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# Long-term viability of a reintroduced population of roe deer *Capreolus capreolus*, in a lowland area of northern Italy

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**Abstract.** In the last decades European roe deer (*Capreolus capreolus*) expanded its range, recolonizing also highly anthropized areas. In northern Italy this species recently reached the Po Plain, suggesting the possibility for the future settlement of a metapopulation in the whole plain. In this process, a key role may be played by internal source areas, such as Ticino Natural Park, where roe deer was reintroduced in 1990s. We carried out drive counts and surveys in a sample area of Ticino NP, in order to investigate about the current status of this population. Population Viability Analysis (PVA) was applied to infer about the population viability and its potentiality as source for dispersals. Our results showed a high-density population ( $30.7 \pm 4.1$  individuals per km<sup>2</sup>), with quite low proportion of juveniles (10.7%), litter size (1.35 fawns/mother) and proportion of successful breeding females (24.6%). Considering the high density of this population, its low productivity is most likely due to the onset of density-dependent factors and the approach to the carrying capacity. Nevertheless, the PVA showed that in the next 30 years this population is likely to remain viable, representing an important potential source for a roe deer expansion in the fragmented lowland area of northern Italy.

**Key words:** population viability analysis, drive counts, population density, population structure, breeding success, metapopulation

## Introduction

In the last decades, the progressive abandonment of crops in upland and mountain areas, together with the introduction of hunting regulations, and environmental management practices, brought to a general recover of European roe deer (*Capreolus capreolus*, L. 1758) populations (Andersen et al. 2000, Gortázar et al. 2000, Acevedo et al. 2005, Burbaite & Csányi 2009, Maillard et al. 2010). This trend continues today with a substantial ongoing expansion of the species both in terms of population density and geographical range (Gortázar et al. 2000, Acevedo et al. 2005, Carnevali et al. 2009).

The European roe deer is an ecotonal species, typically thriving at the edge of woodlands or in glades within forests (Lovari et al. 2017); this high-biodiversity habitat is considered ideal to fulfil the requirements in terms of food resources, thick vegetation cover, and bedding sites for reproduction (Hewison et al. 2001, Acevedo et al. 2005).

However, roe deer is also able to settle down in agricultural landscapes (Acevedo et al. 2005, Dondina et al. 2018), showing a high adaptability that allows it to develop a farmland oriented behaviour strategy (Hewison et al. 2001). More specifically, studies carried out in Norway (Kjøstvedt et al. 1998), Spain (Aragón et al. 1995, Acevedo et al. 2005) and France (Hewison et al. 2001) reported that roe deer colonization of agricultural landscapes might be encouraged by a low degree of anthropization. Recently several roe deer populations have also been reported in densely populated and peri-urban areas (Dandy et al. 2011, Dondina et al. 2018). Undoubtedly the colonization of human-dominated areas poses a number of challenges due to presence of anthropogenic dispersal barriers (Coulon et al. 2004, Kuehn et al. 2006) therefore it is important to predict the possible expansion of the roe deer in these critical areas, in order to elaborate on time specific management plans. Considering the most extensively-

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anthropized regions, as for many European lowland areas, the high fragmentation degree implies that, in such landscapes, a metapopulation structure would be the most suitable option for large wildlife settlement (Elmhagen & Angerbjörn 2001), provided that a sufficient degree of connectivity is guaranteed (Groot Bruinderink et al. 2003, Dondina et al. 2018).

During the last decades, in northern and central Italy, roe deer recolonized its former range in the whole Alps and Apennines through natural expansions of residual populations, immigrations from central Europe and reintroduction programs (Vernesio et al. 2002, Apollonio et al. 2010, Meriggi et al. 2011). Subsequently, after the spontaneous recolonization of foothill areas, the species expanded its range towards lowland, with the occupancy of some areas in the Po Plain, along the tributaries of the River Po (Apollonio et al. 2010, Dondina et al. 2018). A recent study on a Po Plain population of roe deer (in North-western Po Plain, Dondina et al. 2018) reported that, in this highly-anthropized context, the species shows adaptations to non-optimal habitats and human disturbance, moving through the landscape relying on small patches of forest and sub-forest habitats (e.g. small woodlands, hedgerows, reforestations, poplar cultivations, and uncultivated fields) for shelter needs. These findings suggest that the whole Po Plain region may turn out to be suitable for the future settlement of a large roe deer metapopulation, which would allow the species to regain a significant part of its original range in northern Italy.

