

Genetic and viability assessment of a reintroduced Eurasian otter *Lutra lutra* population on the River Ticino, Italy

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Abstract On the River Ticino in northern Italy, a small number of captive Eurasian otters *Lutra lutra*, belonging to the European breeding programme for self-sustaining captive populations, were reintroduced in 1997, after the species had been declared locally extinct in the 1980s. We surveyed for otter signs in 2008, 2010, 2016–2017 and 2018, confirming the presence of what is probably a small population. To assess the abundance and viability of the population, we genotyped fresh spraints collected during the last two surveys, using 11 microsatellite markers, and modelled the population trend using *Vortex*. A minimum of six individuals were identified from 25 faecal samples. The analysis of mitochondrial DNA determined that the reintroduced otters share a transversion that is characteristic of the Asiatic subspecies *Lutra lutra barang*, confirming the contribution of the Asiatic subspecies to the genetic pool of the captive-bred founder population. Population size was consistent with the release of three pairs of otters and all models implied that the number of founders was too small to ensure the long-term survival of the population. Stochastic factors are therefore likely to threaten the success of this reintroduction.

Keywords Eurasian otter, genotyping, inbreeding, Italy, *Lutra lutra*, population viability, reintroduction, River Ticino

Introduction

There is no consensus on how to best measure the success of species reintroductions (Seddon, 1999; Estrada, 2014), although they are generally considered successful if they result in a population that is viable and self-sustaining (Griffith et al., 1989; Caughley & Gunn, 1996; Dodd, 2005) or reaches carrying capacity in the release area (Sarrazin, 2007). The time required for the establishment of a self-sustaining population varies across species (Seddon, 1999; Fischer & Lindenmayer, 2000; Albrecht et al., 2011), and several demographic parameters and stochastic processes need to be assessed to determine whether a population is likely to be viable in the longer term (Sarrazin & Barbault, 1996). In the case of long-lived species such as medium-sized and large mammals, this time is often longer than stakeholders allow for, or than funding is available for monitoring (Sutton, 2015). Therefore, although essential for assessing the efficacy of reintroduction projects of most vertebrates (Semlitsch, 2002; Muths & Dreitz, 2008; IUCN/SSC, 2013), long-term monitoring is rarely achieved (Sutherland, 2000; Estrada, 2014; but see Mason & Macdonald, 2004 and Koelewijn et al., 2010 regarding the Eurasian otter *Lutra lutra*).

The release phase of reintroductions is affected by financial and logistical constraints, and managers' decisions (Converse et al., 2013). In recent decades, the assessment of the size of the founder stock and duration of the release period have been increasingly facilitated by population modelling (Armstrong & Seddon, 2008; King et al., 2013), but the absence of decision analysis (i.e. analysis of management objectives and alternative actions to reach them) may have limited the success of previous reintroduction projects (Converse et al., 2013).

The Eurasian otter is a semi-aquatic mustelid whose populations suffered a sharp decline throughout Europe in the 20th century (Macdonald & Mason, 1994; Ruiz-Olmo et al., 2000). In the second half of the 20th century, the otter became a symbol for nature conservation. In an attempt to prevent its seemingly unavoidable extinction in several countries, the species was included in the European breeding programme for self-sustaining captive populations (Europäisches Erhaltungszuchtprogramm, EEP), involving zoological gardens and specialized breeding centres (Vogt,

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1995). Since the 1980s, the otter has been translocated (UK: Wayre, 1985; Roche et al., 1995; Spain: Fernández-Morán et al., 2002) or reintroduced in several countries (Sweden: Sjöåsen, 1996; The Netherlands: Koelewijn et al., 2010; France: Geboes et al., 2016), and further projects are underway (Denmark: Christiansen, 2017; Japan: Murakami, 2017).

At the end of the 20th century, the Eurasian otter became extinct in the northern and central Italian peninsula (Prigioni et al., 2007). On the River Ticino (northern Italy, Piedmont/Lombardy regions), one of the best conserved lowland riverine habitats of northern Italy, the species was declared extinct in 1985 (Cassola, 1986; Prigioni, 1986), after the last reliable signs of its presence had been recorded in 1980 (Galeotti, 1981). The river was later identified as a potential reintroduction area in the Action Plan for the Conservation of European Otters (Macdonald & Mason, 1990), and deemed suitable for otters by a feasibility study (Prigioni, 1995). In the EEP framework, two breeding centres were built and a reintroduction trialled with the release of one pair of otters (Montanari & Boffino, 2000).

Concern about the reintroduction was raised by the Italian Institute for Wildlife, because analyses of the mitochondrial DNA of the otters hosted in European breeding centres confirmed that individuals of the Asiatic subspecies *Lutra lutra barang* had contributed to the gene pool of the EEP population (the so-called genetic B-line; Randi et al., 2001). In addition, although the European River Otter Studbook was established with the aim to increase the genetic variability of captive-bred otters, a high level of inbreeding was recorded (Randi et al., 2001, 2005).

The reintroduction was interrupted soon after the first release in 1997, mainly because of concerns about the genetic make-up of the captive population. Nonetheless, a second pair of animals was released or escaped in 1998 and a further female with her cub probably escaped before 2000. None of these otters were equipped with radio transmitters; their fate is thus unknown and the precise number of reintroduced animals is uncertain.

Although translocations of large numbers of individuals can have negligible effects on local populations (Arrendal et al., 2004), and some reintroductions have been successful with relatively few founders (*lynx Lynx lynx*: Breitenmoser et al., 1998; fisher *Martes pennanti*: Aubry & Lewis, 2003; Eurasian badger *Meles meles*: Balestrieri et al., 2018), success can be affected by the number of released animals (Griffith et al., 1989). Small founder populations are vulnerable to both demographic and environmental stochasticity (Gilpin & Soulé, 1986) and thus at risk of local extinction. Nevertheless, since 2007 signs of otter presence such as roadkill and spraints have been recorded regularly around a short stretch (5–7 km) of the Ticino River near the release site (Balestrieri et al., 2016). In 2016–2017 otters were recorded by camera traps near the release site on three

occasions, and otter signs were found in 16 out of 32 sampling locations (600 m long river stretches) surveyed (Smirardo et al., 2019). Throughout 2018, a total of 77 km of watercourses were monitored, but only five otter spraints were found, in four surveyed stretches of the river (Tremolada et al., 2020). Compared to the core area of the Italian otter range, marking density around the release site has generally been low (0.15–0.2 vs 3.7 spraints/100 m), suggesting that the local otter population probably consists of few individuals (Prigioni et al., 2006a).

The persistence of the species in the release area despite the small founder population also raised questions about the possible survival of a residual native otter population that was not recorded during the last national census (Prigioni, 1986). To assess the abundance of the otter population on the River Ticino and examine the genetic origin of the reintroduced population, we analysed DNA from faecal samples. This method has proved to be effective for assessing the minimum number of individuals and haplotypes of elusive species such as the Eurasian otter (Dallas et al., 2003; Hung et al., 2004; Kalz et al., 2006; Koelewijn et al., 2010). In addition, we used population viability analysis to determine a posteriori the number of founders and predict the likely fate of the population.

