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ORIGINAL RESEARCH

Sex-specific impact of tooth wear on senescence in a low-dimorphic mammal species: The European roe deer (*Capreolus capreolus*)

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Keywords
Capreolus capreolus; body condition index; dental measurements; tooth wear score; senescence; sexual dimorphism.

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Abstract

Among ungulates, capital breeding males, especially in highly dimorphic species, support higher reproductive costs than females. Roe deer, a relatively monomorphic species, is defined as an 'income breeder', using a concurrent intake of energy from forage to pay for a reproductive attempt. In a Northern Apennines (Arezzo province, Tuscany, Central Italy) population, we detected sexual dimorphism in adult roe deer according to average body mass (males 11% heavier than females), mandible size (male mandibles are 2% longer than female ones), and tooth measurements (first lower molar 10% higher and mandible cheek teeth row 4% longer in males than in females, providing males with a larger surface for chewing). In our study, body mass and body condition of roe deer males decreased with increasing tooth wear as in females. However, males started losing weight at a lower tooth wear level than females; after losing about 15% (~3.2 kg) of body mass they had greater probability of death than females. For low-dimorphic species like roe deer, these findings raise new considerations about the role of sexual dimorphism in feed intake efficiency.

Introduction

Senescence is defined as age-related gradual deterioration of body functions (e.g., Carranza et al., 2004; Ericsson & Wallin, 2001), and results in an increase in the mortality rate at progressively older ages (Finch, 1994). Senescence is pervasive in mammals (see Lemaître et al., 2020; Nussey et al., 2013 for a review), but natural mortality remains low in wild populations, especially at adult stages. This is also the case for ungulates, where the average yearly natural adult survival probability was estimated to be 88% in males (see Williams, 1957 for an analysis among 18 species), and may exceed 95% in females (Gaillard, Festa-Bianchet, Yoccoz, et al., 2000).

Because longevity is a major source of variation in lifetime reproductive success and fitness among long-lived species (Clutton-Brock, 1988; Kirkwood & Rose, 1991), knowledge of the processes driving variation in longevity is essential to understanding population dynamics and evolutionary ecology. Longevity costs of reproduction may vary between sexes (Penn & Smith, 2007) according to differences in energy intake and growth of juveniles, especially in sexually size-dimorphic species (Clutton-Brock, 1991). In this context, the energy costs for males of acquiring mating opportunities can exceed those of lactating females (e.g., Forsyth et al., 2005; Key & Ross, 1999; Lane et al., 2010; McCullough, 1999). In contrast, sexually monomorphic species may be expected to have no or only a small difference in sex-biased survival compared to dimorphic species (Promislow, 1992). However, terminal decline and whether it differs between sexes remain open questions for these species. Research to increase this knowledge base is very important, particularly in ungulates, because their longevity, strong iteroparity, and overlapping generations produce unique patterns of population dynamics and life-history evolution (Gaillard, Festa-Bianchet, Yoccoz, et al., 2000, Gaillard et al., 2001). In addition, this knowledge could be valuable for the adaptive management of hunted species (Festa-Bianchet, 2007).

In this context, European roe deer (*Capreolus capreolus*) represents a perfect case study species. Among deer, it is the most widespread and abundant species in Europe and a highly valued game species for which monitoring of population status is often required (Apollonio et al., 2010).

Considering the provision of energy ('financing'') of reproduction, often described on a continuum ranging from capital to income breeding (see Stephens et al., 2009), the roe deer is defined as an income breeder (Apollonio et al., 2020). Capital breeders, like red deer, build and/or store reserves that allow them to reproduce at a later time (e.g., the following year), whereas income breeders, like roe deer, allocate recently acquired energy from forage directly to reproduction (see Stephens et al., 2009).