The Po Plain is characterized by several small forest residues and few large and continuous forest areas, suggesting that a source-sink dynamic for a roe deer metapopulation (Pulliam 1988) would fit in such a fragmented landscape. Here, the viability of the entire metapopulation would depend on the viability of subpopulations in the largest source areas and on their ability in sustaining sink subpopulations, providing a net flux of dispersing individuals (Pulliam 1988). In particular, the role of source areas for the long-term recolonization of the northern Italy lowlands by the roe deer may be played by the surrounding Alps and Apennines. However, given the large size of the lowland and the presence of several anthropogenic barriers, the presence of further internal source areas in the plain might be critical (Coulon et al. 2006, Breyne et al. 2014). Ticino Natural Park is a wide protected area composed of about 220 km<sup>2</sup> of continuous broad-leaved forest (mainly *Quercus-Carpinetum boreoitalicum*) along the River Ticino, located in the central-western part of the Po Plain. It is the largest

relict lowland forest of northern Italy and a strategic ecological corridor, connecting Alps to Apennines across the Po Plain. The park also hosts a roe deer population, originated from a reintroduction project carried out between the 1991 and the 1995 (Cravin & Roveda 2003). After the post-release survey (Cravin & Roveda 2003), no data were collected on this population, and to date little is known about its current demographic status. The presence of a large and viable roe deer population in Ticino Natural Park may play a key role for the restoration and maintenance of a stable metapopulation in the western and central part of the Po Plain. Hence, a better knowledge of the status of this population is essential not only for its proper management, but also to evaluate its potential role in the stable return of this species over the whole lowland. In this study we collected three years of observation data, in order to estimate 1) density, 2) structure and 3) reproductive parameters of this population, aiming to 4) infer about its long-term viability by means of a Population Viability Analysis (PVA). The possibility of this population to provide a source for dispersing individuals, towards the surrounding farmland, will also be discussed.

## Material and Methods

### Study area

The study was carried out in a 3 km<sup>2</sup> area located on the left side of the lower course of the River Ticino, in Lombardy (northern Italy, 45°24'35" N, 8°50'32" E; Fig. 1). Almost all the study area falls within the boundaries of Ticino Natural Park, and the SCIs (Sites of Community Importance) "Boschi della Fagiana" and "Basso corso e sponde del Ticino" of the Natura 2000 network (Perracino 2010). The surface is covered by lowland broadleaf woodlands (51 %), mostly in the western part along the River Ticino, and crops with rural buildings (49 %) in the eastern portion. Woodlands are composed for the most part by mesophile forest, dominated by oaks (*Quercus robur* and *Quercus petraea*) and common hornbeam (*Carpinus betulus*), characterized by a shrub layer with common hazel (*Corylus avellana*), bird cherry (*Prunus padus*), elder (*Sambucus nigra*) and brambles (*Rubus* spp.). A lesser part is composed of hygrophilous woodlands, with common alder (*Alnus glutinosa*), poplars (*Populus nigra* and *Populus alba*) and willows (*Salix* spp.). The cultivated areas are represented by rotational crops (rice, winter wheat, maize, soy bean, and hay fields). Moreover, a small part of the study area (13 %) is covered by traditional poplar groves and reforestations. The study area is part of a narrow

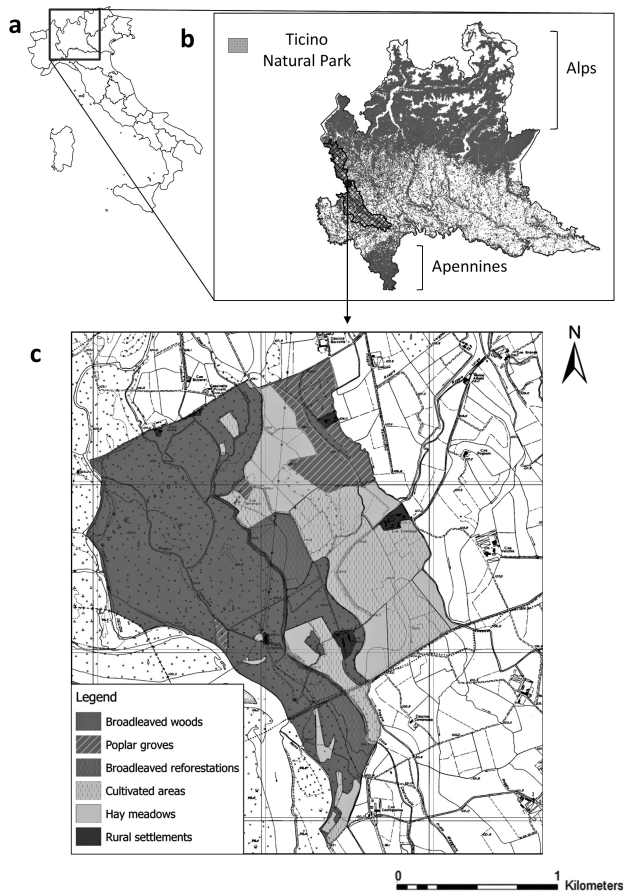
strip of protected woodlands, extended north and southwards, on both banks of the River Ticino (also in Piedmont region), while the surrounding area is an open farmland, rich in small and medium size urban settlements and industrial areas, with a high density of roads and wide irrigation channels.