Methods

Genetic analyses

During the 2016–2018 monitoring surveys, which covered the entire Italian stretch of the River Ticino, we collected 25 sufficiently fresh otter spraints and preserved them in ethanol at -20°C for genetic analyses (Bonesi et al., 2013; Vergara et al., 2014). In addition, using the Wizard Genomic DNA Purification Kit (Promega Italia, Milan, Italy), we extracted genetic material from five tissue samples of otters found as roadkill, to use as controls.

Before DNA extraction, spraint samples were placed in a vacuum to evaporate the remaining ethanol. We extracted DNA with the QIAamp DNA Stool Mini Kit (Quiagen S.r.l., Milan, Italy) and performed genotyping using 11 polymorphic autosomal microsatellite loci, chosen among those reported in previous studies (Dallas & Piernney, 1998; Dallas et al., 1999; Huang et al., 2004, 2005; Hájková et al., 2006; Kalz et al., 2006; Bonesi et al., 2013; Lerone et al., 2014; Vergara et al., 2014), based on expected polymorphisms and size of PCR fragments (Table 1). We split the 11 primer pairs into two multiplex PCRs and labelled them with different fluorescent dyes for distinguishing the amplified fragments. We used the Multiplex PCR Kit (Quiagen S.r.l., Milan, Italy) for PCR amplifications, following the manufacturer's recommendations, except for the final volume, which was reduced to 25 μl . We sequenced PCR amplification fragments using the commercial

TABLE 1 Number of samples successfully amplified (N_S) among the genotyped samples of otter *Lutra lutra* spraint collected on the Ticino River, Italy, during 2016–2018. The table shows amplification success per locus, number (N_A) and size of alleles, and observed and expected heterozygosity (H_O and H_E , respectively).

Locus	N_S	Amplification success (%)	N_A	Size (base pairs)	H_O	H_E
OT-14	8	100.0	1	129	0.00	0.00
OT-04	7	87.5	5	166, 173, 175, 177, 190	0.40	0.34
Lut 453	8	100.0	4	123, 127, 129, 131	0.50	0.48
OT-17	6	75.0	3	147, 151, 172	0.40	0.34
Lut 833	7	87.5	4	154, 163, 165, 175	0.80	0.70
Lut 701	4	50.0	2	207, 247	0.67	0.44
Lut 818	5	62.5	4	140, 156, 160, 170	0.50	0.66
OT-19	5	62.5	3	211, 215, 218	0.40	0.32
Lut 435	8	100.0	3	123, 129, 143	0.50	0.40
Lut 715	6	75.0	1	203	0.00	0.00
Lut 902	3	37.5	2	145, 166	0.67	0.44
Mean	6	76.1	3		0.41	0.35

TABLE 2 Accession number and origin of European and Asian otter sequences downloaded from GenBank (National Center for Biotechnology Information, Bethesda, USA).

GenBank accession no.	Origin	References
LC094961_Lutra_lutra	Unknown	Unpublished
FJ236015_Lutra_lutra	South Korea	Jang et al. (2009)
EF672696_Lutra_lutra	South Korea	Ki et al. (2010)
LC049955_Lutra_lutra	Japan	Waku et al. (2016)
LC049954_Lutra_lutra	Russia	Waku et al. (2016)
LC049953_Lutra_lutra	Unknown	Waku et al. (2016)
LC049952_Lutra_lutra	China	Waku et al. (2016)
LC049378_Lutra_lutra	China	Waku et al. (2016)
LC049377_Lutra_lutra	China	Waku et al. (2016)
MN122838_Lutra_lutra	Denmark	Unpublished
KY117556_Lutra_sumatrana	Thailand	Mohd Salleh et al. (2017)
AY860320_Lutra_lutra_captive_Caramanico	Italy	Ketmaier & Bernardini (2005)

fragment analysis service offered by Macrogen (Seoul, South Korea) and analysed electropherograms using *GeneMarker 2.2.0* (SoftGenetics, State College, USA; see Tremolada et al., 2020 for further details).

We analysed the mitochondrial DNA control region to verify the genetic strain and determine the occurrence of hybridization with a residual native population. We accessed sequences from European and Asian otters from GenBank (National Center for Biotechnology Information, Bethesda, USA; Table 2) to pinpoint a short region amplifiable in poor and degraded faecal mtDNA and capable of identifying the origin of the genotypes.

Using the primers OTTDL3- and OTTDH5- (Mucci et al., 2010), we amplified mtDNA from seven of the 25 samples. The reaction was performed using the following protocol: 2 minutes at 94 °C, followed by 40 cycles of three steps (30 seconds at 94 °C, 30 seconds at 55 °C, and 30 seconds at 72 °C), before a final extension of 10 minutes at 72 °C. We carried out Sanger sequencing using the reverse primer OTTDH5. Amplicons were separated on an ABI 3130XL automated sequencer (Applied Biosystems, Waltham,

USA) and visualized and corrected using *Seqscape 5.0* (Applied Biosystems, Waltham, USA).

We also included a haplotype (GenBank accession no. AY860320) from an individual otter hosted in Caramanico Otter Centre, Italy, as it belonged to the same captive-bred group as the reintroduced individuals. We used the mtDNA extracted from tissues of an individual found as roadkill in the lower valley of the River Ticino in 2007 as a reference sample.

We aligned the results and sequences from GenBank in *Bioedit 7.0* (Hall, 1999) and created a neighbour joining phylogenetic tree (1,000 bootstrap resamples) in *MEGA 7* (Kumar et al., 2016) to identify the maternal lineage of Italian spraint samples. We used a sequence of the hairy-nosed otter *Lutra sumatrana* (GenBank accession no. AY860320) to root the tree.

Population viability analysis

We performed the population viability analysis using *Vortex 10* (Lacy & Pollak, 2018), the most commonly

TABLE 3 Modelling scenarios for assessing the viability of the otter population reintroduced on the River Ticino, with demographic input data for *Vortex* simulations.

