; and 4) Zadarska and Ličko-Senjska county (Croatia): National Park "Paklenica".

Release sites were selected following the protocols of the LIFE Lynx project (LIFE16/NAT/SI/000634) that took into account the presence of other lynx individuals (investigated using camera traps) (Slijepčević et al. 2019), local stakeholder acceptance (Majić-Skrbinšek et al. 2020) and human-induced mortality risks. As the literature review showed no obvious advantages for soft or hard release (Wilson 2018), based on the technical and financial capacities of project partners and hunters' active involvement, a soft release was implemented in Slovenia and a hard release in Croatia.

Predominant habitats in Croatia and Slovenia are rugged karst terrains with mixed forests of European beech and mixed oak forests that dominate at medium and low altitudes in a deep soil and humid slopes, valleys, and canyons. The dominant canopy tree species of the mountain conifer forests are spruce (*Picea abies*), silver fir (*Abies alba*), and black pine (*Pinus nigra*). The Dinaric karst region of Croatia and Slovenia is part of the Dinaric Mountain range, which belongs to the Alpine-Himalayan mountain belt. It spans in NW-SE direction from Slovenia through Croatia and Bosnia and Herzegovina up to Montenegro and northern Albania with altitudes ranging from sea level to 2,600 m a.s.l. (Ozimec et al. 2012). Dominant species of wild ungulates in the study area are roe deer, red deer (*Cervus elaphus*), wild boar (*Sus scrofa*) and chamois (*Rupicapra rupicapra*). There are also a few small and isolated introduced populations of mouflon (*Ovis ammon*) and fallow deer (*Dama dama*). Besides the Eurasian lynx, brown bear (*Ursus arctos*), grey wolf (*Canis lupus*), and golden jackal (*Canis aureus*) are present in the area, as well as several species of smaller carnivores (Ozimec et al. 2014). Climatically, an average annual rainfall of 1,500-2,000 mm characterises this ecoregion with an annual temperature averaging 5-8 °C, ranging from a maximum of 32 °C in July to a minimum of –20 °C in January (Ozimec et al. 2014).

The release sites are all characterised by the *Omphalodo-Fagetum* forests with an additional Mediterranean habitat in National Park Paklenica (Southern Velebit Mt.). The seacoast limits animal movement around the Paklenica canyon from the SW side and agricultural fields and open space from the NE side. The southern side of the mountain Velebit is covered chiefly with dense shrub species typical of Mediterranean scrub. In contrast, the northern side is covered with mixed fir and beech forest (Ozimec et al. 2014).

Material and Methods

Seven lynx were released during 2019 and 2020, but we used telemetry data from six male Carpathian lynx, captured in Romania and Slovakia and released in the Dinarics for reinforcement. The telemetry collar from the seventh lynx, named Pino, stopped working immediately after the release in Croatia, so there were no available data, and Pino's fate is unknown. The six monitored lynx were named Goru, Doru, Alojzije, Catalin, Boris and Maks (Table 1). Five of them (all except Maks) were captured in box traps during the winter (January-March) in 2019 and 2020. They were kept in quarantine enclosures in the country of capture for at least three weeks before transportation to Croatia and Slovenia. Age estimation was done following Marti & Ryser-Degiorgis's (2018) classification while handling animals at the capture sites (Table 1). Lynx Maks was not captured but was found in poor health in the Poľana Protected Landscape Area in Slovakia due to a forelimb fracture. Besides leg injury and malnutrition, the animal was also infested with numerous parasites.

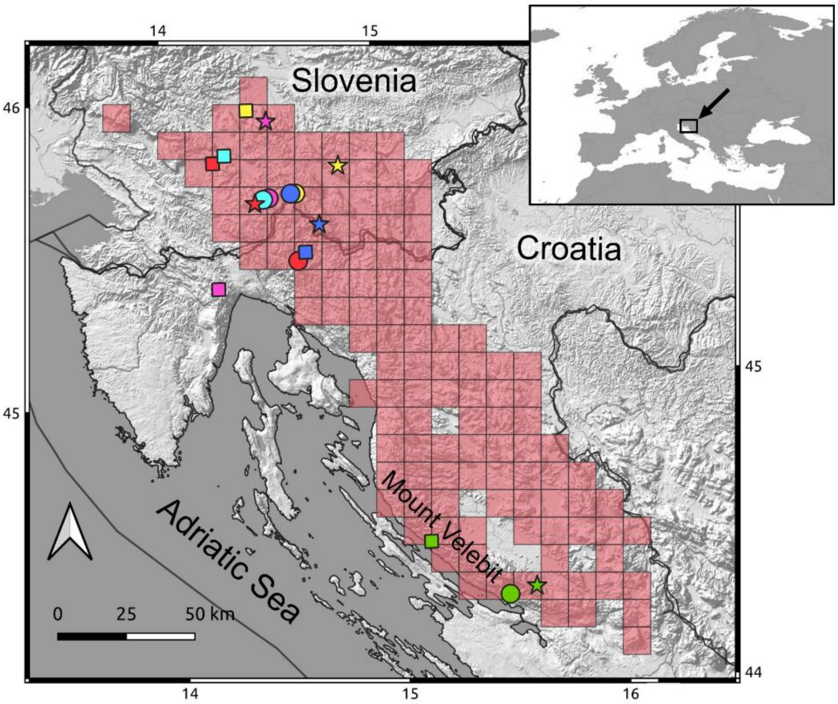

Fig. 1. Red 10 × 10 km quadrants indicate lynx distribution in Slovenia and Croatia. Each colour represents a different lynx individual: Doru (red), Goru (yellow), Alojzije (green), Catalin (rose), Boris (blue) and Maks (cyan). Release sites are presented with circles: hunting ground "Loški Potok" (blue and yellow), hunting ground "Jelen", Snežnik (cyan and rose), National Park "Risnjak" (red) and National Park "Paklenica" (green). Squares represent the farthest location reached from the release site by each lynx. Stars represent the location after which the movement pattern changed, indicating settlement.

Maks was transported and rehabilitated in the National ZOO Bojnice for nine months, after which he fully recovered. The team decided to translocate him to the Dinarics with other animals captured for repopulation within the LIFE Lynx project.

Two lynx (Doru and Alojzije) were hard released in Croatia at two release sites, and the other four were soft released in Slovenia at two release sites (Fig. 1). Due to technical and financial capacities, the hard release was used in Croatia, while in Slovenia animals were kept in an enclosure at the release site for at least two weeks after the transport from the country of origin and before the soft-release. Before the release, animals were equipped with the GPS-GSM-VHF telemetry collars (Vertex Lite, Vectronic Aerospace GmbH). Collars had different GPS schedules, set to collect GPS locations in intervals between 4 and 24 h.