The climate is sub-continental temperate, with annual mean precipitation of 1211.7 mm (mostly in spring and autumn) and mean annual temperature of 11.6 °C, ranging from a mean of 1.7 °C in January to a mean of 21.8 °C in August (1971-2000 data, station of Milan Malpensa, CNMCA 2009). The study area is located less than 2 km south of the release site of the 33 founder roe deer (from Boschi di Carrega Regional Park, South-central Po Plain) and the species presence within the area is reported since 1991 (Cravin & Roveda 2003). The only roe deer potential predator occurring in the study area, is the red fox (*Vulpes vulpes*), which is known to actively prey on fawns within the first 40 days of life (Jarnemo et al. 2004, Panzacchi et al. 2008). There are no other ungulate species in the study area, except for the wild boar (*Sus scrofa*).

### Density and population size

To estimate the roe deer population density and size within the study area, we carried out drive counts for three years (2014-2016). The study area was mapped starting from field surveys and digital cartography DUSAF 4 (ERSAF 2014). Using the software QGIS (Quantum GIS Development Team 2015) we defined eight sample areas (mean surface =  $0.15 \pm 0.06$  km<sup>2</sup>; max = 0.22 km<sup>2</sup>, min = 0.08 km<sup>2</sup>), according to their representativeness of the different categories of wooded areas (woodlands, poplar cultivations, and reforestations) characterizing the study area (Mayle et al. 1999, Sutherland 2006, Borkowski et al. 2011). The wooded sample areas were delimited by wide ditches or canals, forest roads, clearings and open fields. This allowed us to easily detect all the deer that came out of the sample areas (Mayle et al. 1999, Borkowski et al. 2011). All the sample areas were included in the counts for all the three years, except for one that was replaced in the second year, because of the cutting of a poplar grove.

Drive counts were carried out each year in the first two weeks of April. In this period, one month before the breeding season, the species is already homogeneously spread through the habitat, after the winter group breakup (Cibien et al. 1989, Sempéré et al. 1996). Moreover, in early April the vegetation growth is not such as to strongly affect visibility within woodlands. Each drive count was achieved with the collaboration of 30-40 people, among beaters and observers, from 9.30 a.m. till 1.30 p.m., with the assumption that in daylight hours all roe deer returned in wooded areas to rest. The observers were all experts in the recognition of the age and sex of the roe deer and trained with several observation sessions on our population. Moreover among the beaters several expert operators were placed. The beaters were instructed to move very slowly so that the animals would arrive in front of the observers at a moderate speed. Beaters were lined up along one side of the area and on the other sides observers were placed at variable distances depending on the visibility and in such a way to detect all fleeing animals. Flushed deer were counted by observers except for those breaking through the beater's line that were counted by beaters. Observers recorded on a card the deer observations reporting number, sex and age and time of observation. A coordinator collected the same information from the beaters. At the end of each drive the coordinator immediately resolving any possible doubt to reduce the probability of double counts. To increase the accuracy of the method, the drive counts were carried



**Fig. 1.** Lombardy region in northern Italy (a), forest cover in Lombardy and location of the study area within Ticino Natural Park (b), and land use map of the study area (c).

out in two days, one week apart, in order to cover adjacent areas in different times, minimizing the risk of double counts. The chosen days were characterized by clear sky and optimal visibility. The high number of participants allowed displaying a dense beater frontline and a large observer network, so that the possibility of uncounted individuals was negligible. Furthermore, a half of the observers and some beaters were equipped with cameras for a posteriori classification of counted individuals by sex and age. For each year, starting from the counts within sample areas, we provided estimates of population density within the wooded areas, overall population size and population density in the whole study area (including open habitats). Finally we also provided estimates of the mean population density and size over the three years.

#### *Population structure*

We classified the roe deer detected during the drive counts by sex and age using a method based on the evaluation of key body characteristics, commonly in use for roe deer culling (Putman 2005). To assess the sex, we relied on the presence or absence of the antlers and on the rump patch shape (bean-shaped in males, heart-shaped with anal tuft in females). To estimate the age, we considered three age classes: juveniles (< 1 year), sub-adults (< 2 years) and adults (> 2 years). To classify the animals, we relied on the combined evaluation of summer coat change degree (complete in younger animals while only in the initial phase in older ones) and body proportions (overall size and shape, muscular development, relative head length and chest size, neck thickness). To estimate the age of females we also relied either on the shape of the abdomen (convex in nulliparous younger ones, flat after the first birth) and the state of pregnancy. Antler size and shape (number of points and base diameter) are not considered good characteristics for ageing male roe deer, although they might be useful as supplementary indicator (Putman 2005). We thus relied on antlers only in a few cases of uncertainty, when clear snapshots were available.

In order to define the population structure, we calculated, for each year, the proportions of individuals in the different age classes and we tested for significant differences across the three years by the likelihood ratio (LR) test for contingency tables with permutation.

For each year, we also calculated the global and the within age class sex ratios ( $SR = \text{females/males}$ ) (Focardi et al. 2002) and we tested for the deviation from the expected sex ratio of one, by chi-square goodness-of-fit test with permutation. Moreover, we

tested for significant differences in the proportion of sexes across age classes, using the LR test for contingency tables. Finally, for each year, we calculated the spring juvenile/doe ratio (J/D ratio), dividing the number of observed juveniles by the number of observed adult females. Being a synthesis of births, early mortality and winter survival rates of fawns, this index is useful to give insights about population productivity, net of early and winter mortality. All the analyses were performed using the R software version 3.3.3 (R Development Core Team 2017).