The roe deer is a small cervid (adult weight approx. 20–30 kg) with low sexual dimorphism. However, the measurements of sexual dimorphism have been mainly made on the basis of body weight (males are about 10% heavier than females; Andersen et al., 1998) and size (the height at withers in males is about 9% greater than in females; Janiszewski et al., 2011), neglecting differences in other characteristics, such as dental measurements, which may be also important and draw some changes in habitat selection, foraging behaviour and, finally, in individual life history (i.e., body condition and rates of survival and reproduction; Gaillard et al., 1998; Gaillard, Festa-Bianchet, Yoccoz, et al., 2000). Although an earlier study on roe deer used estimates of molar wear for ageing (Hewison et al., 1996), only a few analyses were performed to link patterns of tooth wear to sex or life history theory (Chirichella et al., 2021; Veiberg et al., 2007).

In order to fill this gap, our study aimed to: (1) compare sexual dimorphism in adult roe deer in terms of eviscerated body mass and body condition as well as considering sex-related differences in mandible and teeth measurements; (2) apply a recently developed tooth wear scoring scheme for roe deer females (Chirichella et al., 2021) to males in order to determine if there is the same relationship between body condition and the degree of tooth wear; (3) provide evidence of the role of tooth wear as a proximate cause of senescence determined as body mass (weight) loss in relation to animal (skeletal) size; and (4) select the best proxy of senescence in roe deer males using either tooth wear score or other widely used indices of wear (i.e., the height and the height/breadth ratio of the first lower molar).

Materials and methods

Study area

The study was carried out in the Northern Apennines (Arezzo province, Tuscany, Central Italy, 43°28′N, 11°53′E). Approximately 57% of the territory is above 400 m above sea level (asl), with 7.4% of it being above 1000 m asl. The climate is temperate continental, with a mean temperature ranging from 1.4°C in January to 24.9°C in July.

Roe deer hunting is only allowed in hunting districts: in the Arezzo province, there are 22 hunting districts of about 9500 ha each, subdivided into hunting zones of 109.3 ha ± 1.2 SD (see Figure S1: Online Resource ESM1). The hunting districts are evenly distributed throughout the province and cover an overall area of 114 000 ha. The majority (61%) of this area is forested, predominantly composed of deciduous oaks (*Quercus cerris* and *Q. pubescens*) along with common beech (*Fagus sylvatica*) and sweet chestnut (*Castanea sativa*). The remaining area consists of cultivated fields (29%), scrub or herbaceous vegetation (5%), artificial surfaces (4.6%), and inland wetlands and waters (0.4%).

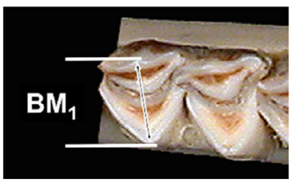
The study area harbours a rich wild ungulate community: beside roe deer which are present in all the hunting districts, there are wild boar (*Sus scrofa*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*), and mouflon (*Ovis gmelini musimon*). Wild boar is homogeneously distributed across the whole province, whereas red deer, fallow deer, and mouflon are more localised (Apollonio & Mattioli, 2006). Predators are primarily grey wolf (*Canis lupus*), with an estimated 25 packs in 2015 (Bassi et al., 2015), and red fox (*Vulpes vulpes*).

Data collection

In all 22 hunting districts of the Arezzo province, roe deer are legally hunted each year from January 1 to March 15 (females and juveniles), from June 15 to July 15 (males), and from August 15 to September 30 (males, females, and juveniles). From 2007 to 2017, a sample of 531 roe deer mandibles was collected from legally shot individuals between January 1 and March 30 (319 adult females) and June 15 to July 15 (212 adult males, all of them harvested before the rut and hence not experiencing seasonal body mass loss; see Apollonio et al., 2020). Hunters prepared all the mandibles of hunted individuals using the hot water maceration method, hand removal of soft tissue and cartilage, and a 35% hydrogen peroxide treatment. Each mandible was registered by the Provincial Government and made available to us for measurements and analyses. Date of culling, sex, body mass, and hunting district were recorded for each roe deer. Body weight used in this study is eviscerated body mass (i.e., weighed without viscera, potential embryos/foetuses carried by females, and flowing blood; BW). For the Arezzo province population, the mean value of eviscerated body mass for adult (i.e., 2 or more years old) females culled in the January–March period was 18.1 kg (Chirichella et al., 2019), and for adult males culled in the June–July period it was 21.6 kg (personal unpublished data, based on 2491 individuals culled between 2012 and 2017).