Biological parameters	Data values			Sources
	Scenario 1	Scenario 2	Scenario 3	
Inbreeding depression	default	default	default	O'Grady et al. (2006)
State variables	default	default	default	
Reproductive system	polygynous	polygynous	polygynous	Kruuk (2006)
Age at first offspring	3 years	3 years	2 years	Kuhn & Jacques (2011)
Max. age of reproduction	10 years	10 years	10 years	Ansorge et al. (1997)
Max. lifespan	12 years	12 years	12 years	Ebenhard (2000)
Max. number of broods per year	1	1	1	
Max. number of progeny per year	3	3	2	2.3–2.6 (Larsson & Ebenhard 1994; Hauer et al., 2002)
Sex ratio at birth	1:1	1:1	1:1	Ansorge et al. (1997)
% breeding at low density	70	70	60	Ansorge et al. (1997), Hauer et al. (2002)
% breeding at carrying capacity	20	20	20	
% adult females breeding	{70 - [(70 - 20) × (N/K) ²] × [N/(1 + N)]}			
Reproductive rates	default	default	default	Normal distribution of the <i>N</i> of offspring per female per brood
Age-specific mortality rates (%)				
0–1 year	24 (F), 34 (M)	52 (F), 52 (M)	17 (F), 17 (M)	Ansorge et al. (1997), Larsson & Ebenhard (1994)
1–2 years	8 (F), 14 (M)	10 (F), 5 (M)	16 (F), 16 (M)	
2–3 years	8 (F), 10 (M)	10 (F), 4 (M)	12 (F), 12 (M)	
> 3 years	8 (F), 6 (M)	3 (F), 2 (M)	12 (F), 12 (M)	
Number of types of catastrophes	1	1	1	Exceptional flood October 2000 (Cattaneo et al., 2000)
Frequency of catastrophic events (%)	3	3	3	Return period: 30–35 years (Cattaneo et al., 2000)
Severity for reproduction and survival (%)	0.2–0.75	0.2–0.75	0.2–0.75	
% of males > 2 years old in breeding pool	70	70	70	
Population size (<i>N</i> ₀)	1 F, 1 M 2 F, 2 M 3 F, 3 M	1 F, 1 M 2 F, 2 M 3 F, 3 M	1 F, 1 M 2 F, 2 M 3 F, 3 M	Uncertainty on the number of reintroduced pairs
Carrying capacity (<i>K</i>)	50	50	50	0.18–0.20 otters/km (Prigioni et al., 2006b)
Supplementation	1 F, 1 M 2 F, 2 M	1 F, 1 M 2 F, 2 M	1 F, 1 M 2 F, 2 M	Eventually escaped individuals (J. Conroy, in: Prigioni et al., 2009)
Initial rate of increase λ	1.14–1.2	1.07–1.13	1.14–1.23	

used package for modelling reintroduced populations (Armstrong & Reynolds, 2012) and guiding conservation initiatives (Hamilton & Moller, 1995; Gaona et al., 1998). *Vortex* is an individual-based, stochastic model that applies Monte Carlo simulations to model the dynamics of populations by stepping through a series of events that describe both deterministic and stochastic processes (Lacy, 1993). Simulations allow the probabilistic assessment of (1) population size and genetic variation of extant populations, (2) extinction risk at specified intervals, and (3) mean time to extinction of those simulated populations that became extinct.

We retrieved biological parameters from the available literature (Table 3; Larsson & Ebenhard, 1994; Ansorge et al., 1997; Ebenhard, 2000; Hauer et al., 2002; Kruuk, 2006; Kuhn & Jacques, 2011), and assessed carrying capacity based on otter density in southern Italy (Prigioni et al.,

2006b). To account for the uncertainty about the number of otter pairs released and the possibility of unintentional supplementation by otters escaped from two enclosures in the 2–3 years following the start of the reintroduction project, we set the population size (*N*₀) at three different values (1–3 pairs) for each scenario and simulated the adding of a pair of individuals per year at time *t*₀ + 1 and *t*₀ + 2 (Table 3). The risk of a catastrophe was set at 3%, assuming that exceptional floods, which may occur at intervals of c. 30–35 years (Cattaneo et al., 2000), could primarily affect the survival of juveniles. For each scenario, we ran 500 simulations over a 100-year period, defining extinction as the point when one sex was eliminated.

We conducted sensitivity tests for uncertain parameters, to examine the effects of a range of values for each parameter on the probability of otter survival. We tested inbreeding depression, quantified as the number of lethal equivalents

TABLE 4 Year of sampling, amplified loci and amplification success for the six otters genotyped.

Locus	2016		2017			2018
	Otter 1	Otter 2	Otter 3	Otter 4	Otter 5	Otter 6
OT-14	129–129	129–129	129–129	129–129	129–129	129–129
OT-04	177–190	177–177		177–177	177–177	166–177
Lut 453	127–131	123–131	127–131	127–127	127–127	127–127
OT-17	151–172	147–151		151–151	151–151	151–151
Lut 833	163–175	154–175		163–175	163–175	165–165
Lut 701	207–247	207–247			247–247	
Lut 818	160–170		140–140		156–170	170–170
OT-19	215–215		215–215	211–215	211–215	215–215
Lut 435	129–143	123–143	129–143	143–143	143–143	143–143
Lut 715	203–203	203–203	203–203	203–203	203–203	203–203
Lut 902	145–166			145–145	145–166	
Amplification success (%)	100.0	72.7	54.5	81.8	100.0	81.8

(alleles that are lethal when homozygous or a collection of genes considered to be equivalent) per diploid individual (0.0–12.58, increments of 0.629), carrying capacity ($K=15–60$, increments of 5), per cent frequency of catastrophic events (1–10%, increments of 1%) and severity of the latter for reproduction (0.1–1.0, increments of 0.1).

Results

Of the 25 faecal samples stored for genetic analyses (21 from monitoring in 2016–2017 and four from 2018), eight (32%) were successfully genotyped, allowing us to identify six individuals (five and one, respectively; Table 4), along a c. 36 km long stretch of the river (Fig. 1). The distance between faecal samples from the same individual was 4–11 km. Two loci were monomorphic and nine polymorphic (mean = 2.9 alleles per locus; maximum = 5 alleles for OT-04), for a total of 32 alleles. Expected heterozygosity (H_e) was $0.35 \pm SE 0.07$, and observed heterozygosity (H_o) was $0.41 \pm SE 0.08$.

We obtained a region of mtDNA 175 base pairs long for three of the seven analysed samples. All individuals shared an identical haplotype (Fig. 2) that was the same retrieved in the otter found as roadkill. All the Asian haplotypes were characterized by a transversion (A instead of G) at position 16.400 of the European lineage (Genebank accession no. MN122838). The same mutation was also found in the haplotype from the River Ticino.

Population size in 2016–2017 was consistent with population viability analysis models with $N_o = 2$ and the supplementation of four individuals in the following 2 years (two per year; scenario 1), despite a high probability of extinction in the first decade post-release (Fig. 3). The reintroduction of such a small number of individuals would be insufficient to ensure the long-term survival of the population ($P_{100} < 0.1$; Fig. 3).

For all scenarios, an initial phase of population growth, with a peak of 4–33 individuals ($< K$) 15–20 years after the reintroduction, was followed by a progressive decline leading to $N_{100} < N_o$ for all models, except for those of scenario 1 based on the concurrent release of 2–3 pairs ($N_{100} = 12$ and 20 individuals, respectively). As expected, for most models the probability of extinction in the first decade post-release was high (c. 70%).