#### *Reproductive parameters*

The reproductive parameters were obtained from data collected in two years (2013 and 2014) of roe deer population survey in the study area. The aim was to count the number of newborns and to estimate the proportion of successful breeding females. The surveys were performed in the post-reproductive period, from August to November, when roe deer fawns begin to follow the mothers in open areas, but the typical winter groups are not formed yet (Sempéré et al. 1996, Linnell et al. 1998), so that, it was easy to assign any observed fawn to its own mother. Data were collected through direct observation from a car of family groups, driving through the whole road network of the study area once a week, at dawn or at dusk. Moreover, monthly night surveys were performed by using a spotlight from a car.

To collect data in the western part of the study area, inaccessible by car, we installed 20 camera traps once a month for 48 hours in randomly selected sites inside the cells identified by a 250-m spaced grid following a tessellation stratified sampling (TSS; Barabesi & Franceschi 2011, Barabesi & Fattorini 2013). To avoid double counts, captures recorded less than 30 minutes apart, were merged into a single observation (Kelly & Holub 2008, Monterroso et al. 2014, Torretta et al. 2017). Observation data of fawns and mothers were used to infer the distribution of litter size in the population and to calculate a mean litter size (mean number of fawns [Fw] per mother [M], Fw/M ratio), net of the losses due to early and winter fawn mortality. Joined with census data, this parameter has been used to estimate the year's reproductive success (RS) of the population, in terms of proportion of successful breeding females over the estimated total number of adult females. We calculated RS by dividing the number of successful breeding females (i.e. the number of litters detected for each year), obtained as the ratio between the year's counted juveniles (J) divided the Fw/M ratio, to the year's total number

of adult females (Fe), as described by the following equation:

$$RS = \frac{J}{\frac{Fw/M}{Fe}}$$

Considering the mildness of the Po Plain winter climate and the absence of large predators, the juvenile winter mortality of this population should be very limited, so that the RS obtained using post-reproductive observations and spring census data, is a good estimation of the true proportion of successful breeding females (net of early and winter mortality).

#### Population viability

To predict the long-term viability of the investigated roe deer population, we performed a PVA starting from count and survey data, using the software VORTEX 10.0 (Lacy & Pollack 2014).

Input data were demographic and reproductive parameters collected from our census and post-reproductive survey, or literature data on other roe deer populations, considering, whenever possible, only populations matching with the one in this study (i.e. populations living in temperate woodlands, high-density populations, and absence of large predators) (Table 3). The species reproductive system is polygynous (Vanpé et al. 2008). The sexual maturity is reached at two years for females, and three for males, while the age of maximum reproduction is 13 years for both sexes, as well as the maximum lifespan (Sempéré et al. 1996). The maximum number of litters per year is one, with a maximum litter size of three fawns (Sempéré et al. 1996). Sex-ratio at birth for this population was unknown, so we choose to set it to 1:1 (50 % males), that is a typical ratio for a healthy roe deer population (Hewison & Gaillard 1996). Without any hint about fertile-age males unfit for reproduction, the proportion of adult males, potentially included in the reproductive pool is assumed to be 100 %. The initial population size and structure, as well as the current proportion of breeding females and the distribution of the number of fawns per litter have been defined from data collected in this study (see the Result section). To define density-dependent changes in the yearly proportion of breeding females, we used the estimated proportion of breeding females at both low and high population density, relying on post-release survey data (Cravin & Roveda 2003) and on our current data. The proportion of breeding females we set up, is a breeding success index based on spring juvenile (almost one year of age) counts, inclusive of fawn's early and winter mortality losses. To overcome this issue, we set fawn

mortality equal to 0, as this rate is already included in the above. For the remaining age classes, we calculated mean mortality rates from data reported in other studies (Gaillard et al. 1993, Marcandalli 2004, Mustoni et al. 2012). Thus, we set a mortality rate of 9.2 % for both sub-adult and adult females, 16.7 % for sub-adult males and 14.6 % for adult males. Since information about the presence of inbreeding depression in our population are lacking, this effects have been excluded from our simulations.