In the laboratory, measurements of mandibles and teeth were done with a digital calliper (±0.01 mm) always by the same researcher (see Table 1 for a description of collected measurements). For the analysed population, the mean lengths of mandible (ML), a proxy of skeletal size, for adult females and males were 155.4 and 158.0 mm, respectively (see De Marinis et al., 2019).

Table 1 Measurements of mandibles and teeth on 531 adult roe deer (319 females, 212 males) shot in the Northern Apennines (Arezzo province, Tuscany, Central Italy) during the regular annual harvest (1 Jan–15 Mar for females; 15 Jun–15 Jul for males) from 2007 to 2017

Measurement	Schematic drawing and name abbreviation	Description
Mandible		
Mandible length		Distance from the anterior margin of the alveolus of I_1 to the posterior margin of the *processus angularis* De Marinis et al. (2019)
Diastema length		Distance from the posterior margin of the alveolus of C to the anterior margin of P_2 Sabalinkiene et al. (2017)
Teeth		
Cheek teeth row length		Distance from the anterior margin of P_2 to the posterior margin of M_3 De Felice et al. (2020)
Height of M_1[a]		Distance from the peak of the mesiobuccal cusp to the enamel/cementum line Chirichella et al. (2021)
Breadth of M_1 [a]		Buccolingual breadth of the mesial cusp Chirichella et al. (2021)

[a]Both HM1 and WM1 were measured 3 times for each individual, and mean values were derived ($\overline{HM_1}$ and $\overline{BM_1}$).

The progressive wearing of the cheek teeth was assessed using a scoring scheme based on 28 objectively described morphotypes on two premolars (P_3 and P_4) and all three molars (M_1, M_2 and M_3; see Chirichella et al., 2021 for details about the schematisation of the wear process, and for nomenclature and terminology of morphotypes of the occlusal surface).

Tooth wear was also estimated using crown height of the first molar (HM_1) and the M_1 height/breadth ratio ($HBRM_1$, defined as the height of M_1 divided by the buccolingual breadth as reported by Ozaki et al., 2007, 2010 in unworn teeth), which are the most widely used indices of tooth wear in wild ruminants (e.g., Ericsson & Wallin, 2001; Fandos et al., 1993; Kojola et al., 1998; Skogland, 1988). Both height and breadth of M_1 were measured three times per each individual and mean values of $\overline{HM_1}$ and $\overline{HBRM_1}$ were derived. The dataset is available as Online Resource ESM2: Appendix S1.

Data analysis

We determined sexual dimorphism in terms of eviscerated body mass and ratio of eviscerated body mass to mandible length (i.e., BW/ML), as a proxy of body condition in roe deer (Blant & Gaillard, 2004; Hanzal et al., 2017; Hewison et al., 1996). Differences between sexes were also evaluated for all the mandible and teeth measurements (shown in Table 1). In particular, $\overline{HM_1}$ and $\overline{HBRM_1}$ were compared between sexes by ANOVA according to tooth wear score classes (i.e., class 1: 0–10; class 2: 11–20; class 3: 21–30; class $4 \geq 31$; see Chirichella et al., 2021). Moreover,

Table 2 Sexual dimorphism in roe deer

Measurements	*F*	*P*	% (M > F)
Body			
Eviscerated body mass (BW[a])	105.41	<0.001	10.6
Body condition index (BW/ML[a])	74.29	<0.001	7.1
Mandible			
Mandible length (ML)	50.39	<0.001	1.7
Diastema length (DL)	1.83	0.177	-
Teeth			
Cheek teeth row length (TRL)	105.60	<0.001	3.9
Height of M_1 ($\overline{HM_1}$[b,c])	12.69	<0.001	10.1
Breadth of M_1 ($\overline{BM_1}$[c])	0.16	0.688	-
Height/breadth ratio ($\overline{HBRM_1} = \overline{HM_1}/\overline{BM_1}$)	12.63	<0.001	10.3

Differences between 319 adult roe deer females and 212 adult males shot in the Northern Apennines (Arezzo province, Tuscany, Central Italy) during the regular annual harvest (1 Jan–15 Mar for females; 15 Jun–15 Jul for males) from 2007 to 2017: (1) body mass (eviscerated body mass, BW; body condition index, BW/ML), (2) mandible size (mandible length, ML; diastema length, DL), and (3) dental measurements (cheek teeth row length, TRL; height of M_1, $\overline{HM_1}$; breadth of M_1, $\overline{BM_1}$). For visualisation of differences, see ESM3: Figure S2.