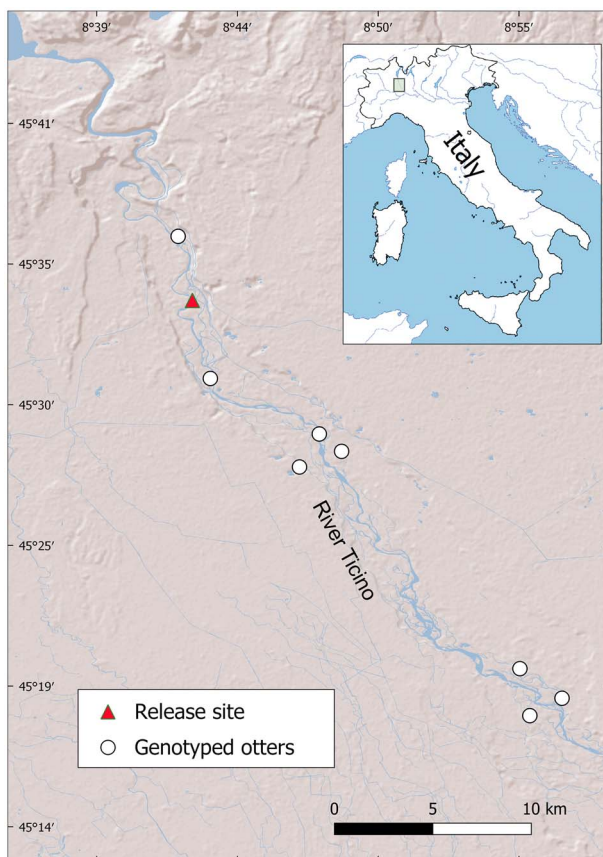


FIG. 1 Distribution of genotyped otter *Lutra lutra* spraints (2016–2018) on the River Ticino, Italy.

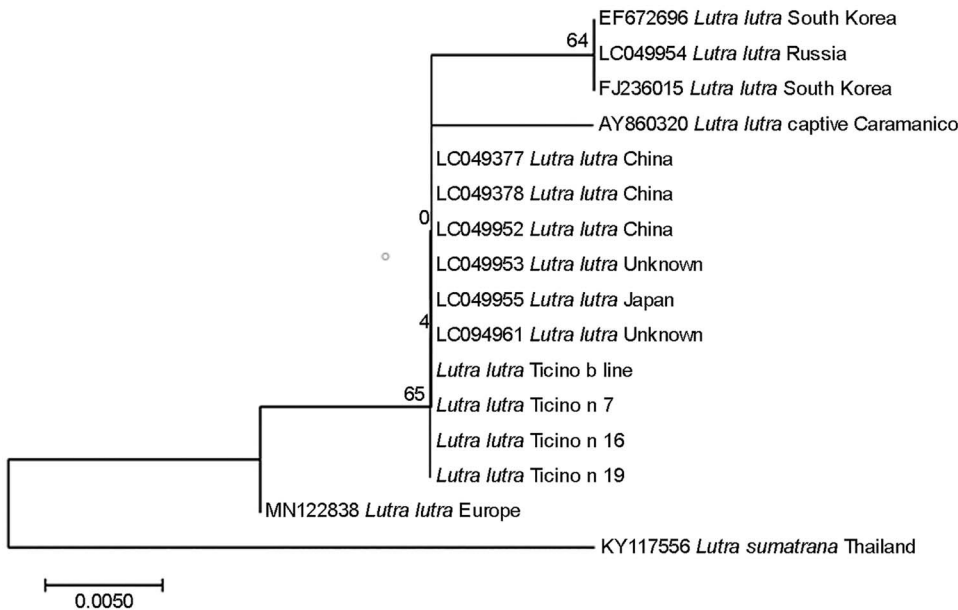


FIG. 2 Neighbour joining tree showing the phylogenetic relationship between otters from the River Ticino (both non-invasive samples and one individual found dead on the road) and European, Chinese and Korean lineages.

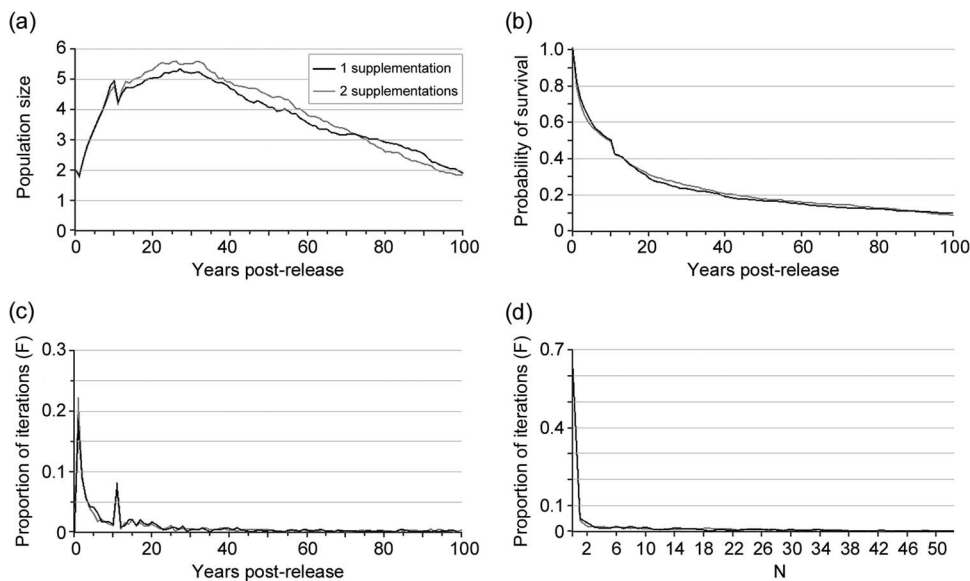


FIG. 3 *Vortex* simulations (scenario 1) for the reintroduced otter population, with initial population size $N_0 = 2$ and the supplementation of four individuals in the following years (two per year): (a) population trend, (b) probability of survival, (c) proportion of iterations (F) that lead to extinction, and (d) proportion of iterations that lead to population size = N , 21 years after the reintroduction.

Sensitivity analyses indicated inbreeding depression as the main cause of variation in the probability of population survival (Fig. 4). For numbers of lethal equivalents below the default value proposed by *Vortex* it is an index of inbreeding depression used by *Vortex*, as added in the Methods (6.29), the probability of survival increased from 12 to 45%.

Discussion

Despite the small number of founder individuals and lack of post-release monitoring, an otter population has survived on the River Ticino since 1997. Signs of otter presence were recorded on a 7 km stretch of the river in 2010 (Prigioni & Balestrieri, 2011), and along 60 and 32 km in

2017 and 2018, respectively. Throughout the study period, the population comprised few individuals. Although the sample size and number of re-captures were insufficient to assess population size effectively, the number of individuals was considerably smaller than the predicted carrying capacity. The minimum number of individuals was consistent with that assessed by the most likely scenario in the population viability analysis, based on the information available: an initial population consisting of only one pair of otters, supplemented with a further pair per year in the following 2 years.