We included in our model the effect of major floods from May to July, as possible catastrophic events which may have relevant effects on this population. Wuczyński & Jakubiec (2013) reported a strong effect of flooding on roe deer fawn survival, while adults were only marginally affected by drowning risk. Based on national flood risk assessment (Po River Basin Authority 1998), major floods within the study area have a return period of 200 years and may affect up to 40 % of the study area surface. So, we set the annual major floods frequency to 0.5 %, while to simulate the effect of newborn losses in the areas at risk, in case of flooding we set the reproduction rate (percentage of breeding females) to drop by 40 % (reproduction: 0.6, survival: 1). The area carrying capacity (*K*) is unknown, thus, we simulated three different scenarios, hypothesizing  $K_1 = N$ ,  $K_2 = 1.5N$  and  $K_3 = 2N$ , where *N* is the initial population size estimated by drive counts. We did not include any harvest or introduction in our simulations. The simulations were run for 30 years, with 1000 iterations, and the extinction threshold was set on the disappearing of one sex (Lacy et al. 2015). For each *K* scenario, we reported a line graph of the mean population size across all the 1000 simulations ( $N[all]$ ) during the time interval, the final mean population size ( $N[all]_{30}$ ), the final probability

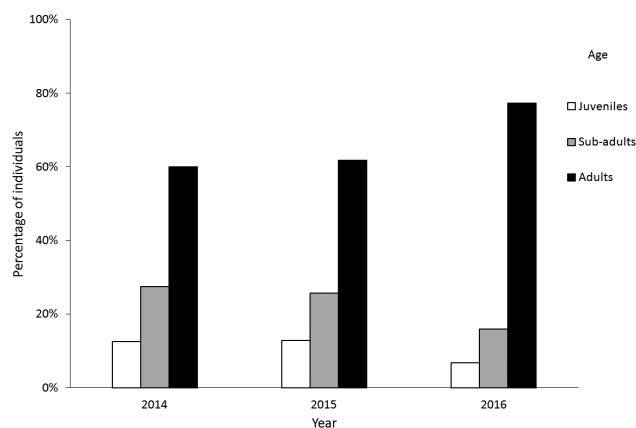
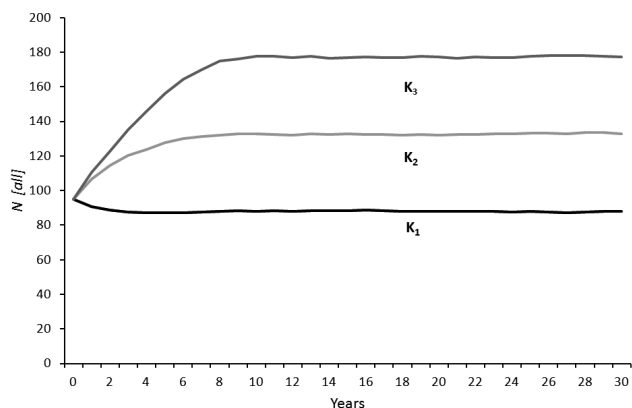


Fig. 2. Population structure for age classes of the reintroduced roe deer population of Ticino Natural Park (northern Italy, 2014-2016).



**Fig. 3.** Mean population size ( $N[all]$ ) across three different scenarios of carrying capacity ( $K_1 = 94$ ,  $K_2 = 141$  and  $K_3 = 188$ ), from 30 years Population Viability Analysis performed on the reintroduced roe deer population of Ticino Natural Park (northern Italy, 2014-2016).

of survival ( $P[ survive ]_{30}$ ) and the mean stochastic growth rate ( $stoch-r$ , calculated after  $K$  truncation) characterizing the simulated population after 30 years.

**Table 1.** Annual variations of global sex ratio F/M (SR) in the reintroduced roe deer population of Ticino Natural Park (northern Italy, 2014-2016).

Year	Females	Males	SR	$X^2$	$P$
2014	25	17	1.47	1.52	0.280
2015	30	21	1.42	1.59	0.262
2016	19	28	0.68	1.72	0.240
Total	74	66	1.12	0.46	0.499

**Table 2.** Annual variations of within-age class sex ratio F/M (SR) in the reintroduced roe deer population of Ticino Natural Park (northern Italy, 2014-2016).

Year	Juveniles			Sub-adults			Adults		
	SR	$X^2$	$P$	SR	$X^2$	$P$	SR	$X^2$	$P$
2014	1.5	0.20	1.000	2.7	2.27	0.227	1.0	0.00	1.000
2015	5.0	2.67	0.219	1.0	0.00	1.000	1.1	1.88	0.229
2016	0.5	0.33	1.000	0.4	1.29	0.453	0.6	1.88	0.229
Total	1.8	1.14	0.424	1.1	0.13	0.856	0.9	0.56	0.520

## Results

### Density and population size

Our density estimates can be considered accurate following the findings of Borkowski et al. 2011: 1) the driven areas covered in each count day were sufficiently isolated from each other to avoid double counting, 2) the number of beaters and observers was sufficient in respect to the driven area surface, 3) the target species is considered solitary thus avoiding the problems of aggregated observations (Hewison et al. 1998), 4) the period in which counts were carried out was at the end of aggregation phase of roe deer, 5) the densities were high or very high enhancing the accuracy of drive counts, and finally 6) the total

surface of driven areas was a very high percentage of the study area (37.5 %).

In 2014, 2015, and 2016 we estimated in the whole study area densities of 26.6, 33.1, and 32.5 roe deer per  $km^2$ , respectively (Table S1). Over the three years, the mean population density was  $30.7 \pm 4.1$  individuals/ $km^2$  while the mean population size was  $94 \pm 12$  roe deer.