[a]Both BW and BW/ML were evaluated in individuals belonging to the first tooth wear score class (i.e., with total wear score ≤ 10).

[b]Differences in HM1 were evaluated only for individuals without wear of M1.

[c]Both HM1 and BM1 were measured three times per each individual and mean values were derived ($\overline{HM_1}$ and $\overline{BM_1}$).

controlling for body size, we tested if males had a larger chewing surface than females by means of linear models testing the effect of sex, ML and their combination on cheek teeth row length (TRL).

BW and BW/ML were related to tooth wear score in 212 adult males and compared to the mean value of eviscerated body mass and the body condition index of adult males in the same population (De Marinis et al., 2019; see Chirichella et al., 2021 for a comparison with adult females in the same population).

Linear regressions were calculated between BW and BW/ML (dependent variables) and (1) the wear scores of each tooth, (2) the sum of the scores obtained by pooling the molariform teeth in different ways (see Table 2 for the complete list of independent variables), (3) the height of M_1 ($\overline{HM_1}$), and (4) the height/breadth ratio of M_1 ($\overline{HBRM_1}$). Models were compared by means of Akaike's Information Criterion for small sample sizes (AICc) and we checked standardised residual plots for assumptions of normality, homoscedascity, and independence (Burnham & Anderson, 2002; Zuur et al., 2010). Differences in body mass, mandible length and body condition index were investigated by ANOVA and Tukey HSD *post hoc* test for consecutive classes of tooth wear score (4 classes). The percentage of weight and body condition loss in relation to increasing tooth wear was calculated (see Chirichella et al., 2021 for a comparison with adult females in the same population).

Statistical analyses were performed using R version 4.0.4 (www.r-project.org; R Core Team, 2021).

Results

We detected sexual dimorphism in adult roe deer in body, mandible, and tooth measurements (Table 2 and Online Resource ESM3: Figure S2). The most pronounced differences were found in eviscerated weight, with males being about 11% heavier than females before losing weight as a result of advanced tooth wear. A similar difference was recorded for the height of the unworn first molar (before the wear started), which was about 10% higher in males than in females. This difference was reflected in sex-related difference in the $\overline{HBRM_1}$, which is also due to the absence of sexual dimorphism in M_1 breadth. The difference in the crown height of M_1 remained constant between males and females until the most advanced stage of tooth wear (i.e., molariform wear score ≥ 31), when the two sexes no longer showed significantly different heights (Fig. 1). Cheek teeth row length was also significantly larger in males than in females (approximately 4%), providing them a larger surface area suitable for chewing (see Online Resource ESM4: Table S1 for major details).

Each evaluated proxy of weight and body condition loss showed a different ability to synthesize information on senescence in roe deer males (Table 3). Molar ($M_1 + M_2 + M_{3total}$) and molariform wear score ($P_3 + P_4 + M_1 + M_2 + M_{3total}$) were best correlated with body mass and body condition (i.e., models with $\Delta AICc < 2$; Table 3, Fig. 2). On the contrary, $\overline{HM_1}$ and $\overline{HBRM_1}$, despite their correlation with M_1 wear score ($r_p = -0.64$, $P < 0.001$ for $\overline{HM_1}$, and $r_p = -0.62$, $P < 0.001$ for $\overline{HBRM_1}$, respectively), were less effective indices to describe the variation in body condition of roe deer males (Table 3).