We defined the early post-release period as three months (91 days) after the release. To analyse the lynx's first behavioural response, we assessed the movement patterns of released animals and their temporal dynamics. We defined straight-line movement and increased distance from the release sites as exploratory movement, while polygonal movement with decreased and stabilised distance indicated a settlement process (Bunnefeld et al. 2011).

The following variables were calculated using QGIS (Geographic information system, Open source geospatial foundation project 2020) (Table 1): 1) time (days) from the release site to initial settlement; 2) straight-line distance (SLD) from the release site to initial settlement; 3) total distance (TD) moved from the release site to initial settlement; 4) average daily distance (DD) (km/days); 5) maximum daily distance (km); 6) maximum aerial distance from release site (km); 7) min-max altitude (m a.s.l.); 8) direction of lynx movement; 9) terrain elevation, slope, forest cover and aspect; 10) time (days) until first kill detection; 11) the average number of kill sites during exploration movement; 12) the average number of kill sites during settlement.

Table 1. Data on movement and predation for six translocated male Eurasian lynx during 91 days after their releases. SLD – straight-line distance from the release site in kilometres, TD – total distance from the release site to initial settlement in kilometres, DD – daily distance.

Lynx	Estim. age (years)	Release site	Release date	Days until initial settlement	SLD (km)	TD (km)	DD (km/days)	Max. daily distance (km)	Max. aerial distance from release site (km)	Min-max altitude (m a.s.l.)	Days until first kill	Total number of kill sites during exploration	Average days between kill sites during exploration	Average days between kill sites during settlement
Doru	4	Risnjak NP, CRO	4.5.2019	43	18	205	2.4	10.8	50.4	536-1,357	7	6	10.75	10.66
Goru	5	Loški Potok, SLO	14.5.2019	17	15	256	2.8	17.1	35.2	347-964	1	2	8.5	8.2
Alojzije	4	Paklenica NP, CRO	13.3.2020	42	8	255	2.7	9.8	35.1	476-1,637	3	2	7	6.75
Catalin	5	Snežnik, SLO	31.3.2020	20	24	358	4.2	14.4	34.2	340-1,434	6	4	10	8.87
Boris	1-2	Loški Potok, SLO	28.5.2020	15	14	97	1.1	7.8	20.6	316-1,160	10	2	7.5	5.2
Maks	2	Snežnik, SLO	23.6.2020	1	N/A*	N/A*	1.7	9.2	19.8	542-1,165	2	N/A	N/A*	6.9

*Lynx Maks did not show exploratory movement after release.

Straight-line distance (SLD) from the release site to initial settlement represents the aerial distance between the location of the release site and the location closest to the release site, after which the animal showed polygonal movement that indicated settlement.

For calculating the distance between daily locations, we chose 91 locations for each lynx taken on consecutive days at 24 h intervals. We chose 24 h intervals because, on some collars, that was the minimum scheduled interval. There were cases when the collar failed to fix the location, so we interpolated to 24 h intervals.

The total distance (TD) measures the sum of aerial distances between the starting location and the following location for the movement path of a given individual. Average daily distance (DD) measures the sum of aerial distances between two consecutive locations divided by the number of days in which this distance was attained. In comparison, the maximum daily distance represents the highest distance between two consecutive locations. The maximum aerial distance from the release site (km) presents the farthest overall location from the release site reached by each lynx.

The direction of movements was defined by calculating the bearing between two consecutive locations. Next, terrain characteristics were calculated in QGIS using a 5 km buffer around the animal's GPS locations (chosen as an assumed maximal distance an animal can perceive from a fixed point) (Boitani & Fuller 2000). We then took 5,000 random points inside the buffer for which we calculated aspect, slope angle and elevation from a dem30 map, in 1 × 1 km resolution (Gesch et al. 1999).

We compared (a) the aspect frequency of the actual terrain used by the six lynx (5,000 random points inside a 5 km buffer around all lynx locations) with the aspect frequency of a universal terrain. Universal terrain is a hypothetical terrain with the same aspect frequency in all compass directions from 0° to 360°. Next, to compare between the use and availability of the terrain; (b) we overlapped the frequency of movement direction of the lynx with an aspect frequency of the actual terrain. The goal was to define whether the terrain aspect influences lynx exploratory movement. We used R package Overlap (Ridout & Linkie 2009) to calculate the coefficient of overlapping (Table 2). The smaller the coefficient (a) is, the more the actual terrain differs from universal terrain, i.e. elongates in a specific direction. The smaller the coefficient (b) is, the more lynx movement follows the mountain direction, while a higher coefficient indicates that the lynx movement is more perpendicular to the mountain. To test this presumption, we also (c) overlapped the frequency of lynx movement direction with an aspect frequency of universal terrain and compared the coefficients ((a) *vs.* (c)). If (a) is smaller than (c), it confirms that lynx movement follows the mountain direction and *vice versa*. For calculating the coefficient of overlapping, we used Dhat 1 estimator because we had less than 50 locations (movement directions) for each lynx. We standardised the variables aspect and converted the degrees of the terrain aspect and lynx movement direction (0-360°) to a normalised 0-1 range.

Kill sites were detected using three GPS collar locations in a period longer than 30 h within 300 m, following the GPS location cluster analysis described by Krofel et al. (2013). Forest cover analyses were done using the CORINE Land Cover inventory for 2018 (Büttner et al. 2021). All shapefiles were projected as the HTRS96/UTM zone 33N coordinate system.

Results

Six male lynx were monitored with GPS-GSM-VHF telemetry collars throughout the early post-release period (i.e. three months after the release). All animals settled during this period, on average 23 days (SD = 16.5) after the release.

Doru

Lynx Doru (Fig. 2) was hard-released at 707 m a.s.l., surrounded by mountain ridgelines above 1,000 m a.s.l. The first day after the release, Doru moved 3.8 km and climbed to 1,180 m a.s.l. For the next two weeks, he stayed within 6.2 km from the release site keeping his average altitude at 1,074 m a.s.l. He started distancing himself gradually on the 17th day, with the peak on the 60th day when he was 50 km away from the release site and was stopped by the A1 Ljubljana-Koper highway in Slovenia. From there, he started showing settlement indicating polygonal movement (Fig. 1). Average altitude during his exploratory movement was 1,021 m a.s.l. and 796 m a.s.l. during settlement indicating movement. His average speed showed no difference in exploratory movement (N = 2.2 km/daily) *vs.* moving in his home range (N = 2.5 km/daily) in the monitored period. His first kill site was detected seven days after the release while the average time between kill sites during the exploration phase was 10.75 days.

