

DNA fingerprinting of the otter population in the National park Goričko

Hana Jontez, Simona Sušnik, Jakob Smole,
Marjana Hönigsfeld Adamič and Peter Dovč

*University of Ljubljana, Biotechnical Faculty, Department of Animal
Science, Groblje 3, SI-1230 Domžale, Slovenia*





Introduction

- Otter population in Slovenia as well as in other parts of Europe was considered to be critically endangered at the end of the last century, but thanks to efficient conservation actions, it started to expand again.
- The Goričko regional park hosts the most connected, vital otter population in Slovenia.
- **LIFE AQUALUTRA project:**
 - **Conservation of the otter population (*Lutra lutra*) in Goričko;**
 - *action: Estimation of the size of the otter population, its structure and distribution in the target area by regular monitoring and a DNA fingerprinting method*

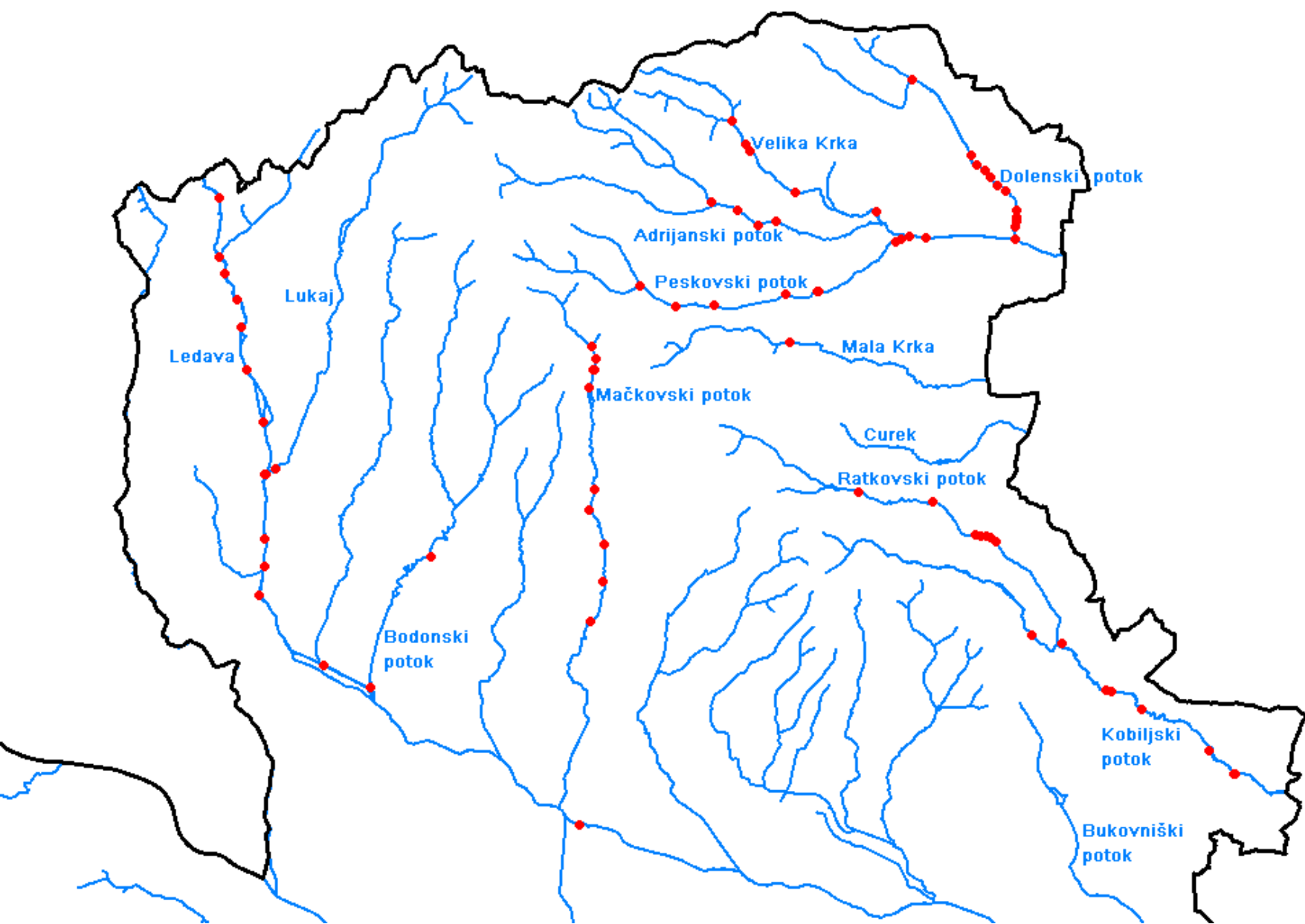




Sampling

- Fresh spraints were collected during three seasons at different locations in Goričko. Spraints were conserved in EtOH and stored at -20° C.







DNA isolation

- DNA isolation from spraints is known to be difficult. Several protocols were tested on otter spraints, but, as already reported ([Lampa et al., 2007](#)), QIAamp DNA Stool Kit (Qiagen) turned out to be the most efficient one and was therefore used for DNA isolation.





Methodology I

