Assessment of otter population size: non-invasive genetic sampling and snow tracking


Photo: L. Votocek

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Population size

- very important population parameter, but very difficult to obtain

  - spraint numbers
  - holts counting
  - radio-tracking + Zn injection
  - visual census
  - snow or mud tracking
  - camera trapping
  - infrared counters

- non-invasive genetic sampling (NGS)

Photo: J. Rolecek

Aims & Objectives

1. Assessment of otter population size by snow tracking and NGS at two study sites (different habitats).

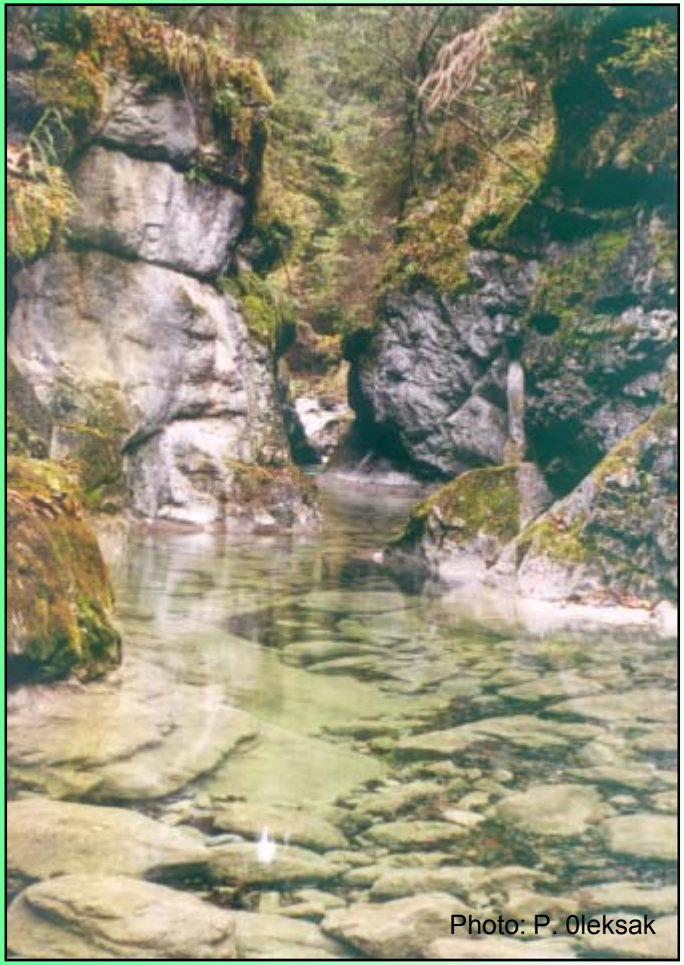
2. Estimation of NGS error rates.

3. Comparison and evaluation of NGS and snow tracking.

4. Recommendations for more efficient and reliable use of the methods.

Study sites (100 km^2)

1. Mountain/sub-mountain area – Slovensky Raj National Park, Slovakia



2. Fishpond area – Trebonsko PLA & BR, Czech Republic

Faecal sampling

- spraints, spraints with jelly, anal jellies
- cold months of 2003-2004
- GPS location
- 1-4 sub-samples
- buffers or 96% ethanol
- portable coolbox

Snow tracking

- fresh snow + most water bodies frozen
- SR: February 2003, TR: January 2004
- 8-16 experienced field surveyors

Methods (NGS)

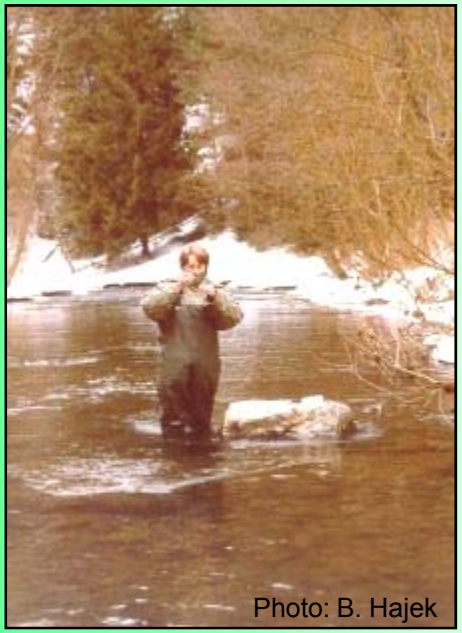


Photo: B. Hajek

collection of fresh (≤ 18 hours) samples

(storage at -20 °C or -80 °C)

↓

DNA extraction

(stool kits – Qiagen, Invitek)


Photo: J. Bryja

↓

hot-start PCR

(multiple-tubes approach, Taberlet *et al.* 1996)