Faecal samples collected in 2016–2017 were stored for 1 year before extraction, whereas those found in 2018 were all analysed irrespective of how fresh they were. Despite

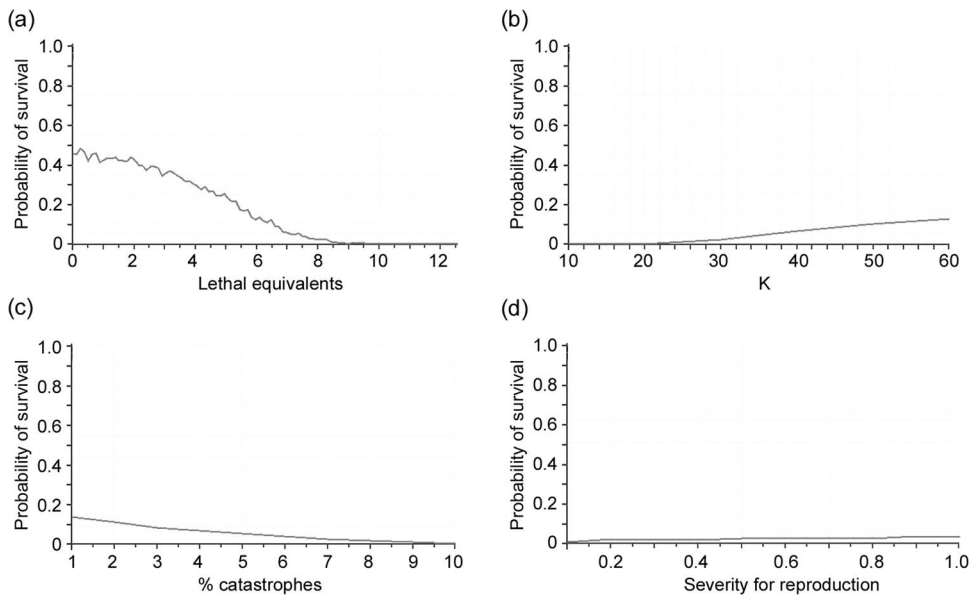


FIG. 4 *Vortex* sensitivity analysis, modelling the variation in the probability of survival of the reintroduced otter population for four uncertain parameters: (a) the number of lethal equivalents (increments of 0.629), (b) carrying capacity K (increments of 5), (c) per cent catastrophes (increments of 1%), and (d) severity for reproduction (increments of 0.1).

this difference in the treatment of samples from different years, genotyping was reasonably successful, and our success rate of 32% was in the range of that reported in previous studies (e.g. 14%, Lanszki et al., 2008; 21%, Ferrando et al., 2008; 41%, Prigioni et al., 2006b; 73%, Janssens et al., 2008). Throughout the otter's European range, H_o is 0.35–0.69, and the mean number of alleles per locus is 4.9, ranging from 2.6 in southern Italy to 6.8 in Norway (Mucci et al., 2010). Although these numbers cannot be directly compared, as four of our microsatellites differed from those used by Mucci et al. (2010), both the heterozygosity and polymorphism recorded in the Ticino population were as low as those reported for relict populations of southern Italy (Verduci, 2019), and consistent with the small founder population.

Although in the mid 1990s the EEP coordinator considered it unlikely that Asian otters had contributed to the programme's captive population (Vogt, 1995), mtDNA analysis confirmed the suspected B-line. Although otter signs are conspicuous, small isolated populations can remain undetected for several years (Gariano & Balestrieri, 2018). Our findings do not support the hypothesis that a residual, autochthonous population persisted that may have been reinforced by the reintroduction programme.

Since reintroduction, the size of the otter population probably fluctuated randomly on the edge of extinction, between 2–3 and <10 individuals, unable to spread throughout the aquatic habitats of the River Ticino valley. As the river has been judged suitable for otters by three different analyses (Prigioni, 1995; Montanari & Boffino, 2000; Prigioni & Balestrieri, 2011), the insufficient number of founders may be a major cause of low population growth.

All models in the population viability analysis suggest that the number of founders was also too few to ensure the long-

term survival of the population; the simultaneous release of three pairs of otters would have increased population size and probability of persistence, although the risk of extinction in the short term would still have been high. Similarly, the extinction probability of a larger group of 10 otters in Upland, Sweden, has been reported to be 49% within 50 years (Ebenhard, 2000). Such small groups also tend to lose half of the heterozygosity over 50 years, decreasing population fitness (Ebenhard, 2000; Reed & Frankham, 2003).

Inbreeding depression plays a major role in genetic stochasticity and increases extinction risk for most threatened mammals (O'Grady et al., 2006). Consistently, according to population viability analysis, inbreeding depression is the factor that may contribute most to the variation in the extinction risk of the small populations on the River Ticino.

Considering that the genetic variability of the founder population was less than expected by EEP managers (Randi et al., 2001), there is reason to assume that the number of lethal equivalents was higher than the default value used by *Vortex*. Under such conditions, stochastic events such as reproduction failure or road mortality would be expected to drive this small isolated population to extinction.

There has been debate concerning a possible reinforcement of the reintroduced otter population (Balestrieri et al., 2016). Although B-line otters have also been reintroduced in the UK and the Netherlands (Duplaix & Savage, 2018), the confirmed genetic composition of the Ticino population is a major hurdle to its reinforcement with wild-caught otters, for example from Austria, where otters are widespread and perceived as a threat by fish farmers (Kranz, 2000). On the other hand, although in the 2000s the population was completely isolated, recent expansion of otter populations in the French (Barthelemy & Arthur, 2018), Austrian (Kranz, 2018) and Swiss Alps (Angst, 2018)

suggests that the River Ticino could be a potential route for future recolonization of northern Italy, and supports the need for further conservation actions. As a first step, any reinforcement project would require a new feasibility study and modelling of the release phase, to assess the number of individuals needed to achieve population viability in the long term.

The reintroduction project attempted on the River Ticino faced several of the 18 key problems described by Macdonald (2009). In particular, it was not coordinated on a national level, the genetic origin of reintroduced animals was not considered, the number of individuals released was insufficient and there was no post-release monitoring. Despite this, in the last 2 decades the reintroduction has been considered a success, and a small population of otters persists on the river. Our findings highlight the need for long-term monitoring and viability analysis of reintroduced populations, as even positive trends in the first years post-release do not guarantee success in the longer term (Short, 2016).

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Author contributions Research conception: AB, PT; fieldwork: AB, PB; genetic analysis: LG, FV, EG, EC, NM, CM; data analysis: AB, LG, FV, PB; writing: AB, PT, NM.

Conflicts of interest None.

Ethical standards The research was non-intrusive and had the necessary approval and permits from the administration of Parco Lombardo della Valle del Ticino. The research conforms to the standards set out by the British Sociological Association and the Oryx guidelines on ethical standards.