Tooth wear score classes were related to significant loss of body mass and body condition (ANOVA: $F_{3,208} = 17.75$, $P < 0.001$ for BW; $F_{3,208} = 17.55$, $P < 0.001$ for BW/ML), while no relation was found with mandible length ($F_{3,208} = 0.93$, $P = 0.43$) (for details, see Table 4). Body mass and body condition decreased respectively by 15.0% and 14.3% in those males with the most advanced stage of tooth wear compared to individuals experiencing minor tooth wear (Table 4), clearly illustrating that tooth wear was the proximate cause of body weight loss. Eviscerated weight was under the

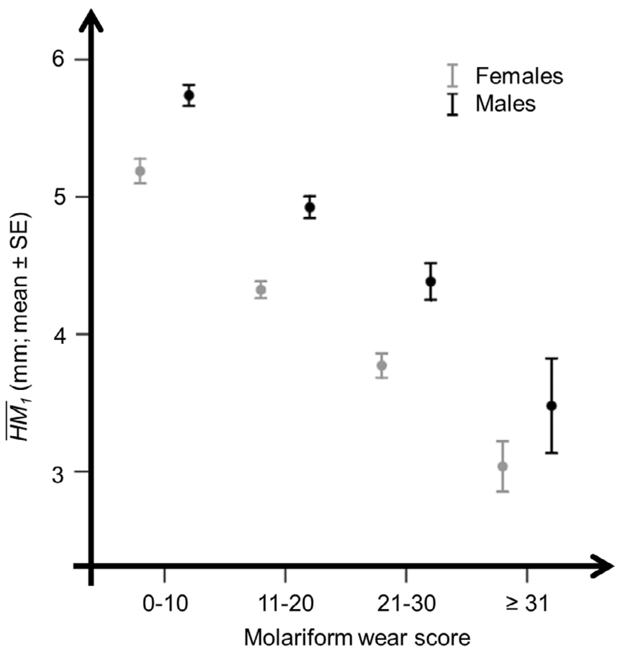


Figure 1 Differences in height of M_1 between sexes. Mean and standard errors (SE) of M_1 height (in mm) for 531 adult roe deer (319 females, 212 males) shot in the Northern Apennines (Arezzo province, Tuscany, Central Italy) during the regular annual harvest (1 Jan–15 Mar for females; 15 Jun–15 Jul for males) from 2007 to 2017 according to consecutive molariform wear score classes.

mean population value when the molars wear score exceeded a value of 10. Individuals in the 11–20 tooth wear score class had one or both infundibula worn away in M_1. With increasing tooth wear scores above this level, body masses were more and more frequently found to be below the population average (R^2 = 0.22; see Fig. 2). This process was associated with the progressive disappearance of infundibula in M_2 and M_3.

Sexual differences in BW versus molariform wear score are highlighted in Fig. 3, showing the value at which tooth wear level is no longer able to sustain the physical condition of individuals (i.e., tooth wear score of 10 for males and 20 for females).

Discussion

Body mass and size of the European roe deer may vary widely depending on geographical, climatic, and environmental conditions (e.g., Janiszewski et al., 2009; Petelis & Brazaitis, 2003; Pettorelli et al., 2002; Wajdzik et al., 2016). This study takes advantage of the possibility of obtaining a large number of measurements within a single population (i.e., a wild population with a uniform management regime and experiencing similar climatic/environmental conditions) in order to evaluate the sexual dimorphism and tooth wear processes in this widespread species.

In general, the body mass of European roe deer increases in males up to about 3 years of age (Mysterud & Østbye, 2006; Nussey et al., 2011), while by 2 years of age, females have reached approx. 95% of their maximum body mass (Gaillard, Festa-Bianchet, Delorme, et al., 2000). As reported by Andersen et al. (1998) for this species, males are consistently about 11% heavier than females, but in the analysed population their mandible length, a good proxy for body size (Blant & Gaillard, 2004; Hanzal et al., 2017; Hewison et al., 1996), is only about 2% longer. Despite this very small difference, the height of the first molar (before wear has started) showed a similar sexual dimorphism as for body mass (about 10%). This difference, together with the longer cheek teeth row (approx. 4%), provides males a