Goru

Lynx Goru (Fig. 3) was released after an 18-day stay at the soft-release enclosure at 894 m a.s.l. The first day after the release, Goru made 4.8 km, crossed the border to Croatia and stayed in that range at 916 m a.s.l. for three days, after which he returned to Slovenia. He reached the farthest point from the release site eight days post-release and then, after reaching the lowland agricultural landscape around Ljubljana, he turned East. His average altitude during exploratory movement was 712 m a.s.l. and 672 m a.s.l. during settlement indicating movement. His average speed was 5.9 km/daily and dropped to an average of 2.1 km/daily after he started showing polygonal movement. His first kill site was detected already the first day after the release while the average time between kill sites during the exploration phase was 8.5 days.

Alojzije

Alojzije (Fig. 4) was hard-released inside the Paklenica canyon at 624 m a.s.l. For the first three days, he stayed less than 400 m from the release site and for two weeks within 6 km from the release site with an average altitude of 807 m a.s.l. and a maximum of 1,308 m a.s.l. He reached the maximum distance from the release site on the 33rd day when he was 35 km away from the release site. His average altitude during exploratory movement was 882 m a.s.l. and 792 m a.s.l. during the settlement indicating movement. His average speed was 2.2 km/daily while in exploratory movement *vs.* 3.3 km/daily after he started showing polygonal movement. His first kill site was detected three days after the release, while the average time between kill sites during the exploration phase was seven days.

Catalin

Lynx Catalin (Fig. 5) was released after a 24-days stay at the soft-release enclosure at 979 m a.s.l. Catalin started to move away from the release site from the first day post-release and climbed to 1,434 m a.s.l. In the next few days, he continued his exploratory movement and distanced for 15.2 km from the release site, crossing the border to Croatia. After two weeks, he returned to Slovenia and was 32 km away from the release site when he travelled 14 km in one day, which was his maximum daily distance in the monitoring period. He reached his maximum distance from the release site on the 66th day when he was already showing polygonal movement. His average altitude during the exploratory movement was 782 m a.s.l. and 709 m a.s.l. during the settlement indicating movement. While in exploratory movement, Catalin's average speed was 9.1 km/daily, which dropped to an average of 2.8 km/daily after he started showing polygonal movement. His first kill site was detected six days after the release, while the average time between kill sites during the exploration phase was ten days.

Boris

Lynx Boris (Fig. 6) was released after a 28-day stay at the soft-release enclosure at 894 m a.s.l. The next day, he climbed to 1,097 m a.s.l. and stayed under 3 km distance for the first week after the release and started moving farther on the 10th day when he travelled 6 km in a day and soon afterwards crossed the border to Croatia. He reached the farthest point from the release site on the 50th day of the monitoring period. His average altitude while engaged in exploratory movement was 941 m a.s.l. and 571 m a.s.l. His

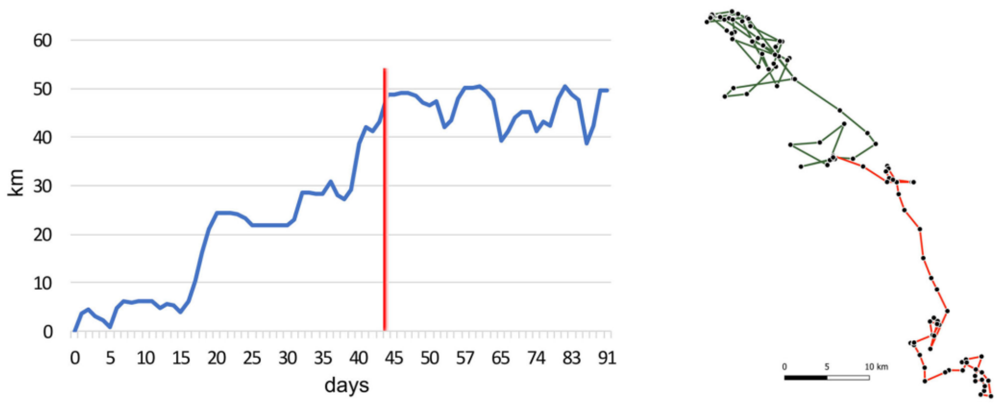

Fig. 2. Left: movement (kilometres over time) for male lynx Doru with mixed movement pattern line – indicating exploratory movement pattern and after the red line the start of settlement pattern. Right: Doru's exploratory movement (red) and settlement indicating movement (green).

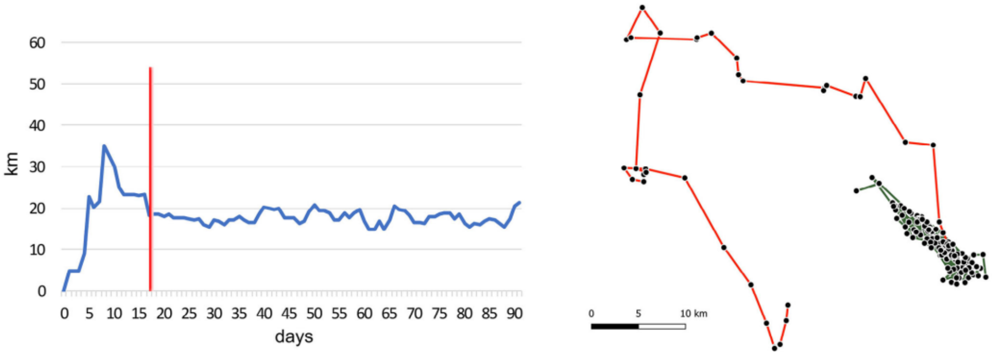

Fig. 3. Left: movement (kilometres over time) for male lynx Goru with mixed movement pattern line – indicating exploratory movement pattern and after the red line the start of settlement pattern. Right: Goru's exploratory movement (red) and settlement indicating movement (green).

average speed was 1.8 km/daily during exploratory movement and 1.1 km/daily after showing polygonal movement. His first kill site was detected ten days after the release, while the average time between kill sites during the exploration phase was 7.5 days.