↙ ↘

microsatellites – 5 + 4 loci

(Dallas & Piertney 1998)

Lut-SRY/914

(Dallas *et al.* 2000)

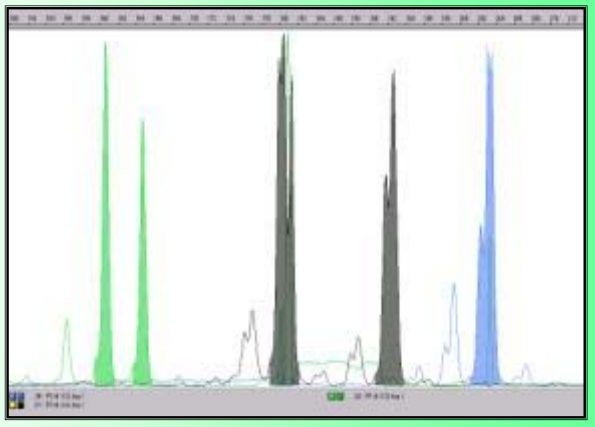
↓ ↓

fragment analysis - ABI Prism 310 a 3130 Genetic Analyzers

Quality and reliability of NGS data

- **probability of identity** (GEMINI software)

6 loci	SR: $PI = 1.395 \times 10^{-3}$	$PI_{sibs} = 4.791 \times 10^{-2}$
	TR: $PI = 2.514 \times 10^{-4}$	$PI_{sibs} = 2.148 \times 10^{-2}$
10 loci	SR: $PI = 5.846 \times 10^{-6}$	$PI_{sibs} = 3.665 \times 10^{-3}$
	TR: $PI = 1.211 \times 10^{-5}$	$PI_{sibs} = 5.144 \times 10^{-3}$



- multiple-tubes approach: 6 PCR+ for homozygotes, 3 PCR+ for heterozygotes

- **PCR success rate 71%**
- **successfully genotyped 59% of samples** (82% anal jellies, 58% spraints with jelly, 46% spraints)
- **allelic dropout = 18%** (loci 12-24%, samples 0-100%)
- **false alleles = 3%**