### Population structure

The percentage of juveniles resulted quite low through the three years, decreasing from 12.5 % (2014) and 12.8 % (2015), to 6.8 % (2016). The percentage of sub-adults was higher in the first two years (27.5 % in 2014, 25.6 % in 2015) and lower in the last one (15.9 % in 2016). Conversely, adults were more than half of the population in all the three years, increasing from 60 % (2014) and 61.7 % (2015), to 77.3 % in 2016. However, the differences between years were not significant (LR = 2.48, df = 4,  $P = 0.671$ ) (Fig. 2). A total of the 150 roe deer were counted over the three years of census; of these, 140 were sexed, while 10 remained indeterminate (Table S2).

The global SR was slightly biased towards females, both considering the three years pooled and separately, except for the 2016. However, in all the cases the deviation from the expected value of one was not

significant (Table 1). Considering the three age classes separately, the SR was not significantly different from the expected value of one, both considering the three years pooled or separately (Table 2).

Finally, in each year, the SR was not significantly different between age classes (2014: LR = 1.66, df = 2,  $P = 0.531$ ; 2015: LR = 2.41, df = 2,  $P = 0.362$ ; 2016: LR = 0.25, df = 2,  $P = 0.862$ ). The J/D ratio was quite constant across the three study years (2014: 0.417, 2015: 0.400, 2016: 0.385).

### Reproductive parameters

In two years of post-reproductive survey (2013 and 2014) we observed a total of 17 does followed by litter

**Table 3.** Values of the main parameters used for the Population Viability Analysis carried out on the reintroduced European roe deer population of Ticino Natural Park (northern Italy, 2014-2016).

Parameters	Values	Source
Number of iterations per scenario	1000	Lacy et al. (2015)
Years of simulation	30	-
Reproductive system	Polygynous	Sempéré et al. (1996)
Age of first offspring males	3	Vanpé et al. (2008)
Age of first offspring females	2	Sempéré et al. (1996)
Age of maximum reproduction (m/f)	13	Sempéré et al. (1996)
Maximum lifespan	13	Sempéré et al. (1996)
Maximum number of broods per year	1	Sempéré et al. (1996)
Maximum number of progeny per brood	3	Sempéré et al. (1996)
Sex-ratio at birth – in % of males	50	Hewison & Gaillard (1996)
Distr. of n. of offspring per female per litter	1 fawn: 64.7 %, 2 fawns: 35.3 %	Survey data
Percentage of breeding females (high density)	29.6 %	Census and survey data
Percentage of breeding females (low density)	71.2 %	Cravin & Roveda (2003)
Allee effect (A) – Steepness parameter (B)	0, 1	Extrapolated data from Cravin & Roveda (2003)
Fawn mortality	0 (included in % breeding females and fawn counts)	See text
Sub-adult and adult females' mortality (SD)	9.2 % (4.5)	Mean values from: Gaillard et al. (1993), Marcandalli (2004), Mustoni et al. (2012)
Sub-adult males' mortality (SD)	16.7 % (11.17)	
Adult males' mortality (SD)	14.6 % (2.96)	
Frequency of major summer floods	0.5 %	Po River Basin Authority (1998)
Severity of major summer floods	Reproduction: 0.6, Survival: 1	See text
Percentage of males in the reproductive pool	100 %	See text
Initial population size	94	Census data
Initial population structure	Fawns 10.7 % (m: 3.9 %, f: 6.8 %) Sub-adults 23 % (m: 10.6 %, f: 12.4 %) Adults 66.3 % (m: 35.8 %, f: 30.5 %)	Census data

(11 singleton litters, 64.7%, and six twin litters, 35.3%). No cases of triplets were recorded within the population. Overall, a total of 23 fawns and 17 does followed by litter were counted, giving a mean litter size (Fw/M ratio) of 1.35 fawns per mother. Reporting these data to the spring census results we calculated a mean RS of 29.6 % (SD = 1.15), that is the average value, estimated for the three years, of the percentage of successful breeding females in the population (2014: juveniles = 5, adult females = 12, percentage of successful breeding females = 30.8 %; 2015: juveniles = 6, adult females = 15, percentage of successful breeding females = 29.6 %; 2016: juveniles = 5, adult females = 13, percentage of successful breeding females = 28.5 %).

### *Population viability*

Relying on count and survey results, we run the PVA considering an initial population size of  $n = 94$  individuals, equal to the average estimates of the three years population size. Consequently, the habitat carrying capacity of the three hypothesized scenarios have been set to  $K_1 = 94$ ,  $K_2 = 141$  and  $K_3 = 188$  respectively. The age-sex structure assigned to the initial population was equal to the average values calculated over the three years: 10.7 % juveniles (3.9 % males, 6.8 % females), 23 % sub-adults (10.6 % males, 12.4 % females), 66.3 % adults (35.8 % males, 30.5 % females). We set up the distribution of the number of fawns per litter to 64.7 % single births, 35.3 % twin births and 0 % triple births.