Maks

Lynx Maks (Fig. 7) was released after a 20-day stay at the soft-release enclosure in the area of Snežnik, Slovenia (979 m a.s.l.). The first three days after the release, he stayed within 2 km of the release site and climbed to 1,153 m a.s.l. On the third day, he started distancing gradually and 18 days post-release he reached 19.8 km from the release site when the A1 Ljubljana-Koper highway stopped him. His average altitude was 774 m a.s.l. His average speed was 1.7 km/day. Maks showed only polygonal movement between the release site and the highway inside the monitoring period (91 days), so we have not registered clear exploratory movement behaviour. His first kill site was detected two days after the release and the average time between kill sites was 6.9 days.

First kill sites of all animals were detected in the first ten days (SD = 1.7), on average, 3.4 days after the release. The average number of days between the kill sites for all lynx during exploration was 8.75 days, while after settlement, the average was 7.76 days between kill sites (Table 2). The main exploratory movement direction of the released animals was NW-SE, corresponding to the orientation of the predominant ridgelines of the Dinaric Mountain range (Fig. 8.)

Forest cover was primarily uniform, and five out of six lynx moved on a terrain with a high percentage of forest cover, while Alojzije used terrain covered with forests and shrublands, typical habitat for the southern part of mountain Velebit, where he was released and established a territory (Table 2).

When we compared the coefficients of the overlap between the direction of movement of the lynx and the aspect of the natural and universal terrain for all released animals, the coefficient of overlap was smaller in the actual terrain than in the universal terrain (Table

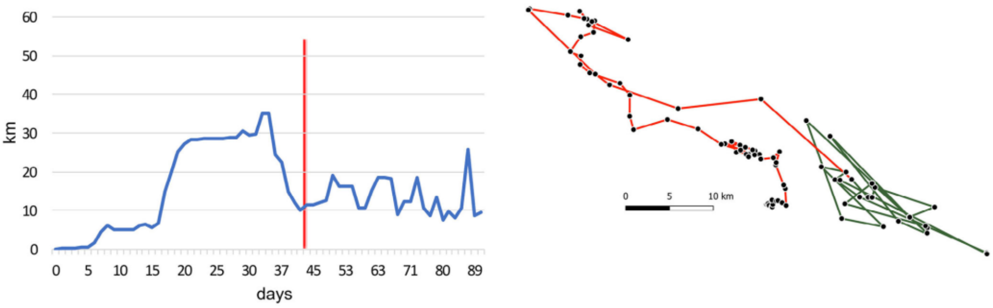

Fig. 4. Left: movement (kilometres over time) for male lynx Alojzije with mixed movement pattern line – indicating exploratory movement pattern and after the red line the start of settlement pattern. Right: Alojzije's movement during exploratory movement (red) and settlement indicating movement (green).

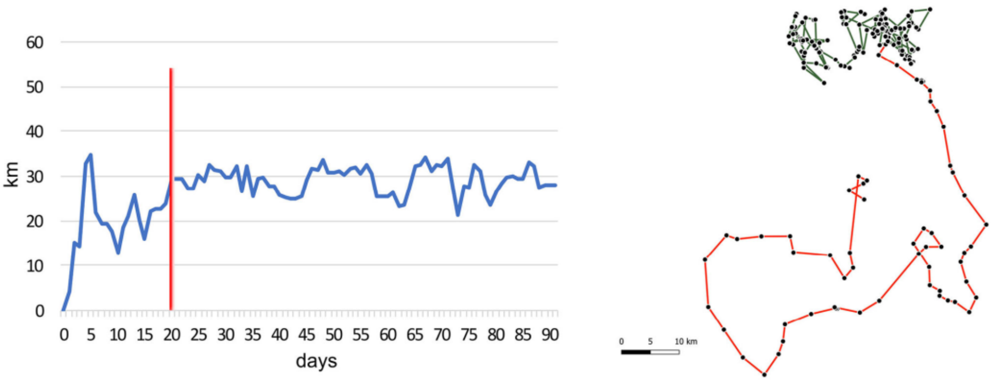

Fig. 5. Left: movement (kilometres over time) for male lynx Catalin with mixed movement pattern line – indicating exploratory movement pattern and after the red line the start of settlement pattern. Right: Catalin's movement during exploratory movement (red) and settlement indicating movement (green).

2). This finding indicates that all lynx movement directions matched the actual terrain's directions. The largest difference between the coefficient of the natural and universal terrain was recorded in the case of lynx Alojzije, which could be explained by the fact that the terrain he used for exploratory movement was the steepest (Table 2). Fig. 9 compares the frequency of Alojzije's movement directions with frequencies of the aspect of the actual surrounding terrain. The observed low overlap indicates that Alojzije avoids going up or down the slopes (Fig. 9).

Discussion

Information on Eurasian lynx exploratory movements after translocation is rare in the scientific literature (Vandel et al. 2006) and found only in the grey literature, such as project reports in the respective countries' native languages (Ryser et al. 2004). Once released into the wild, animals can choose to stay near the release site or to move away from it (Berger-Tal & Saltz 2014). Homing behaviour is typical in re-introductions, when animals tend to travel towards the direction of their capture sites after release (Rogers 1988). Such behaviour has been interpreted as a rejection of the forced dispersal and typically results in low site fidelity, i.e. animals are unwilling to settle in the new area (Miller et al. 1999).

Personality may also play an important role in the movement response (Spiegel et al. 2017, Rueda et al. 2021), as well as the survival of reintroduced individuals (Bremner-Harrison et al. 2004). Five out of six lynx from our study showed exploratory movement behaviour after the release. The six lynx established their territories after an average of 23 days (SD = 16.5) post-release. They settled on average 15.8 km (SD = 5.8) from the release sites,

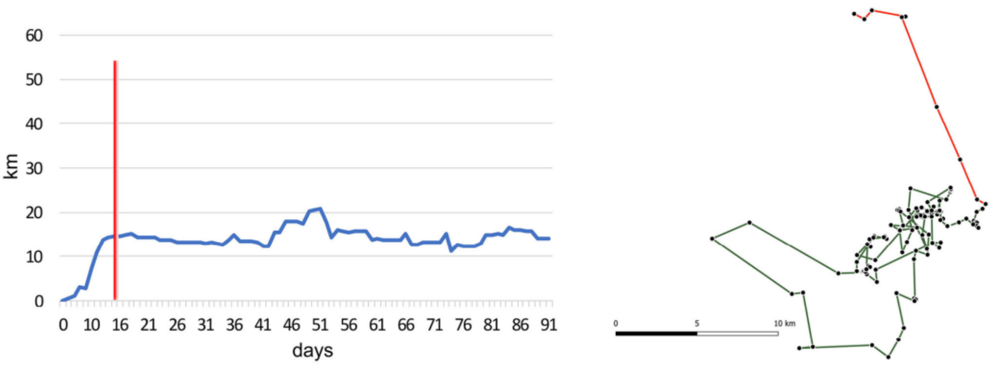

Fig. 6. Left: movement (kilometres over time) for male lynx Boris with mixed movement pattern line – indicating exploratory movement pattern and after the red line the start of settlement pattern. Right: Boris's movement during exploratory movement (red) and settlement indicating movement (green).