- In order to produce comparable genetic data, we have chosen 13 microsatellite loci that were used in the majority of otter studies in Europe:

Lut833, Lut832, Lut818, Lut782, Lut733, Lut717, Lu715, Lut701, Lut615, Lut604, Lut457, Lut453, Lut435 (*Dallas & Piertney, 1998*).
- The protocol for microsatellite amplification has been optimized on one DNA sample, isolated from otter tissue (muscle), in order to avoid possible inhibition in DNA samples isolated from spraints, and to test the robustness of PCR reaction (quantity of DNA, quantity of primers, annealing temperature).





Methodology II

- Genotyping of spraint samples requires re-amplification procedures due to low DNA concentrations and presence of inhibitors that prevent efficient amplification in the first reaction.
- Multiplex PCR was optimized with DNA from tissue sample. Two to four microsatellite loci were amplified in multiplex reactions. The second step of amplification was performed on a single microsatellite locus and multiple loci were loaded onto ABI3130 capillary electrophoresis.
- ZFX/ZFY region, amplified using ZFXRb/ P1-5EZ primer pair and cut with restriction enzyme *BsmI* was used for sexing (Mucci & Randi, 2007).





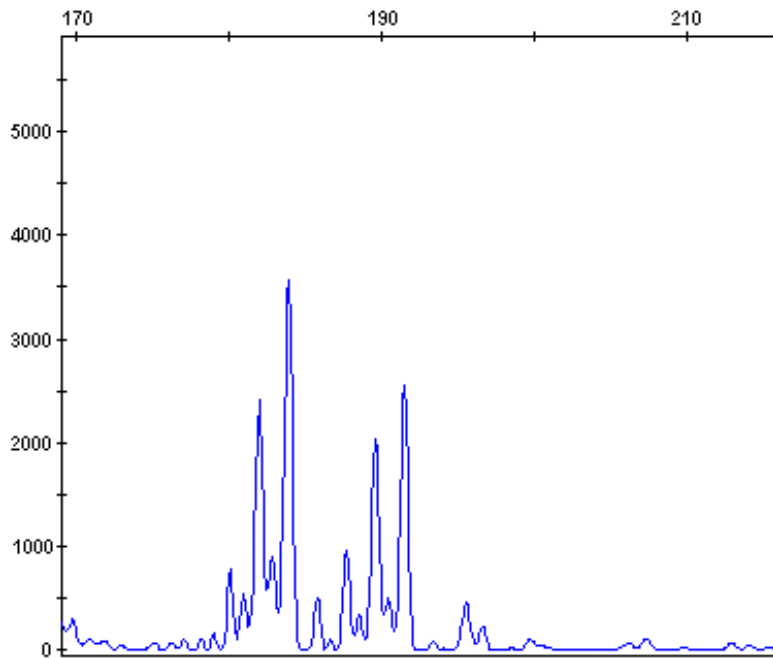
Extraction efficacy

Season	No.	No. isolated	Successful PCR
05-06	94	35	11
06-07	102	40	11
summer 07	19	18	10
winter 07-08	39	39	15
<i>total</i>	254	132	47
		51,97%	35,61%