The current percentage of breeding females in the population was set to the average value of 29.6 % found through our surveys. The percentage of breeding females at low density was set to the average value of 71.2 %, calculated from reproductive data of 1992 (66.7 %), 1994 (85.7 %) and 1995 (61.1 %) (Cravin & Roveda 2003). The steepness parameter of the density-dependent function was set to  $B = 1$ . All the PVA input parameters used for this study are summarized in Table 3. The results of the simulation (Fig. 3) showed that, regardless the  $K$  scenario, the mean population size over the next decades is generally likely to stabilize around a value lower than  $K$ , with a  $P[\text{survive}]_{30} = 100$  %. Hypothesizing that the initial population have already reached the habitat carrying capacity (scenario  $K_1$ ), we found a moderate population decrease in the first four years, followed by a stabilization of the mean population size around 90 individuals ( $N[\text{all}]_{30} = 88.40$ ,  $\text{SE} = 0.24$ ,  $\text{stoch-r} = -0.003$ ). Conversely, assuming a carrying capacity equal to  $1.5N$  (scenario  $K_2$ ), population increased, settling around a mean number of 130 individuals after nine years ( $N[\text{all}]_{30} = 132.41$ ,  $\text{SE} = 0.33$ ,  $\text{stoch-r} = 0.011$ ). When  $K$  is assumed to be twice the current population size (scenario  $K_3$ ), the increasing trend of the population was more marked, reaching a mean number of about 180 individuals after 30 years ( $N[\text{all}]_{30} = 177.85$ ,  $\text{SE} = 0.42$ ,  $\text{stoch-r} = 0.021$ ).

## Discussion

### *Density and population size*

The mean population density estimated within the whole study area over the three years, is quite high compared to densities reported in the review by Melis et al. (2009) for other predator-free roe deer populations in Europe (mean density: 14.85 individuals/km<sup>2</sup>). Moreover, considering individuals resting in wooded areas only, we obtained densities (see Table S1) very close to the highest density value reported in literature for this species (53.8 individuals/km<sup>2</sup> in northern Italy, Focardi et al. 2002). The high density characterizing the investigated population may be due to the favourable environmental conditions of this area. The study area and in general the Ticino Natural Park matches with the optimal habitat for the roe deer in many ways. Firstly, the spatial pattern of alternated wooded and open areas provides a wide availability of ecotonal areas that have been related to smaller home-range size (Lovari et al. 2017). The presence of multi-age woodland areas, moving from mature broadleaved forests to early stage mixed-tree reforestations provides the area with high habitat heterogeneity and

may be related to higher roe deer density (Gill et al. 1996). Furthermore, lowland riparian woodlands are optimal habitats for this species because of their plant diversity (Barančeková 2004), which includes most of the roe deer preferred species: brambles, ivy (*Hedera helix*), hawthorn (*Crataegus monogyna*), dogwoods (*Cornus* spp.) and common hornbeam (Tixier et al. 1997). Finally, positive aspects are also the absence of large predators or competitors in the study area (Melis et al. 2009) and a milder climate compared to other regions of Europe (Gaillard et al. 1993). The relative stability of the density estimates obtained over the three years of counts suggest that, about twenty years after its reintroduction, this roe deer population may be approaching the carrying capacity.

### *Population structure*

The first feature arising in the structure of the investigated population is the quite low proportion of juveniles as for other roe deer populations with matching characteristics in northern Italy (Bontardelli et al. 2003). As a consequence, we recorded a progressive increase of the proportion of adults through the three years. The percentages of sub-adults observed in 2014 and 2015 are higher compared to the open population of Boschi di Carrega (range: 13.70-20.30 %, Bontardelli et al. 2003), while they decreased in 2016 to typical values. In open roe deer populations, it is supposed that a long-range dispersal of juveniles and sub-adults occurs at the beginning of the territorial and reproductive phase in spring (Wahlström & Liberg 1995). The high proportion of sub-adults observed in 2014 and 2015 may indicate a substantial difficulty to disperse into the surrounding farmland. Conversely, the 2016 decrease may be a direct consequence of the low proportion of juveniles, observed in the two previous years. We did not find any significant bias of observed sex ratio, both globally and within each age class. These results are consistent for a roe deer population, in absence of large predators and harvesting, not showing evident signs of environmental stress (e.g. severe winter conditions or lack of resources), which would be reflected in a higher mortality of adult and sub-adult males (Gaillard et al. 1993), and, potentially, in an increasing productivity (Focardi et al. 2002, Bontardelli et al. 2003).

The low percentage of juveniles and J/D ratio values indicates that this population is affected by low productivity. The estimated J/D ratio were, in fact, lower than the value of 0.75, reported by Focardi et al. (2002) for a high-density population in Italy, and

0.80 reported by Kałuziński (1982) for a harvested population in Poland. However, it was comprised within the range of values reported for roe deer populations in Great Britain (0.14-0.84, Ratcliffe & Mayle 1992).

Also the percentage of twin litters found in this population is very low compared to the average for roe deer populations in temperate Europe (e.g. 59 %, Nikolandić & Degmečić 2007; 72 %, Macdonald & Johnson 2008), suggesting that either high embryo and early fawn mortality factors are at work in this population.