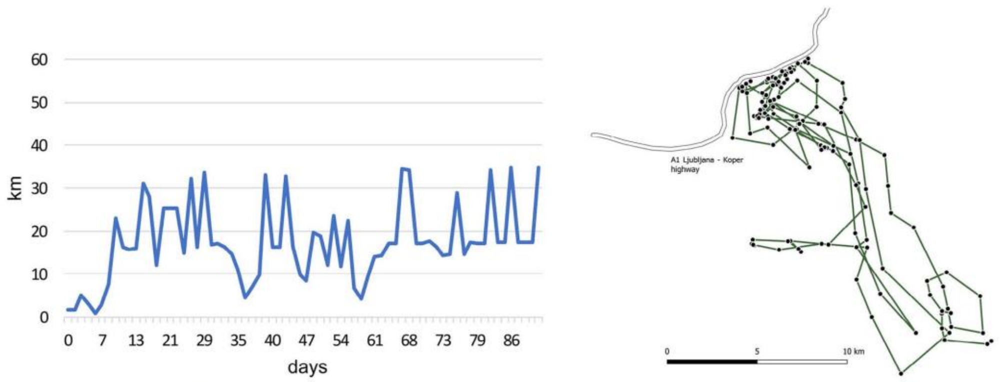

Fig. 7. Left: movement (kilometres over time) for male lynx Maks with settlement indicating movement pattern line. Right: Maks's movement after release showing polygonal movement.

which corresponds to the results from the Swiss project report on the exploration movement of lynx translocation in north-eastern Switzerland (Ryser et al. 2004). First kill sites of all animals were detected on average 3.4 days after the release. The average time between kill sites after settlement was 7.76 days (SD = 1.91) which corresponds to the kill rate of resident Dinaric lynx (Krofel et al. 2013). Unfortunately, data on lynx prey densities are unavailable for the entire study area. However, our results concerning the kill rates of released lynx did not indicate the lack of prey availability around the release areas. Although it is outside of the period which is the focus of this study, we emphasise that Boris and Maks left their first

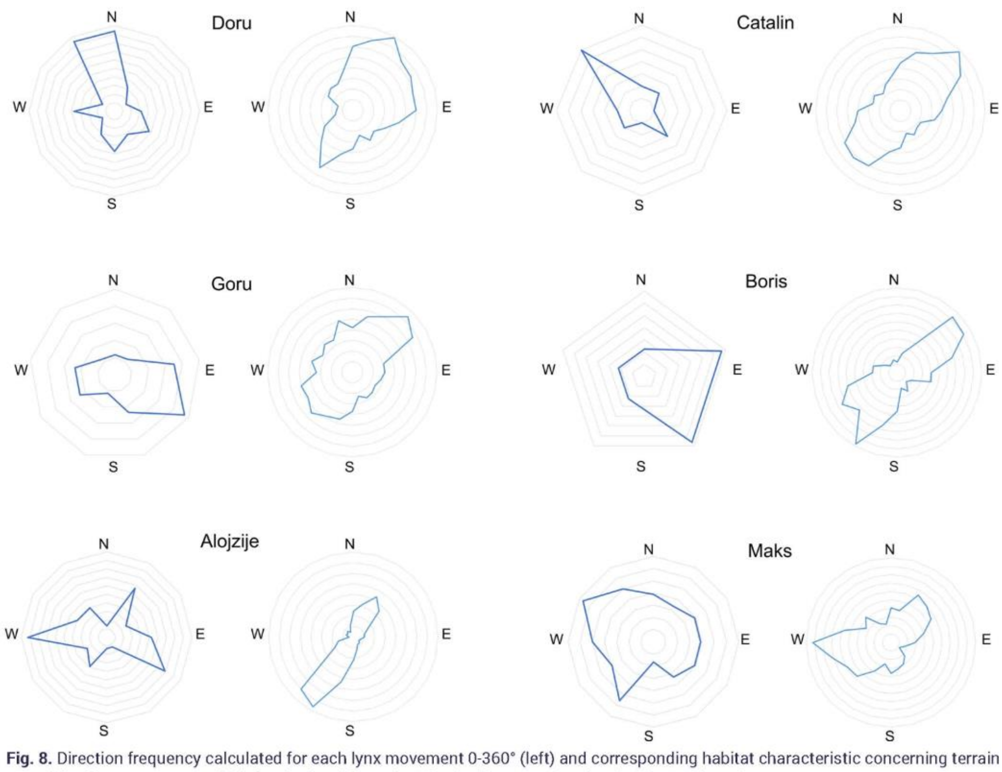

Fig. 8. Direction frequency calculated for each lynx movement 0-360° (left) and corresponding habitat characteristic concerning terrain exposition frequency 0-360° (right) calculated from the 5 km buffer around each animal's GPS locations.

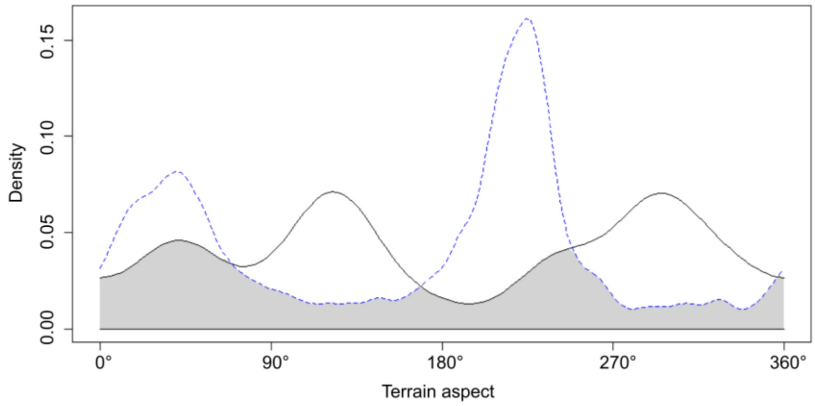

Fig. 9. Graph showing the overlap of lynx movement direction with the aspect of surrounding terrain in the case of lynx Alojzije. Blue intermittent line – terrain aspect; solid black line – lynx movement aspect. The peaks on the intermittent line in the area of 40° (NE) and 200° (SW) show that the slopes are oriented in these directions, i.e. that the mountains spread in the opposite direction of 130° (SE) and 280° (NW). The peaks on the solid line in the area 110° (SE) and 290° (NW) show the most common directions of lynx movement. The grey area of the overlap presents the value of the overlapping coefficient.

territory 102 days (Boris) and 92 days (Maks) after the release and started a second exploratory movement. Boris settled approximately two months later in Croatia, 64 km of straight-line distance from his first territory, while Maks moved 18 km north and stayed again close to the highway for two months before he managed to cross it (Krofel et al. 2021).