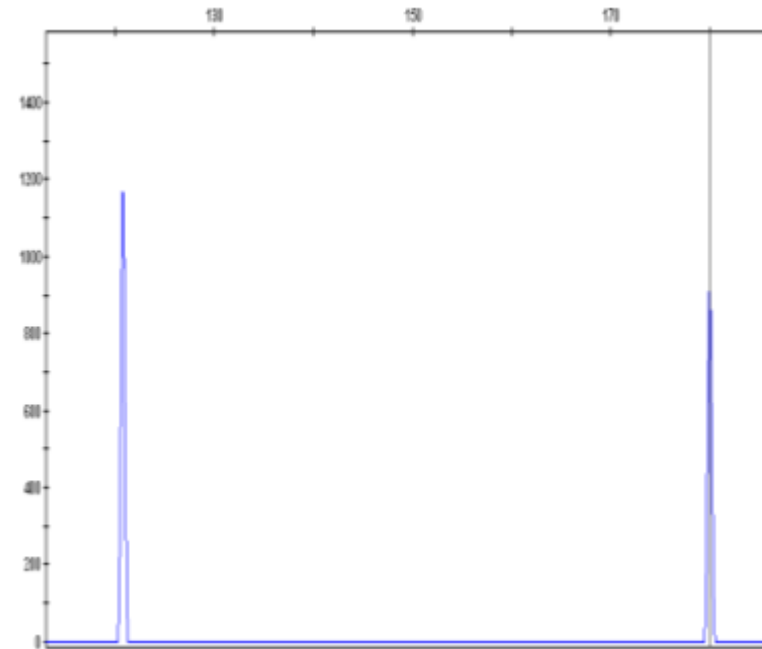


Microsatellite typing

Electropherogram showing two alleles (183bp, 191bp; heterozygote) of locus Lut457.



Electropherogram showing two fragments (119bp, 178bp) of the ZFX/ZFY region (XY, male).





Results

- The total of 18 genotypes were found in the investigated population during the three seasons of sampling.
- The relative abundance of genotypes in the analysed samples ranged from single appearance (16 genotypes) to seven fold appearance (1 genotype).
- The average number of alleles per locus was 7.75, ranging from 3 alleles at the locus Lut453, to 11 alleles at the loci Lut833 and Lut701.
- The ZFX/ZFY marker revealed sex ratio of 10 females vs. 7 males.





Allelic diversity

Locus	Lut717	Lut833	Lut818	Lut715	Lut701	Lut832
Allele No.	8	11	9	10	11	8
Allele range	181-203	143-169	154-178	192-214	186-208	170-192

Locus	Lut782	Lut733	Lut453	Lut604	Lut435	Lut457
Allele No.	5	10	3	6	6	6
Allele range	185-193	159-179	122-126	129-139	122-134	179-191

Sex ratio 10F:7M





Genotypes I

Season	Watercourse	Location	Date	Sex	Name
season 06-07	Ledava	Večeslavci	25.01.2007	f	Angelca
winter 07-08	Ledava	Nuskova	18.12.2007	m	Cene
summer 07	Peskovski potok	Izliv v Krko	26.09.2007	m	Čuftek
winter 07-08	Kobiljski potok	Cerkev Kobilje	14.01.2008	f	Ditka
season 06-07	Ledava	Brana	25.01.2007	f	Katka
season 06-07	Kmetov potok	Moščanci	20.12.2006	f	Krasna
summer 07	Dolenski potok	Hodoško jezero, čistilna naprava	13.08.2007		noname1
summer 07	Ledava	Brana	02.10.2007		noname2
season 05-06	Mačkovski potok	Avtobusna postaja pred Vanečo	20.04.2006		noname3
season 06-07	Ledava	Brana	06.12.2006		noname4
season 06-07	Ledava	Brana	08.12.2006		noname5
season 05-06	Peskovski potok	Izliv P.p. v Krko	04.01.2006	m	Oto
season 06-07	Ledava	Brana	25.01.2007	m	Pučko
season 05-06	Dolenski potok	3.most pod Hodoškim jezerom	31.03.2006	m	Rene
summer 07	Dolenski potok	Železniški podhod	02.10.2007	f	Rezi
winter 07-08	Ratkovski potok	Prosenjakovci center	01.12.2007	f	Suzi
season 06-07	Ledava	Brana	23.01.2007	f	Kunigunda
	Ledava	Kopališče Murska Sobota	26.01.2007		
season 05-06	Adrijanski potok	Sojkini	28.02.2006	f	Jagoda
winter 07-08	Kobiljski potok	Motvarjevci 97	17.01.2008		
season 05-06	Ledava	Nuskova	06.01.2006	m	Bine
	Velika Krka	OŠ Šalovci	02.03.2006		