Table 3 Relation between male roe deer body condition, tooth wear score and M_1 measurements

	BW (kg)				BW/ML (kg/mm)			
Independent variables	AICc (Rank; Value)	R^2 adj.	*F*	*P*	AICc (Rank; Value)	R^2 adj.	*F*	*P*
Tooth wear scores								
P_3	10; 953.2	0.048	11.649	0.001	9; −1193.9	0.040	9.903	0.002
P_4	5; 936.9	0.118	29.290	<0.001	4; −1210.5	0.113	27.841	<0.001
M_1	3; 915.4	0.203	54.884	<0.001	3; −1230.5	0.193	51.401	<0.001
M_2	4; 935.8	0.123	30.558	<0.001	4; −1210.5	0.113	27.790	<0.001
M_3	9; 947.9	0.071	17.230	<0.001	7; −1203.2	0.082	19.789	<0.001
$M_{3\ (Additional\ Distal\ Element)}$	7; 939.9	0.074	17.731	<0.001	12; −1184.4	0.057	13.588	<0.001
$M_{3\ total}$	6; 938.1	0.113	27.635	<0.001	6; −1208.9	0.106	25.992	<0.001
Premolars ($P_3 + P_4$)	8; 944.3	0.087	21.170	<0.001	8; −1202.5	0.078	18.968	<0.001
Molars ($M_1 + M_2 + M_{3total}$)	**1; 912.3**	**0.218**	**59.384**	**<0.001**	**1; −1232.9**	**0.202**	**54.847**	**<0.001**
Molariforms ($P_3 + P_4 + M_1 + M_2 + M_{3total}$)	**2; 914.0**	**0.209**	**56.587**	**<0.001**	**2; −1231.0**	**0.194**	**51.920**	**<0.001**
M1 measurements								
$\overline{HM_1}$	11; 962.5	0.078	18.730	<0.001	11; −1186.8	0.008	2.762	0.098
Height/breadth ratio ($\overline{HBRM_1}$)	12; 962.6	0.005	2.034	0.115	10; −1186.9	0.008	2.801	0.096

Main parameters of linear regressions between eviscerated body mass (BW in kg; on the left) and the ratio of eviscerated body mass to the mandible length (BW/ML in kg/mm; on the right) as dependent variables, and tooth wear scores, M_1 height ($\overline{HM_1}$ in mm) and height/breadth ratio of M_1 ($\overline{HBRM_1}$) for 212 roe deer males shot in the Northern Apennines (Arezzo province, Tuscany, Central Italy) during the regular annual harvest (15 Jun–15 Jul) between 2007 and 2017. Best models (i.e., models with ΔAICc < 2) are highlighted with bold characters.