Before he was soft-released, Maks spent nine months in the rehabilitation centre in Slovakia and 20 days in the enclosure at the release site in Slovenia. Unfortunately, our sample size was too low to statistically analyse the influence of hard *vs.* soft release (and time spent in the enclosure) on the exploratory movement of the released animals. Also, it is hard to hypothesise whether his behaviour was affected by the rehabilitation process, his young age or the A1 Ljubljana-Koper highway that limited his exploration. However, this individual and others released confirmed that the A1 Ljubljana-Koper highway presents a significant anthropogenic barrier (Krofel et al. 2006, Skrbinšek & Krofel 2008) for lynx movement from the Dinaric Mountains towards the Alps. Zimmermann et al. (2007) study showed that

Table 2. Terrain characteristics and coefficients of overlapping for six reintroduced lynx.

	Elevation average (min-max)	Slope	% under forest	Dhat1 (b)	Dhat1 (a)	Dhat1 (c)
Alojzije	811 (1-1,653)	8.6	52.5	0.64	0.54	0.82
Boris	830 (309-1,207)	6.7	96.4	0.73	0.58	0.65
Catalin	791 (301-1,707)	4.9	95.5	0.88	0.69	0.77
Doru	967 (328-1,628)	5.0	94.3	0.84	0.81	0.83
Goru	626 (268-1,180)	4.2	96.0	0.82	0.65	0.75
Maks	745 (426-1,241)	4.0	99.7	0.82	0.81	0.9

a – coefficient of overlapping between aspect frequency of real terrain with aspect frequency of universal terrain. b – coefficient of overlapping between frequency of movement direction of lynx with an aspect frequency of real terrain. c – coefficient of overlapping between frequency of movement direction of lynx with an aspect frequency of universal terrain.

subadult lynx have a low capability to move through unfavourable habitats and cross linear barriers such as fenced highways. Lynx Maks reached the highway 18 days post-release and was forced to stay on the terrain south of the highway with several unsuccessful attempts of crossing, which likely influenced his settlement behaviour. He crossed the highway four months after reaching this barrier for the first time and soon after undertook a second exploratory movement (Krofel et al. 2021). The other two translocated lynx that reached this highway (Doru and Catalin) never managed to cross it. Such poor connectivity presents a threat to populations already fighting with inbreeding. Creation and maintenance of functional linkages between remnant populations should become a conservation priority, and resources must be dedicated to enhancing habitat networks that allow natural gene flow among populations. Given the results presented in this study, we urge responsible institutions to prioritise the construction of wildlife passes suitable for lynx on the A1 Ljubljana-Koper highway, specifically at the section identified as an essential wildlife corridor in this study (highway section Unec-Postojna).

Besides the barriers, landscape heterogeneity significantly shaped exploratory movement directions and provided an efficient tool for understanding the decision-making of the animals. Our results showed a preference for NW-SE exploratory movement direction, corresponding to the orientation of the predominant ridgelines of the Dinaric Mountain range. Zimmermann et al. (2007) showed the same exploratory movement pattern in the Jura Mountains that run SW-NE, which appeared to shape the exploratory movements of subadult lynx. On the contrary, in the north-western Swiss Alps, where parallel ridgelines are not present, movement directions were oriented randomly (Zimmermann et al. 2007). Furthermore, when we compared the coefficients of the overlap between the direction of movement of the lynx and the aspect of the universal and actual terrain, in the case of all released animals, the coefficient of overlap was smaller in the actual terrain than in the universal terrain. From this, we concluded that the lynx chose to move along the mountain range and not perpendicular to the mountain, i.e. they avoided moving uphill and downhill. The largest difference between the actual and universal terrain coefficient was recorded in the case of lynx Alojzije, which could be explained by the fact that the terrain he was using for exploratory movement was the steepest. We are aware that the orientation of the mountain range consequently affects the orientation of the roads, water streams and saddles which can also affect space use and lynx movement direction; however, the lack of such data impaired us from using parameters other than terrain characteristics presented in our study. Nevertheless, we encourage researchers to include more parameters in future studies to describe and quantify local relief.

Compared to other studies (e.g. Nathan 2008, Bunnefeld et al. 2011), our results showed apparent changes in lynx movement behaviour, which we used as an indication of the settlement process of individual animals. Once the animal's daily distance from the release site stabilised and the animal started moving in a polygonal pattern, the graphic presentation of TD showed a clear difference in exploratory movement TD and home range TD. The observed difference showed that all six lynx had established temporary home ranges. However, two of them later abandoned them and moved further (likely due to the presence of another territorial male known from a camera trapping survey (Krofel et al. 2021). Berger-Tal & Saltz (2014) explained the same pattern in theory, where they presented how the knowledge gained in unfamiliar novel environments should be followed by a subsequent change in an animal's movement behaviour, making movement behaviour an excellent indicator of the establishment process.

In-depth analysis of the early movement behaviour of translocated lynx enabled us to describe the average time until territory establishment, their movement and feeding patterns and helped us identify potential habitat connectivity problems. One of the biggest challenges when planning re-introduction is to ensure that released animals will settle within the desired area. Among our translocated animals, the maximum aerial distance reached from the release site was 50 km. At the same time, the Swiss results showed that an individual could move up to 60 km in the early post-release period (Ryser et al. 2004). Therefore, we advise that release locations are at least 60 km away from the borders of the targeted settlement area, such as national borders or protected areas, i.e. where monitoring of released individuals will not be compromised. Also, finding a balance between the quality and duration of telemetry monitoring is challenging. More GPS locations will provide better insight into animal movement, monitoring of their predation, and enable potential reactions if problems arise. But more locations will decrease battery life duration and will jeopardise possible termination of telemetry monitoring. Our finding that released lynx established their territory on average 26 days post-release can help plan the optimal collar schedule. We suggest that for the first 30-45 days after the release, collars are programmed to establish more frequent locations to monitor exploratory movement and fewer locations to enable prolonged monitoring and reduce risks connected to recapturing the animal to change the collar. We recognise the limitations of our study regarding the relatively small all-male sample size, but this study has already highlighted valuable theoretical and practical results. As the Dinaric lynx population reinforcement within the LIFE Lynx project will proceed until the year 2023, further analyses are planned based on a larger and more diverse sample size regarding sex and age structure, as well as a more extended monitoring period.