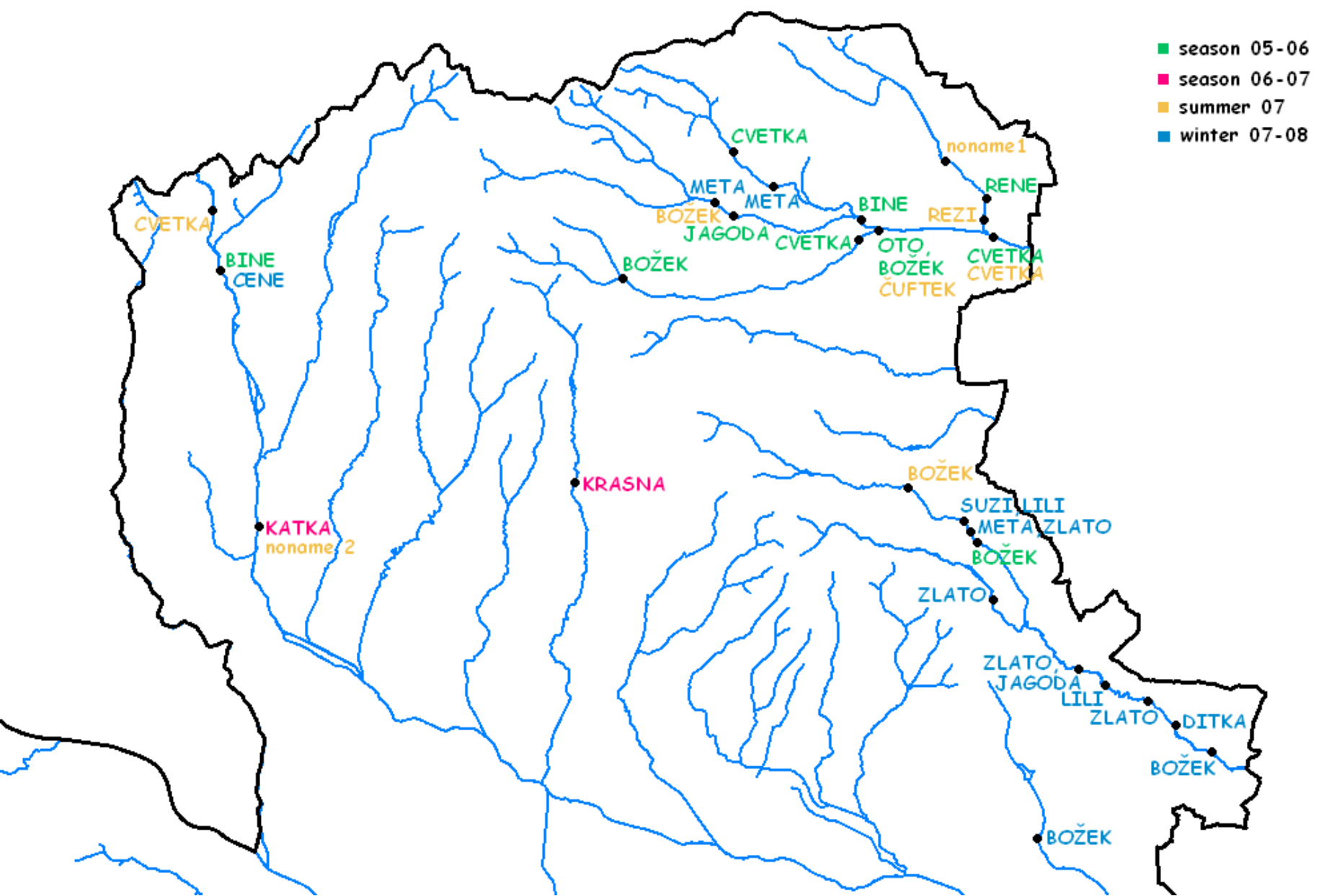


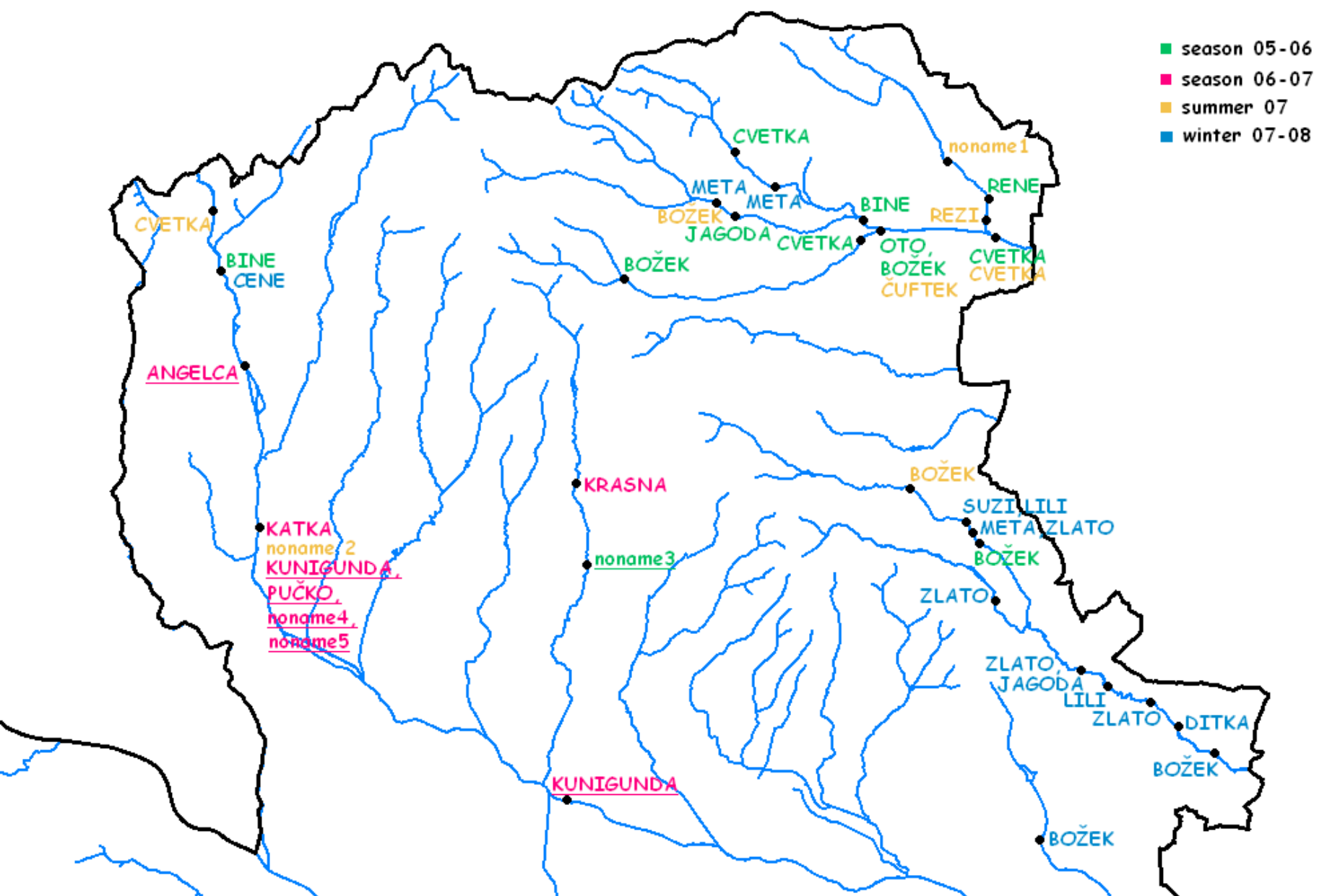


Genotypes II

Season	Watercourse	Location	Date	Sex	Name
winter 07-08	Ratkovski potok	Prosenjakovci center	01.12.2007	f	Lili
	Kobiljski potok	Gramoznice Kobilje	16.01.2008		
winter 07-08	Adrijanski potok	Skrlemkini	26.11.2007	f	Meta
	Velika Krka	Odcep za Budince	28.11.2007		
	Ratkovski potok	Odcep za Čikečko vas	16.01.2008		
winter 07-08	Kobiljski potok	Kobilje 134	14.01.2008	m	Zlato
	Kobiljski potok	Čikečka vas	14.01.2008		
	Ratkovski potok	Odcep za Čikečko vas	15.01.2008		
	Kobiljski potok	Motvarjevci 94	16.01.2008		
season 05-06	Velika Krka	Krplivnik	05.04.2006	f	Cvetka
	Peskovski potok	Šalovci 16	05.04.2006		
	Velika Krka	Most nad Lenaršičevim mlinom	13.04.2006		
	Velika Krka	Krplivnik	13.08.2007		
summer 07	Ledava	Žaga Sotina	17.08.2007		
season 05-06	Peskovski potok	Višnja	06.01.2006	m	Božek
	Peskovski potok	Izliv P.p. v Krko	04.03.2006		
	Ratkovski potok	1.most za Prosenjakovci	17.03.2006		
summer 07	Ratkovski potok	Časarjev mlin	12.10.2007		
	Adrijanski potok	Skrlemkini	16.10.2007		
winter 07-08	Kobiljski potok	Malo Kobilje	15.01.2008		
	Bukovniški potok	Dobrovnik	16.01.2008		