References

- ALBRECHT, M.A., GUERRANT JR, E.O., MASCHINSKI, J. & KENNEDY, K.L. (2011) A long-term view of rare plant reintroduction. A response to Godefroid et al. 2011: How successful are plant reintroductions? *Biological Conservation*, 144, 2557–2558.
- ANGST, C. (2018) *Status of the Eurasian Otter in Switzerland*. Eurasian Otter in the Alps: Towards a Common Strategy Across Boundaries Congress, 10–12 October 2018. Gran Paradiso National Park, Valsavarenche, Italy.
- ANSORGE, H., SCHIPKE, R. & ZINKE, O. (1997) Population structure of the otter, *Lutra lutra*; parameters and model for a Central European region. *Mammalian Biology*, 62, 142–151.
- ARMSTRONG, D.P. & REYNOLDS, M.H. (2012) Modelling reintroduced populations: The state of the art and future directions. In *Reintroduction Biology: Integrating Science and Management* (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 175–221. Blackwell Publishing, Chichester, UK.
- ARMSTRONG, D.P. & SEDDON, P.J. (2008) Directions in reintroduction biology. *Trends in Ecology & Evolution*, 23, 20–25.
- ARRENDAL, J., WALKER, C.W., SUNDQVIST, A.K., HELLBERG, L. & VILA, C. (2004) Genetic evaluation of an otter translocation program. *Conservation Genetics*, 5, 79–88.
- AUBRY, K.B. & LEWIS, J.C. (2003) Extirpation and reintroduction of fishers (*Martes pennanti*) in Oregon: implications for their conservation in the Pacific states. *Biological Conservation*, 114, 79–90.
- BALESTRIERI, A., REMONTI, L. & PRIGIONI, C. (2016) Towards extinction and back: decline and recovery of otter populations in Italy. In *Problematic Wildlife: A Cross-Disciplinary Approach* (ed. F.M. Angelici), pp. 91–105. Springer International Publishing, Cham, Switzerland.
- BALESTRIERI, A., TIROZZI, P., AGOSTANI, E. & SAINO, N. (2018) Long-term assessment of the success of a European badger reintroduction. *Mammalia*, 82, 410–413.
- BARTHELEMY, V. & ARTHUR, C. (2018) *The Situation of the Otter in France. Which Future for the Species in the French Alps?* Eurasian Otter in the Alps: Towards a Common Strategy Across Boundaries Congress, 10–12 October 2018. Gran Paradiso National Park, Valsavarenche, Italy.
- BONESI, L., HALE, M. & MACDONALD, D.W. (2013) Lessons for the use of non-invasive genetic sampling as a way to estimate Eurasian otter population size and sex-ratio. *Acta Theriologica*, 58, 157–168.
- BREITENMOSE, U., BREITENMOSE-WÜRSTEN, C. & CAPT, S. (1998) Reintroduction and present status of the lynx (*Lynx lynx*) in Switzerland. *Hystrix*, 10, 17–30.
- CASSOLA, F. (1986) *La Lontra in Italia*. Serie Atti e Studi no. 5, WWF-Italia, Rome, Italy.
- CATTANEO, M., MAIONE, U., MIGNOSA, P. & TOMIROTTI, M. (2000) L'evento di piena dell'ottobre 2000 sul bacino del Ticino. *L'Acqua*, 6, 7–16.
- CAUGHLEY, G. & GUNN, A. (1996) *Conservation Biology in Theory and Practice*. Blackwell Science, Cambridge, UK.
- CHRISTIANSEN, S.D. (2017) *An investigation of the foundation for reintroducing the Eurasian otter (Lutra lutra) in northern Zealand*. Degree thesis, Department of Geosciences and Natural Resource Management, University of Copenhagen, Copenhagen, Denmark.
- CONVERSE, S.J., MOORE, C.T. & ARMSTRONG, D.P. (2013) Demographics of reintroduced populations: estimation, modeling, and decision analysis. *The Journal of Wildlife Management*, 77, 1081–1093.
- DALLAS, J.F., BACON, P.J., CARSS, D.N., CONROY, J.W.H., GREEN, R., JEFFERIES, D.J. et al. (1999) Genetic diversity in the Eurasian otter *Lutra lutra*, in Scotland. Evidence from microsatellite polymorphism. *Biological Journal of the Linnean Society*, 68, 73–86.
- DALLAS, J.F., COXON, K.E., SYKES, T., CHANIN, P.R.F., MARSHALL, F., CARSS, D.N. et al. (2003) Similar estimates of population genetic composition and sex ratio derived from carcasses and faeces of Eurasian otter *Lutra lutra*. *Molecular Ecology*, 12, 275–282.
- DALLAS, J.F. & PIERTNEY, S.B. (1998) Microsatellite primers for the Eurasian otter. *Molecular Ecology*, 7, 1248–1251.
- DODD JR, C.K. (2005) Population manipulations. In *Amphibian Declines: The Conservation Status of United States Species* (ed. M.J. Lannoo), pp. 265–270. University of California Press, Berkeley, USA.
- DUPLAIX, N. & SAVAGE, M. (2018) *The Global Otter Conservation Strategy*. IUCN/SSC Otter Specialist Group, Salem, USA.
- EBENHARD, T. (2000) Population viability analyses in endangered species management: the wolf, otter and peregrine falcon in Sweden. *Ecology Bulletin*, 48, 143–163.