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Author Contributions

I. Topličanec and T. Gomerčić conceived and designed the study. All authors participated in fieldwork and data collection while I. Topličanec and T. Gomerčić analysed the data. I. Topličanec and M. Sindičić led the writing, while M. Krofel, R. Černe and S. Blašković improved the manuscript with their valuable comments.

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5. DISCUSSION

Scientific data plays a crucial role in wildlife population management as it provides critical information that affects decision-making, policy formulation, and allocation of resources. It also allows for comparison between different study areas, robust monitoring of population size, distribution, and trends over time. These informations are essential for understanding the current status of a population and identifying changes that may require intervention (RUNGE et al 2011). For instance, wildlife protection laws and conservation actions rely on accurate data to develop strategies such as habitat restoration, captive breeding programs, or reintroduction and repopulaton efforts whose aim is to improve the viability of endangered or declining populations (ARMSTRONG et al. 2008).

For two decades now, camera trapping has been extensively used to detect the presence of animals in defined areas. This method is considered as the most effective and cost-efficient as it is non-invasive, causes minimal disturbance for wildlife, allows intensive and prolonged data collection over large and remote areas, and provides objective records of an animal's presence. Therefore, it is of particular interest in research of rare and elusive species such as the lynx whose consistent coat patterns enable the identification of individuals (Paper I and II). Nevertheless, when employing photographic surveys such as camera trapping for estimating population abundance, it is important to accurately and consistently identify individuals in images to prevent estimation biases (JOHANSSON et al. 2020). This is why the optimisation of methods for lynx identification and classification of coat patterns (Paper I) included a strict protocol of two or sometimes three researchers working independantly to minimize possible errors. Furthermore, the quantification method of phenotypic traits developed in Paper I allowed for objective description of individuals and their morphology, reducing bias caused by human subjectivity, skill and experience. Using ImageJ program, we were able to measure the patterns and quantify four different lynx coat traits. Coats of small spots were easily distinguished from coats of big spots, which differed significantly in spot size and density (Figures 3 and 5 – Paper I), although these two patterns proved as the most difficult for researchers involved in this study to distinguish by eye. Our findings in the quantitative analysis of coat patterns subsequently supported the visual assignment of four types of coat patterns in the temporal analysis of the frequencies of coat patterns in Eurasian lynx population from Croatia. The overall frequencies of the four coat patterns in our Croatian lynx population showed the trend big spots > rosettes > small spots > no spots, and this trend was the same when we examined data from 1978-1999 period. However, the absolute frequencies of some patterns did change substantially between 1978-1999 and 2000-2019. Coats without spots vanished completely from the population, and coats with rosettes became much less frequent. This recent data on the lynx with rosettes, which account for fewer than 9% of Croatian lynxes, also showed that they are confined to the area south of the Zagreb - Rijeka highway which implicates the detection of important border for wildlife (Figure 6 - Paper II and Figure 7 – Paper III). Intensive camera-trapping in 2018-2020 failed to detect any lynxes with rosettes in Croatia in area northwest of the Zagreb - Rijeka highway (Paper I and II). Significant inbreeding and low population size were genetically proven in Croatian lynx population (SINDIČIĆ et al. 2013), but to be sure that inbreeding plays the key role in the phenotypic change we observed, further genetic analyses should be conducted. Unfortunately, coat patterns of the six animals that founded the Dinaric lynx population in 1973 are unknown, but now we will have new opportunity to observe the changes in frequency of coat patterns after new individuals entered the closed population. To save the Dinaric population from extinction, from 2019 to 2023 eighteen lynxes from Romania and Slovakia were released into Croatia and Slovenia (Paper III). Since 8 of these animals have coats without patterns, it will be interesting to evaluate their influence on the frequency of coat patterns in the Dinaric population in the future.

Prior to the translocations within the LIFE Lynx project, wide scale camera trapping study needed to be implemented to collect scientifically based data on abundance and distribution of the local population. Since the reintroduction of lynx to Slovenia in 1973, lynx monitoring in Croatia was mainly limited to the mortality records (FRKOVIĆ 2001). Only in the early 2000s research and monitoring of various aspects of lynx biology and ecology started (GOMERČIĆ et al. 2009; GOMERČIĆ et al. 2010, KUSAK 2012). Although one of the goals of Croatian lynx management plan for the period 2010 – 2015 was to establish a national monitoring system (SINDIČIĆ et al. 2010), this was achieved only in 2018 as a combined effort of the LIFE Lynx project implementation (SINDIČIĆ et al. 2018), lynx monitoring in protected areas (especially National park Plitvice lakes and Nature park Velebit), cooperation with numerous hunting grounds and environmental impact assesment studies implemented by the company Geonatura Ltd. Since at the beginning of this study almost 10,000 km^2 was considered as potential lynx distribution area in Croatia (SINDIČIĆ et al. 2010), the first challenge was to establish monitoring of an elusive species over such a large area. There are no specific recommendations how to design the study for estimating lynx populations using camera trapping since it is influenced by various factors such as the size of the study area, resources and research goals. However, some general guidelines exist based on scientific research and best practices (Hočevar et a. 2020). Researchers typically conduct pilot studies to determine the optimal approach for their study area and objectives. WEINGARTH et al. (2015) advise that when establishing monitoring in a new area, a survey should be carried out for as long as possible and then optimize the methodology for future monitoring based on the collected data. Therefore, a monitoring system over the entire assumed area of lynx distribution in Croatia was established. Camera traps were active throughout the year, to record as many different individuals as possible and get a basic, but scientifically based, insight into the population demography.