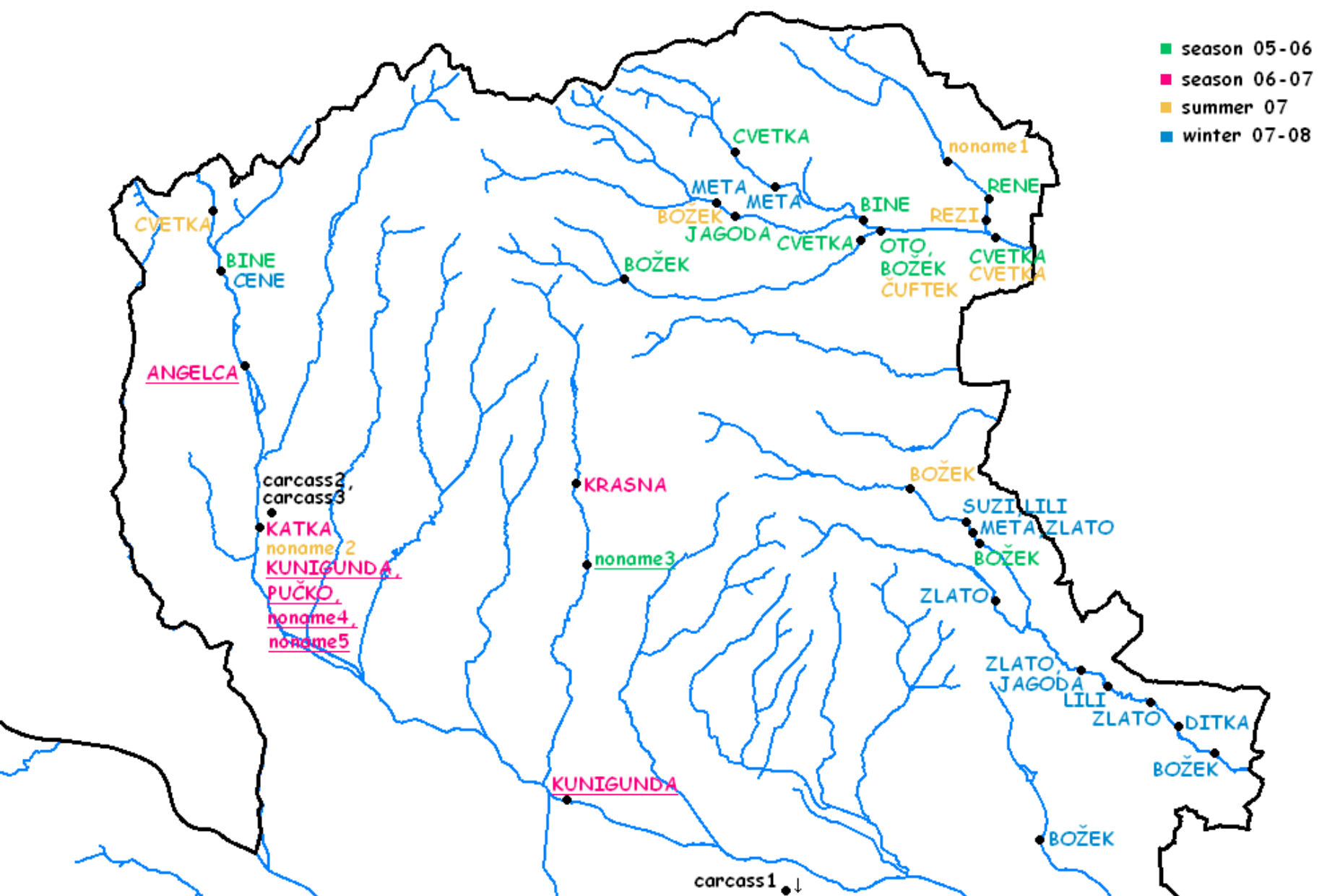






At least 6 new genotypes were found in the Western area...
(additional preliminary results)





Three additional genotypes from found carcasses...





Discussion

- **DNA isolation**
- PCR amplification was possible in more than 50% samples from summer 07. The reason could be spraint activity, which is mostly in May-June and October.
- Samples from the winter period 07-08 reached almost 40% success and indicate the importance of sample freshness.