- ESTRADA, J. (2014) *Finding correlations among successful reintroduction programs: an analysis and review of current and past mistakes*. MSc thesis, University of Michigan, Ann Arbor, USA.
- FERNÁNDEZ-MORÁN, J., SAAVEDRA, D. & MANTECA-VILANOVA, X. (2002) Reintroduction of the Eurasian otter (*Lutra lutra*) in northeastern Spain: trapping, handling, and medical management. *Journal of Zoo and Wildlife Medicine*, 33, 222–227.
- FERRANDO, A., LECIS, R., DOMINGO-ROURA, X. & PONSÀ, M. (2008) Genetic diversity and individual identification of reintroduced otters (*Lutra lutra*) in north-eastern Spain by DNA genotyping of spraints. *Conservation Genetics*, 9, 129–139.
- FISCHER, J. & LINDENMAYER, D.B. (2000) An assessment of the published results of animals relocations. *Biological Conservation*, 96, 1–11.
- GALEOTTI, P. (1981) *Parco Ticino. I Mammiferi*. Biblioteca Parco Ticino, Gruppo Fabbri Ed., Milan, Italy.
- GAONA, P., FERRERAS, P. & DELIBES, M. (1998) Dynamics and viability of a metapopulation of the Endangered Iberian lynx (*Lynx pardinus*). *Ecological Monographs*, 68, 349–370.
- GARIANO, P. & BALESTRIERI, A. (2018) “Otter, come out!”: taking away the stone on the southernmost Italian *Lutra lutra* population. *Ecoscience*, 25, 287–294.
- GEBOES, A.-L., ROSOUX, R., LEMARCHAND, C., HANSEN, E. & LIBOIS, R. (2016) Genetic diversity and population structure of the Eurasian otter (*Lutra lutra*) in France. *Mammal Research*, 61, 121–129.
- GILPIN, M.E. & SOULÉ, M.E. (1986) Minimum viable populations: processes of species extinction. In *Conservation Biology: The Science of Scarcity and Diversity* (ed. M.E. Soulé), pp. 19–34. Sinauer, Sunderland, USA.
- GRIFFITH, B., SCOTT, J.M., CARPENTER J.W. & REED, C. (1989) Translocation as a species conservation tool: status and strategy. *Science*, 245, 477–480.
- HÁJKOVÁ, P., ZEMANOVÁ, B., BRYJA, J., HÁJEK, B., ROCHE, K., TKADLEC, E. & ZIMA, J. (2006) Factors affecting success of PCR amplification of microsatellite loci from otter faeces. *Molecular Ecology Notes*, 6, 559–562.
- HALL, T.A. (1999) *Bioedit*: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
- HAMILTON, S. & MOLLER, H. (1995) Can PVA models using computer packages offer useful conservation advice? Sooty shearwaters *Puffinus griseus* in New Zealand as a case study. *Biological Conservation*, 73, 107–117.
- HAUER, S., ANSORGE, H. & ZINKE, O. (2002) Mortality patterns of otters (*Lutra lutra*) from eastern Germany. *Journal of Zoology*, 256, 361–368.
- HUANG, C.-C., HSU, Y.-C., LEE, L.-L. & LI, S.-H. (2005) Isolation and characterization of tetramicrosatellite DNA markers in the Eurasian otter (*Lutra lutra*). *Molecular Ecology Notes*, 5, 314–316.
- HUNG, C.-M., LI, S.-H. & LEE, L.-L. (2004) Faecal DNA typing determines the abundance and spatial organization of otters (*Lutra lutra*) along two stream systems in Kinmen. *Animal Conservation*, 7, 301–311.
- IUCN/SSC (2013) *Guidelines for Reintroductions and Other Conservation Translocations*. Version 1.0. IUCN Species Survival Commission, Gland, Switzerland.
- JANG, K.H., RYU, S.H. & HWANG, U.W. (2009) Mitochondrial genome of the Eurasian otter *Lutra lutra* (Mammalia, Carnivora, Mustelidae). *Genes & Genomics*, 31, 19–27.
- JANSSENS, X., FONTAINE, M.C., MICHAUX, J.R., LIBOIS, R., DE KERMAON, J., DEFOURNY, P. & BARET, P.V. (2008) Genetic pattern of the recent recovery of European otters in southern France. *Ecography*, 31, 176–186.
- KALZ, B., JEWGENOW, K. & FICKEL, J. (2006) Structure of an otter (*Lutra lutra*) population in Germany—results of DNA and hormone analyses from faecal samples. *Mammalian Biology*, 71, 321–335.
- KETMAIER, V. & BERNARDINI, C. (2005) Structure of the mitochondrial control region of the Eurasian otter (*Lutra lutra*; Carnivora, Mustelidae): patterns of genetic heterogeneity and implications for conservation of the species in Italy. *Journal of Heredity*, 96, 318–328.
- KI, J.-S., HWANG, D.-S., PARK, T.-J., HAN, S.-H. & LEE, J.-S. (2010) A comparative analysis of the complete mitochondrial genome of the Eurasian otter *Lutra lutra* (Carnivora; Mustelidae). *Molecular Biology Reports*, 37, 1943–1955.
- KING, T., CHAMBERLAN, C. & COURAGE, A. (2013) Assessing reintroduction success in long-lived primates through population viability analysis: western lowland gorillas *Gorilla gorilla gorilla* in Central Africa. *Oryx*, 48, 294–303.
- KOELEWIJN, H.P., PÉREZ-HARO, M., JANSMAN, H.A.H., BOERWINKEL, M.C., BOVENSCHEN, J., LAMMERTSMA, D.R. et al. (2010) The reintroduction of the Eurasian otter (*Lutra lutra*) into the Netherlands: hidden life revealed by noninvasive genetic monitoring. *Conservation Genetics*, 11, 601–614.
- KRANZ, A. (2000) Otters (*Lutra lutra*) increasing in Central Europe: from the threat of extinction to locally perceived overpopulation? *Mammalia*, 64, 357–368.
- KRANZ, A. (2018) *Recolonization of the Austrian Alps by Otters and Conflicts*. Eurasian Otter in the Alps: Towards a Common Strategy Across Boundaries Congress, 10–12 October 2018. Gran Paradiso National Park, Valsavarenche, Italy.
- KRUUK, H. (2006) *Otter: Ecology, Behaviour and Conservation*. Oxford University Press, New York, USA.
- KUHN, R. & JACQUES, H. (2011) *La Loutré d'Europe*. Encyclopédie des Carnivores de France, SFPEM, Bourges, France.
- KUMAR, S., STECHER, G. & TAMURA, K. (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.
- LACY, R.C. (1993) VORTEX: a computer simulation model for population viability analysis. *Wildlife Research*, 20, 45–65.
- LACY, R.C. & POLLAK, J.P. (2018) *Vortex: A Stochastic Simulation of the Extinction Process*. Version 10.3.1. Chicago Zoological Society, Brookfield, USA.
- LANSZKI, J., HIDAS, A., SZENTES, K., RÉVAY, T., LEHOCZKY, I. & WEISS, S. (2008) Relative spraint density and genetic structure of otter (*Lutra lutra*) along the Drava River in Hungary. *Mammalian Biology*, 73, 40–47.
- LARSSON, K. & EBENHARD, T. (1994) Isolerade delpopulationer av utter: en sarbarhetsanalys. *Världsnaturfondens Rapport Serie*. WWF, Stockholm, Sweden.
- LERONE, L., DE CASTRO, G., IMPERI, F., MARRESE, M., CARRANZA, M.L., SCORPIO, V. & LOY, A. (2014) Population structure and range dynamics of an isolated otter population in Italy: lessons from the field. *IUCN OSG XII International Otter Congress*, 1–15 August 2014, Rio de Janeiro, Brazil.
- MACDONALD, D.W. (2009) Lessons learnt and plans laid: seven awkward questions for the future of reintroductions. In *Reintroduction of Top-Order Predators* (eds M.W. Hayward & M. Somers), pp. 411–448. John Wiley & Sons, Hoboken, USA.
- MACDONALD, S.M. & MASON, C.F. (1990) Action plan for European otters. In *An Action Plan for Their Conservation* (eds P. Foster-Turley, S.M. Macdonald & C.F. Mason), pp. 29–40. An IUCN Publication c/o Chicago Zoological Society, Brookfield, USA.
- MACDONALD, S.M. & MASON, C.F. (1994) *Status and Conservation Needs of the Otter (Lutra lutra) in the Western Palearctic*. Nature Environment Series, 67, Council of Europe, Strasbourg, France.