Given the high density found in our study area, the low productivity found in this population is likely to be related to the onset of density-dependent regulation, as a result of the approaching to the habitat carrying capacity (Sinclair et al. 2006).

In fact, high densities in roe deer populations result in the reduction of female home-range size (Kjellander et al. 2004), and, consequently, in a global decrease of food availability, which negatively affect fecundity and fawn early survival (Hewison & Gaillard 2001, Pettorelli et al. 2001). Specifically, a lower body mass of pregnant females, due to an inadequate food intake in winter, is related to a lower embryo implantation success (Hewison & Gaillard 2001) and smaller litter size (Nilsen et al. 2004). The availability of food in spring and summer also affect the early survival, growth and future reproductive performance of fawns (Gaillard et al. 1997).

Furthermore, it should not be excluded the possible role of density – independent factors affecting this population. In particular, a major concern for the conservation of ungulate populations comes from disturbance and aggressions by roaming dogs (Sime 1999, Focardi et al. 2009). In our study area, Cravin & Roveda (2003) reported 12 cases of roe deer predation ascribed to roaming dogs in the post-release stage (1991-1996), amounting to the 27 % of the total losses (second death cause).

Finally, we cannot exclude the effect of high fawn predation rate by red fox (Jarnemo et al. 2004), fawn death and bedding sites reduction due to hay mowing (Jarnemo 2002, Cravin & Roveda 2003) and the possibility of locally high poaching levels.

#### *Population long-term viability*

The PVA analysis yielded positive results about the viability of the investigated population during the next 30 years. For all the hypothesized  $K$ -level scenarios, the mean probability of extinction resulted null.

Our simulations resulted in a population characterized

by a high resilience against demographical and environmental stochastic events (e.g. random fluctuations of birth rates, floods in reproductive season), able to maintain its viability with an increasing or stable population size, depending on the  $K$ -level. On one hand, these results are consistent because they suggest that the reproductive success observed is not so reduced to drive population decreasing in the short-term. On the other hand, it is also necessary to point out that our PVA was partially based on parameters obtained indirectly or by other works, and that it has been run without including an important factor such as the inbreeding depression. The roe deer population of Ticino Natural Park descending from a few founder individuals of a long-time isolated population in its turn, fits the typical profile of a population with possible a high degree of inbreeding and, thus, potentially affected by inbreeding depression (Slate et al. 2000, Allendorf et al. 2013). Although hard to quantify, the inbreeding depression (Frankham 2010) and its relationship with reproductive success may have a critical influence on population viability.

#### **Conclusions**

In our study area, we found a large and well-structured European roe deer population, showing typical signs of breeding success decrease due to high population density. However, PVA results do not show any substantial threat to this population viability within next 30 years, so that this population satisfy the stability requirements for a potential lowland source population.

As several studies stated that the roe deer shows a high variability in demographic performance at local scale (e.g. Gaillard et al. 1998), we believe that extending further surveys to other areas would be useful to achieve a complete overview of the roe deer population status in the whole Ticino Natural Park. Preliminary results from on-going research indicate that the species range in the park might not be substantially changed in the last twenty years, with high densities limited to a few kilometres around the original release area and significantly lower densities in the rest of the protected area (unpublished results, April 2018).

If confirmed, this pattern may highlight the presence a strong barrier effect due to crosscutting infrastructures, such as railways, high-traffic roads (Coulon et al. 2004, Kuehn et al. 2006) and concrete-lined canals (Peris & Morales 2004).

The concrete possibility for Ticino Natural Park to play the role of an effective source area in a

lowland metapopulation depends both on internal defragmentation actions and on measures to enhance connectivity between the park and other woodland patches scattered across the Po Plain (Dondina et al. 2018).

To enhance roe deer dispersal through Po Plain an appropriate management strategy may be the improvement of the landscape connectivity by ensuring a strategic spatial arrangement of stepping stones (orchards, groves hedgerows or uncultivated patches) (Dondina et al. 2018) and the adoption of easy expedient for wildlife crossing, such as providing escape ramps on steep canals and opening available road service underpasses. The increase of connectivity and the exchange of dispersing individuals would be useful in a double-way, to sustain the recolonization of the Po Plain by the species, and to allow an inverse genetic flow

(Rosenberg et al. 1997) working as genetic rescue against the risk of inbreeding depression (Allendorf et al. 2013) in the Ticino Natural Park population. We encourage the use of the information we provided, as reference for further reintroduction of roe deer populations in other areas, where new source populations are needed to sustain the recolonization of the original geographic range of this species.

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#### Supplementary online material

**Table S1.** Sample areas characteristics and density results of the drive counts for the reintroduced European roe deer population of the Ticino Natural Park (northern Italy, 2014–2016).

**Table S2.** Age-sex structure results of the drive counts for the reintroduced European roe deer population of the Ticino Natural Park (northern Italy, 2014–2016) (<https://www.ivb.cz/wp-content/uploads/De-Pasquale-Table-S1-S2.xlsx>).