- The overall success of DNA isolation is comparable to results reported in the literature.
- Problems with allele dropout: case of *Lut 715*.





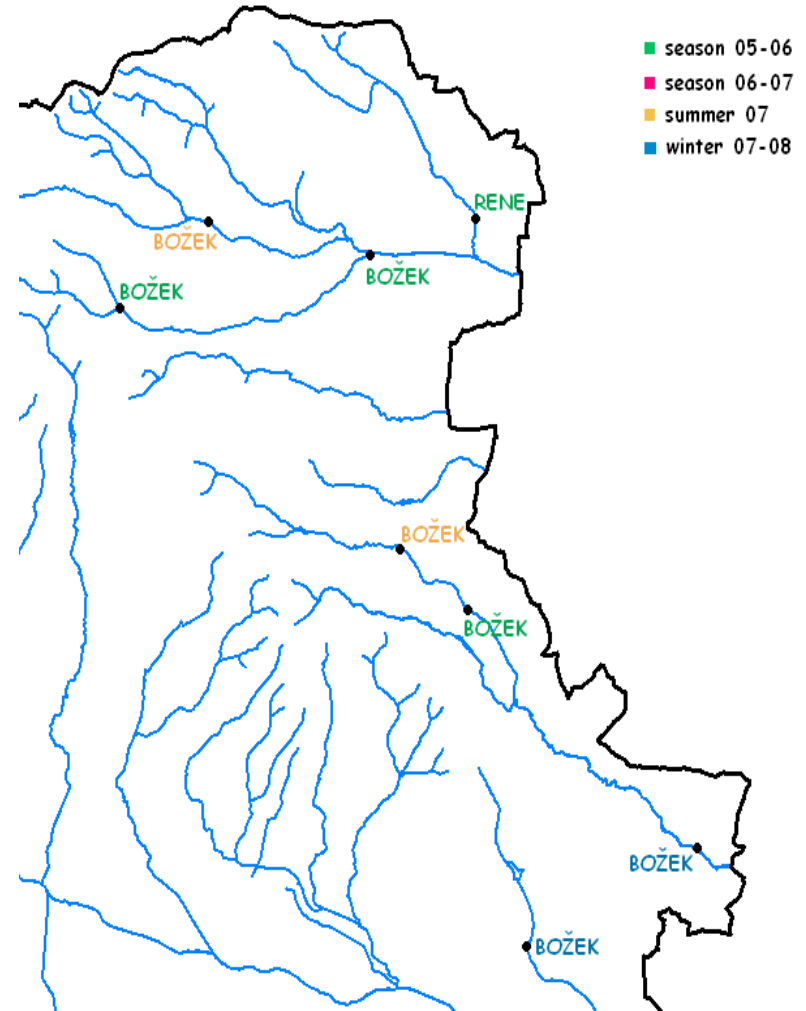
Discussion

- **Density and territory occupancy of otter samples**
- Spraint density can be used as an indicator for the population size of otters.
- Some samples were found on relatively large area; indication for extensive migrations.
- Three carcasses and spraint genotypes did not match, indicating that density of spraint sampling did not reach saturation yet.