- MASON, C.F. & MACDONALD, S.M. (2004) Growth in otter (*Lutra lutra*) populations in the UK as shown by long-term monitoring. *Ambio*, 33, 148–52.
- MOHD SALLEH, F., RAMOS-MADRIGAL, J., PEÑALOZA, F., LIU, S., MIKKEL-HOLGER, S.S., RIDDHI, P.P. et al. (2017) An expanded mammal mitogenome dataset from Southeast Asia. *GigaScience*, 6, 1–8.
- MONTANARI, M. & BOFFINO, G. (2000) *Il Ritorno della Lontra. 1990–2000, il Progetto di Reintroduzione nel Parco Naturale della Valle del Ticino Piemontese*. Parco Ticino Piemontese e Regione Piemonte, Italy.
- MUCCI, N., ARRENDAL, J., ANSORGE, H., BAILEY, M., BODNER, M., DELIBES, M. & RANDI, E. (2010) Genetic diversity and landscape genetic structure of otter (*Lutra lutra*) populations in Europe. *Conservation Genetics*, 11, 583–599.
- MURAKAMI, T. (2017) *Shiretoko Otter Reintroduction Feasibility Study*. Technical report, Shiretoko Museum and Shiretoko Nature Foundation, Shari, Japan.
- MUTHS, E. & DREITZ, V. (2008) Monitoring programs to assess reintroduction efforts: a critical component in recovery. *Animal Biodiversity and Conservation*, 31, 47–56.
- O'GRADY, J., BROOFE, B.W., REED, D.H., BALLON, J.D., TONKYN, D.W. & FRANKHAM, R. (2006) Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation*, 133, 42–51.
- PRIGIONI, C. (1986) La lontra in Lombardia e nelle province di Asti, Alessandria, Parma e Piacenza. In *La Lontra in Italia* (ed. F. Cassola), pp. 25–28. Serie Atti e Studi no. 5, WWF-Italia, Rome, Italy.
- PRIGIONI, C. (1995) Guidelines for the feasibility study of reintroduction of the otter (*Lutra lutra*) in Italy: the project of the Ticino valley (north-western Italy). In *Proc. II It. Symp. On Carnivores* (ed. C. Prigioni). *Hystrix*, 7, 255–264.
- PRIGIONI, C. & BALESTRIERI, A. (2011) *Piano di Monitoraggio dei Vertebrati Terrestri di Interesse Comunitario (Direttive 79/409/CEE e 92/43/CEE) in Lombardia. Monitoraggio dei Mammiferi: Lontra Lutra lutra*. Technical Report, University of Pavia, Pavia, Italy.
- PRIGIONI, C., BALESTRIERI, A. & REMONTI, L. (2007) Decline and recovery in otter *Lutra lutra* populations in Italy. *Mammal Review*, 37, 71–79.
- PRIGIONI, C., BALESTRIERI, A., REMONTI, L., SGROSSO, S. & PRIORE, G. (2006a) How many otters are there in Italy? *Hystrix*, 17, 29–36.
- PRIGIONI, C., REMONTI, L., BALESTRIERI, A., SGROSSO, S., PRIORE, G., MUCCI, N. & RANDI, E. (2006b) Estimation of European otter (*Lutra lutra*) population size by fecal DNA typing in southern Italy. *Journal of Mammalogy*, 87, 855–858.
- PRIGIONI, C., SMIROLODO, G., REMONTI, L. & BALESTRIERI, A. (2009) Distribution and diet of reintroduced otters (*Lutra lutra*) on the River Ticino (NW Italy). *Hystrix*, 20, 45–53.
- RANDI, E., DAVOLI, F. & PIERPAOLI, M. (2001) *Diversità Genetica in Popolazioni di Lontra in Europa*. III Convegno SMAMP, La Lontra in Italia: Distribuzione, Censimenti e Tutela, Montella, Italy.
- RANDI, E., MUCCI, N., ARREL, G., BAILEY, M., BONDER, M., DALLAS, L. et al. (2005) *Assessing the Patterns of Genetic Diversity in Otter Populations in Europe*. Proclamation of the European Otter Workshop, Padula, Italy.
- REED, D.H. & FRANKHAM, R. (2003) Correlation between fitness and genetic diversity. *Conservation Biology*, 17, 230–237.
- ROCHE, K., HARRIS, R., WARRINGTON, S. & COPP, G.H. (1995) Home range and diet of re-introduced European otters *Lutra lutra* (L.) in Hertfordshire rivers. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 5, 87–96.
- RUIZ-OLMO, J., LAFONTAINE, L., PRIGIONI, C., LÓPEZ-MARTÍN, J.M. & SANTOS-REIS, M. (2000) Pollution and its effects on otter populations in south-western Europe. In *Proceedings of the First Otter Toxicology Conference* (eds J.W.H. Conroy, P. Yoxon & A.C. Gutleb). *Journal of the International Otter Survival Fund*, 1, 63–82.
- SARRAZIN, F. (2007) Introductory remarks: a demographic frame for reintroduction. *Ecoscience*, 14, iii–v.
- SARRAZIN, F. & BARBAULT, R. (1996) Reintroduction: challenges and lessons for basic ecology. *Tree*, 11, 474–478.
- SEDDON, P.J. (1999) Persistence without intervention: assessing success in wildlife re-introductions. *Trends in Ecology and Evolution*, 14, 503.
- SEMLITSCH, R.D. (2002) Critical elements for biologically based recovery plans of aquatic-breeding amphibians. *Conservation Biology*, 16, 619–629.
- SHORT, J. (2016) Predation by feral cats key to the failure of a long-term reintroduction of the western barred bandicoot (*Perameles bougainville*). *Wildlife Research*, 43, 38–50.
- SJÖÅSEN, T. (1996) Survivorship of captive-bred and wild-caught reintroduced European otters *Lutra lutra* in Sweden. *Biological Conservation*, 76, 161–165.
- SMIROLODO, G., BALESTRIERI, A., PINI, E. & TREMOLADA, P. (2019) Anthropogenically altered trophic webs: alien catfish and microplastics in the diet of Eurasian otters. *Mammal Research*, 64, 165–174.
- SUTHERLAND, W.J. (2000) *The Conservation Handbook: Research, Management and Policy*. Blackwell Science, Oxford, UK.
- SUTTON, A.E. (2015) Leadership and management influence the outcome of wildlife reintroduction programs: findings from the Sea Eagle Recovery Project. *PeerJ*, 3, e1012.
- TREMOLADA, P., SMIROLODO, G., VERDUCI, F., GATTI, E., BOGGIONI, P., GIANFRANCESCO, L. et al. (2020) The otter population of the River Ticino (N Italy) 20 years after its reintroduction. *Journal of Mountain Ecology*, 13, 51–62.
- VERDUCI, F. (2019) *Metodi di censimento genetico applicati alla lontra eurasiatica (Lutra lutra): stima delle dimensioni delle popolazioni dei fiumi Ticino (Lombardia) e Lese (Calabria)*. MSc thesis, Università degli Studi di Milano, Milan, Italy.
- VERGARA, M., RUIZ-GONZÁLEZ, A., LÓPEZ DE LUZURIAGA, J. & GÓMEZ-MOLINER, B.J. (2014) Individual identification and distribution assessment of otters (*Lutra lutra*) through non-invasive genetic sampling: recovery of an endangered species in the Basque Country (Northern Spain). *Mammalian Biology*, 79, 259–267.
- VOGT, P. (1995) The European Breeding Programme (EEP) for *Lutra lutra*, its chances and problems. In *Proceedings II It. Symp. Carnivores* (ed. C. Prigioni). *Hystrix*, 7, 247–253.
- WAKU, D., SEGAWA, T., YONEZAWA, T., AKIYOSHI, A., ISHIGE, T., UEDA, M. et al. (2016) Evaluating the phylogenetic status of the extinct Japanese otter on the basis of mitochondrial genome analysis. *PLOS ONE*, 11, e0149341.
- WAYRE, P. (1985) A successful reintroduction of European otters. *Oryx*, 19, 137–139.