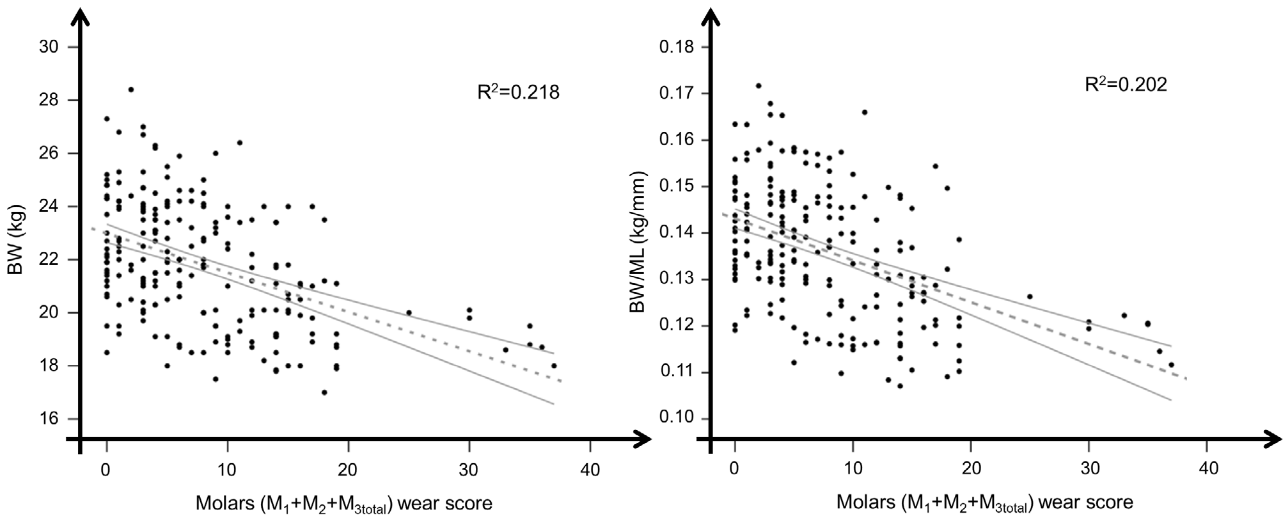


Figure 2 Eviscerated body mass (BW in kg; left panel) and the ratio of eviscerated body mass to mandible length (BW/ML in kg/mm; right panel) as dependent variables in relation to molars wear score in 212 roe deer males shot in the Northern Apennines (Arezzo province, Tuscany, Central Italy) during the regular annual harvest (15 Jun–15 Jul) from 2007 to 2017. R^2 of the linear regression (dotted grey line) is reported.

Table 4 Eviscerated body mass, mandible length and a body condition index in different classes of the molariforms tooth wear score in roe deer males

		Tooth wear score classes			
					
		0–10 (*N* = 128)	11–20 (*N* = 57)	21–30 (*N* = 19)	≥31 (*N* = 8)
Eviscerated body mass (BW; kg)	Min	17.5	17.8	17.0	18.0
	Mean	22.6	21.1[a]	19.8	19.2
	Max	28.4	26.4	24.0	20.1
	% loss	-	6.6	12.4	15.0
Mandible length (ML; mm)	Min	148.6	150.4	152.2	152.1
	Mean	157.2	157.8	157.6	160.6
	Max	167.9	167.4	166.6	166.3
	% loss	-	-	-	-
Body Condition Index (BW/ML; kg/mm)	Min	0.11	0.11	0.11	0.11
	Mean	0.14	0.12[b]	0.12	0.12
	Max	0.17	0.15	0.15	0.13
	% loss	-	14.3	14.3	14.3

Minimal, average and maximal values of eviscerated body mass (BW; in kg), mandible length (ML; in mm) and a body condition index (BW/ML; in kg/mm) of 212 roe deer males shot in the Northern Apennines (Arezzo province, Tuscany, Central Italy) during the regular annual harvest (15 Jun–15 Jul) between 2007 and 2017 according to different classes of the molariforms' tooth wear score. Percentage of weight loss and body condition loss were calculated referring to individuals with minor tooth wear, that is, those belonging to the score class 0–10, and according to the differences in consecutive tooth wear score classes ($F_{3,208}$ = 17.75, P < 0.001 for BW; $F_{3,208}$ = 17.55, P < 0.001 for BW/ML). Tukey HSD *post hoc* test results are indicated by superscripts – means within a row not sharing the same superscript differ significantly. No differences in mandible length ($F_{3,208}$ = 0.93, P = 0.43) for consecutive tooth wear score classes were revealed. Pictures show the mean wear condition of each tooth wear class.

larger and more durable surface suitable for chewing. Therefore, if the tooth wear process in males and females were characterised by similar timing and pattern, males would have a longer life expectancy, being able to sustain their physical condition for a longer time. Indeed, teeth should increase in size in relation to body size if they are to maintain performance and durability (see Fortelius, 1985; Lucas, 2004). However, this is not the case in males of polygynous ungulates, such as red deer, in which tooth