Discussion

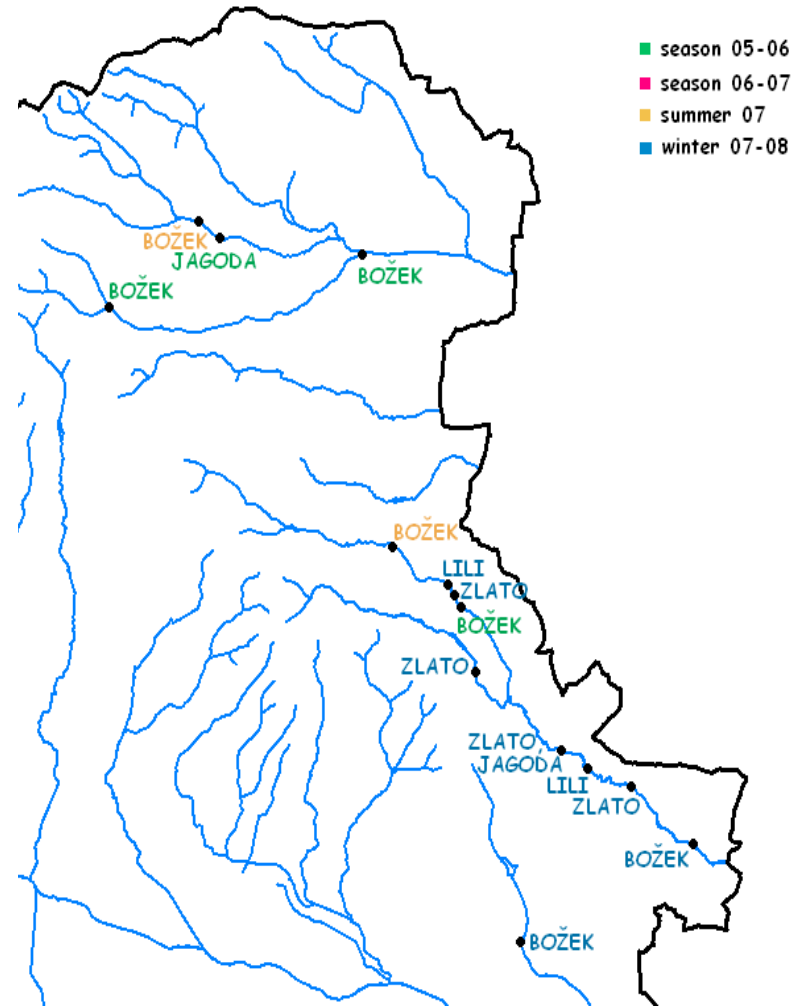
- **Relatedness of otter samples: an example of Božek's “family”**
- Božek (♂) can be spotted through all seasons (except season 06-07) and covers very large area.
- He is probably related to Rene (♂, brother?) (season 05-06), and Katka (♀) who is now on the west side of the territory.





Discussion

- **Relatedness of otter samples: an example of Božek's "family"**
- Božek is most likely the father of Zlato (♂) and Lili (♀), who might be brother and sister. They are most likely cubs, appearing only in the winter 07-08, in the territory size typical for female individuals.
- Jagoda (♀) could be their mother or aunt.



A photograph of three brown bears in a natural setting. They are surrounded by tall, vibrant green grass that reaches up to their chests. In the background, a body of water is visible, with a piece of driftwood floating on the surface. The bears have thick, brown fur and are looking towards the camera. A semi-transparent grey rectangular box is overlaid in the center of the image, containing the text 'THANKS FOR YOUR ATTENTION!' in a bold, black, sans-serif font.

THANKS
FOR YOUR ATTENTION!