Within this study (Paper II), it was estimated that 89 - 108 adult lynxes were present in the period 2018-2020 which presents the first published scientifically – based estimation of lynx population size in Croatia. The actual lynx number is more likely to be closer to older estimation of 130 lynxes (FIRŠT et. al., 2005), then to later estimation of 40 - 60 individuals (SINDIČIĆ et al. 2010). Both the estimation from 2005 by FIRŠT et al. and estimation from 2010 by SINDIČIĆ et al. were based on expert opinion and not on the analysis of sistematically collected data. So this wide variation in estimates illustrates the importance of properly designed and performed monitoring system. Thus, we cannot state that there was an increase in lynx population size in Croatia when we compare this study with past results, but the discrepancies are the product of methods used. Future important steps in monitoring lynx populations include implementing a methodology that allows the use of spatial capture recapture models. However, these first scientifically based data on minimum population size and local population distribution (Paper II) provided a better understanding of the post-release behaviour of animals released as part of the population reinforcement process (Paper III). The study presented in Paper III showed that it is possible to successfully translocate adult lynx individuals to an area where territorial lynx are already present. All six monitored lynx that were realeased within the distribution area of the existing population, sucessfully established their initial territories on average 23 days (SD =16.5) post- release within the area of resident lynx distribution (Paper II, Figure 1 – Paper III). In addition, analysis of the behaviour of translocated lynx in the early period after the release showed obvious changes in lynx movement patterns, which was used as an indicator of the settlement process of individual animals. This also allowed to study feeding patterns and helped identify potential habitat connectivity issues. The results from the Paper III showed a preference for the NW-SE exploratory movement direction, which corresponds to the orientation of the predominant ridge lines of the Dinaric Mountains. ZIMMERMANN et al. (2007) demonstrated the same exploratory movement pattern in the Jura Mountains, that run SW-NE, which appeared to shape the exploratory movements of subadult lynx. In contrast, in the northwestern Swiss Alps, where there are no parallel ridge lines, movement directions were randomly oriented (ZIMMERMANN et al. 2007). Moreover, it appeared that lynx preferred to move along the mountain range rather than perpendicular to it, i.e. they avoided moving uphill and downhill. The first kill sites of all animals were discovered an average of 3.4 days after release. The average time between kill sites after establishment was 7.76 days (SD =1.91), which is consistent with the kill rate of resident Dinaric lynx (KROFEL et al. 2013). Movements of three monitored lynx also confirmed that the A1 Ljubljana – Koper highway presents an important anthropogenic barrier (KROFEL et al. 2006, SKRBINŠEK et al. 2008) for wildlife and divides the Dinaric area populated by lynx from the Alpine area where there is no resident lynx population. To illustrate this further, the example of lynx Maks showed how this individual reached the highway in 18 days post-release and was forced to stay on the terrain south of the highway with several unsuccessful attempts of crossing. Maks finally crossed the highway, 4 months after reaching this barrier for the first time, and soon after went in a second exploratory movement. This second exploratory movement was likely influenced by the presence of another territorial male known from camera trapping survey (KROFEL et al. 2021). On September 27, 2021, Maks's telemetry collar stopped emitting signal and he was not recorded with camera traps, indicating he might no longer be alive. Other two translocated lynx that reached the highway (Doru and Catalin), never managed to cross it. Such poor connectivity presents a threat for populations already fighting with inbreeding. Creation and maintenance of functional linkages between remnant populations should become a conservation priority and resources need to be dedicated to enhancing habitat networks that would allow natural gene flow among populations.

Finally, one of the greatest challenges when planning translocations is ensuring that released animals settle in the desired area. For animals translocated in this study, the maximum aerial distance reached from the release site was 50 km, while Swiss results showed that an individual could move up to 60 km in the early post-release period (RYSER et al. 2012). Therefore, release sites should be at least 60 km away from the boundaries of the targeted settlement area. Of course, there are limitations to this study related to the relatively small all-male sample size, but this study has already provided valuable theoretical and practical insights. Close monitoring of small, isolated populations threatened by inbreeding, such as the Dinaric, is of great importance for the future conservation of this large carnivore species.

6. CONCLUSIONS

1. Quantification of coat pattern types demonstrated that coats with small spots differed significantly in spot size and density when compared to other three lynx coat pattern types.
2. The absolute frequencies of lynx coat pattern types changed significantly between the years 1978-1999 and 2001-2019: 90% of lynx recorded in the period 2001-2019 had spotted coat patterns, coats with rosettes became significantly less common, while coats without spots disappeared completely from the population.
3. The size of the lynx population in Croatia was at least 89 individuals in the period 2018-2020.
4. The permanent distribution of lynx was confirmed in a total area of 7100 km^2 in Primorsko – Goranska and Ličko – Senjska counties, in the southern part of Karlovac County and in the northeastern part of Zadar County
5. Released lynx from the Carpathians have successfully settled in the area where the presence of territorial resident lynx has been recorded.
6. Release sites should be at least 60 km away from the boundaries of the targeted settlement area.
7. The knowledge gained in this study has provided valuable insight into the Dinaric lynx population and contributed to a more effective conservation strategy for this and future reinforcement programmes.
8. This doctoral dissertation confirmed the hypothesis that the status of the autochthonous population affects the reinforcement success, primarily trough the influence on survival rate of released animals during the migration. During the early post-release study all of the released animals survived and established initial territory. But later on, one of the studied lynxes, Maks, was forced to leave the established territory, probably being pushed out by a more dominant resident male. He set to another exploratory movement and even crossed the notorious Ljubljana – Koper highway, which represent an obstacle for wildlife and divides the Dinaric area populated by lynx from the Alpine area where there is no resident lynx population. During this second exploratory movement, Maks's telemetry collar stopped emitting signal and he was not recorded with camera traps, indicating he might no longer be alive. This prolonged exploratory movement faced Maks with additional threats, which have negatively affected his integration into the population.

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8. BIOGRAPHY OF THE AUTHOR WITH BIBLIOGRAPHY OF PUBLISHED WORK

Ira Topličanec was born in Zagreb on October 2nd, 1992. After completing XVIII Gymnasium in a bilingual program in Zagreb in 2011, she enrolled in the Faculty of Veterinary Medicine University of Zagreb. During studies, she interned at a clinic for wild and exotic animals in Reunion Island, France in 2016, and at the National Veterinary School of Toulouse, France, in 2017. In January 2018, she graduated from the Faculty of Veterinary Medicine University of Zagreb and earned the title of Doctor of Veterinary Medicine. Since 2018 she is working as project assistant on the European project LIFE Lynx (LIFE16 NAT/SI/000634). In 2018, she enrolled in doctoral studies in Veterinary Sciences. Upon the invitation of the American organization Blackfoot Challenge, she spent two weeks in the state of Montana for the purpose of exchanging experiences in working with large carnivores and presented preliminary results of her doctoral research at the University in Montana, a leading institution in wildlife education and research in North America. As a lynx expert, she participated in the development of the Expert Background for the development of a Lynx Management Plan and development of the national lynx monitoring program, within the project "Development and implementation of monitoring programs for large carnivores with capacity building of stakeholders for the monitoring and reporting system." She is author of 7 internationally peer-reviewed scientific papers and presented her work at 17 scientific conferences.

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