Assessment of population size: NGS

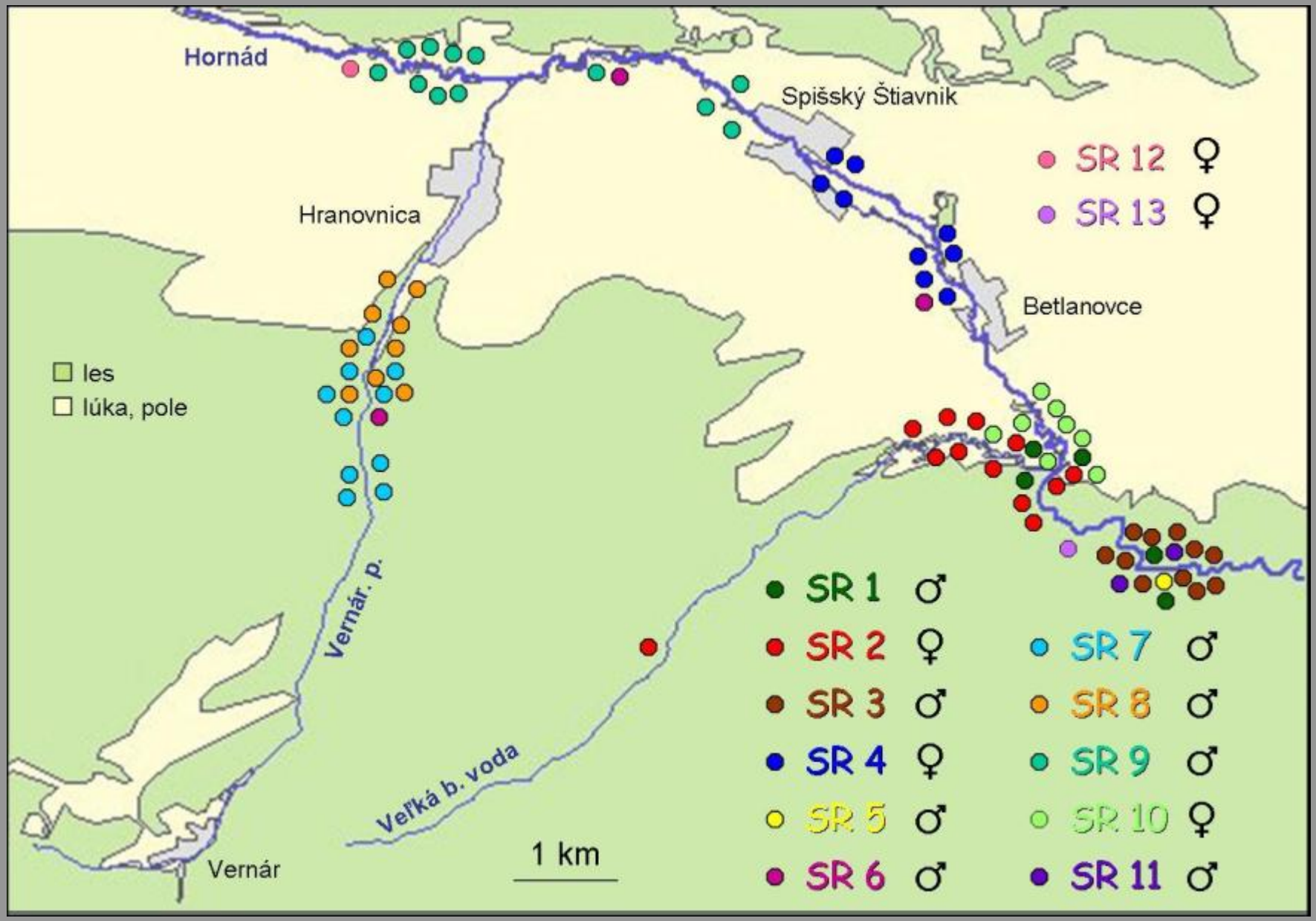
(1) Slovensky raj

- 13 individuals identified; 7 males and 6 females
- no. positive samples per individual 1-23 (mean 9.6, SD 7.32)
- 2 individuals based on a single sample
- 63% samples successfully genotyped
- 1-MM: 0
- 2-MM: 1 (+ sex)

Assessment of population size: NGS

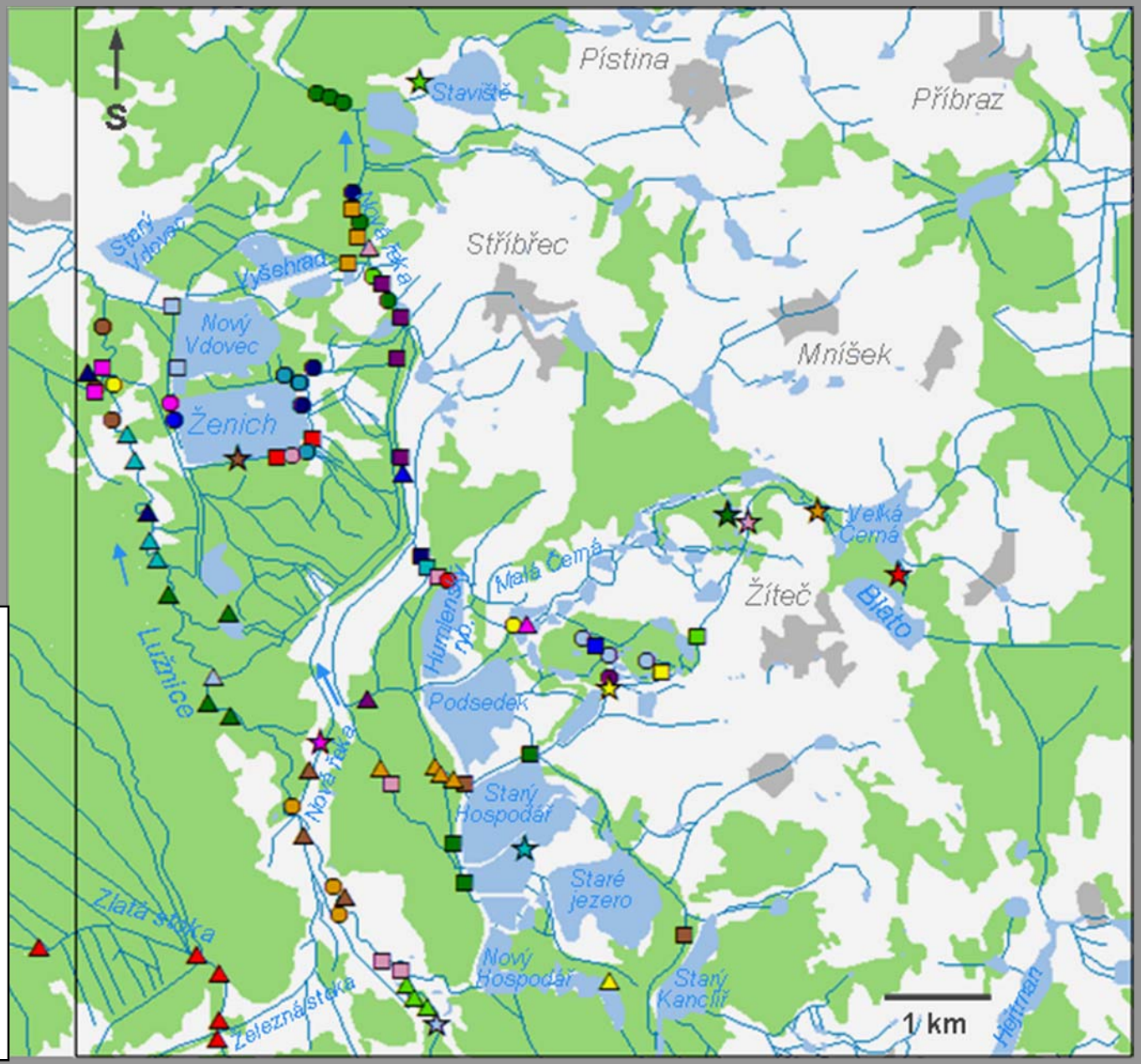
(2) Trebonsko

- 50 individuals identified; 29 males and 21 females
- no. positive samples per individual 1-13 (mean 2.8, SD 2.58)
- 26 individuals based on a single sample
- 55% samples successfully genotyped
- 1-MM: 1 (+ sex)
- 2-MM: 6 (3 + sex)



- 13 individuals at ca. 50 km watercourse

- 50 individuals at 100 km²



TR1	TR18	TR34
TR2	TR19	TR35
TR3	TR20	TR36
TR4	TR21	TR37
TR5	TR22	TR38
TR6	TR23	TR39
TR7	TR24	TR40
TR8	TR25	TR41
TR9	TR26	TR42
TR10	TR27	TR43
TR11	TR28	TR44
TR12	TR29	TR45
TR13	TR30	TR46
TR14	TR31	TR47
TR15	TR32	TR48
TR16	TR33	TR49
TR17	TR34	TR50

Assessment of pop. size: NGS + CMR

- CAPWIRE (Miller *et al.* 2005) – „capture-mark-recapture“ based program for NGS

  - even capturability (ECM model) vs. capture heterogeneity (TIRM model)

SR: **point estimate = 13 indiv.** **$CI_{95\%}$ = 13 indiv.**

TR: **point estimate = 81 indiv.** **$CI_{95\%}$ = 55-89 indiv.**

- CAPWIRE – discrete calculations – short periods of intensive sampling (assumption of „population closure“)

SR: **point estimate = 11 indiv.** **$CI_{95\%}$ = 11 indiv.**

TR: **point estimate = 76 indiv.** **$CI_{95\%}$ = 49-96 indiv.**

Assessment of pop. size: snow tracking

- SR: 10-12 indiv.
- TR: 38 indiv. (Roche & Roche 2004)

Comparison of methods

	Genotypes	CAPWIRE (95% CI)	Snow tracking
SR	11[a]	11 (11-11)[a]	10-12
	13[b]	13 (13-13)[b]	
TR	46[a]	76 (49-96)[a]	38
	50[b]	81 (55-89)[b]	

[a]Estimate under „population closure“

[b]Estimate over entire study period

Discussion

- NGS: potential overestimation (genotyping errors!!, migration?), underestimation? (low success rate, inadequate sampling)
- snow tracking: both over- and underestimation (problems with distinguishing tracks of different individuals, decreased movement on the surface under extreme conditions?)
- NGS time-consuming and costly, but provides more information: individual and sex identification, pop. size estimate, estimates of relatedness, genetic variability and structure (gene flow, spatial structure, effective pop. size,…)
- snow tracking provides information on reproduction, but increasing problems with snow (global climate change)

Conclusions

- similar estimates in simple linear habitats
- in complex habitats with high otter density NGS revealed the presence of higher number of individuals (46-50 genotypes) than snow tracking (38 indiv.)
- CMR-based estimate even higher (76-81 indiv.)
- NGS: strict control of genotyping errors is very important!!

➔ **combination of field and genetic methods**

(suggested also by Arrendal *et al.* 2007)

Suggestions and recommendations

Potential approaches to increase efficiency of NGS:

- proper collection and storage of samples (e.g. Hajkova *et al.* 2006, Lampa *et al.* 2008; future testing?) → very fresh samples, cold weather, jelly samples,…
- use of real-time PCR to assess otter DNA concentration in spraints (selection of high quality samples, optimisation of number of PCR repetitions)
- multiplexing of several loci → decreasing time- and financial costs of analysis (also saving DNA)
- others (e.g. multiplex pre-amplification?, etc.)

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Thank you for your attention…
Photo: B. Zemanova