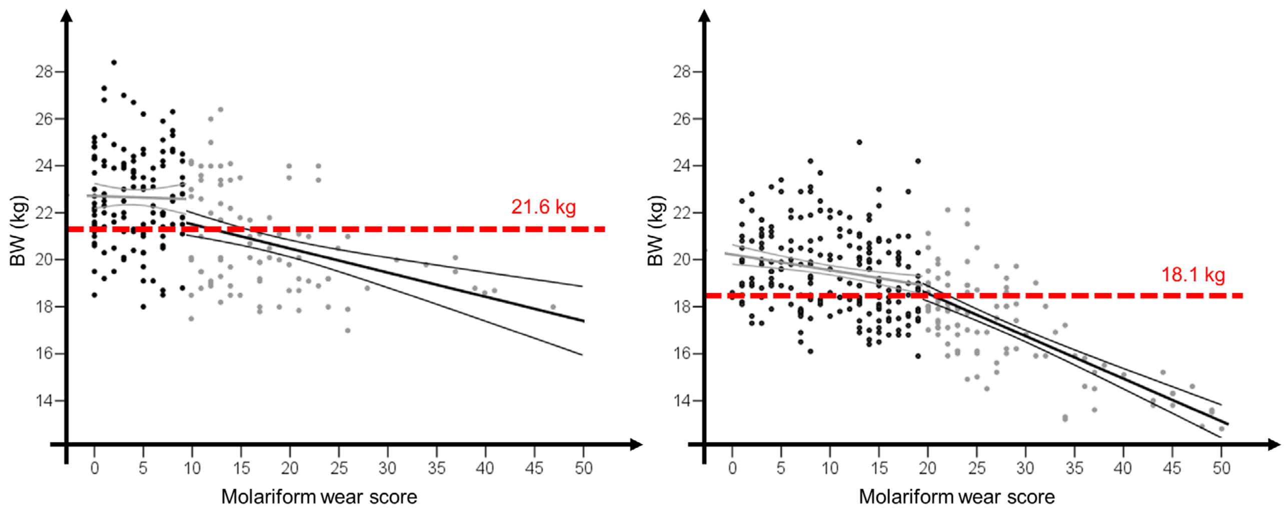


Figure 3 Eviscerated body mass (BW in kg) in relation to molariform wear score in 212 roe deer males (left panel) and 319 females (right panel) shot in the Northern Apennines (Arezzo province, Tuscany, Central Italy) during the regular annual harvest (1 Jan–15 Mar for females; 15 Jun–15 Jul for males) from 2007 to 2017. Linear regressions are reported for animals with a molars wear score below (grey line) or above (black line) the value at which tooth wear level is no longer able to sustain the animal's physical condition (i.e., tooth wear score of 10 for males and 20 for females, respectively). Mean population values of BW are highlighted with red dot lines.

size and durability have not evolved at the same rate as body mass (Carranza et al., 2004; Carranza & Pérez-Barbería, 2007; Loe et al., 2003).

Our data showed that the body mass and body condition of roe deer males decreased with increasing tooth wear score. However, once they have reached tooth wear values that are half of those of females (10 vs. 20; see Chirichella et al., 2021 for a comparison), they are no longer able to support the necessary intake of energy. From this threshold onward, body masses were increasingly below the population average, suggesting the need for a better preserved chewing capacity in males. Moreover, males wore down their teeth more intensely than females during their life (males have 10% higher molars); after losing about 15% (≈3.2 kg) of body mass, they have greater probability of death (i.e., we did not find males with >15% of body mass below the mean population level) than females (see Chirichella et al., 2021).

In mammals, the energy costs for males of acquiring mating opportunities can exceed those of lactating females (Key & Ross, 1999). This has also been verified in ungulates, especially those characterised by high polygyny and capital breeding males that build and store reserves to be used during the rutting period, independently of food availability (Lane et al., 2010; Mysterud et al., 2004). In these species, males, which show no parental care, have a relevant reproductive investment during the rut, because once conception is ensured males have no further involvement in reproduction. Roe deer belong to the opposite end of the capital income breeder continuum (Apollonio et al., 2020), allocating concurrent intake of energy from food directly to reproduction. Indeed, roe deer males are long-term territory holders, but females are solitary, so territorial males have access only to one or few females at a time, that is, only to the females either present in their territory (Kurt, 1991; Lieberg et al., 1998) or visiting them during reproductive excursions (Debeffe et al., 2014). All this reflects the low level of polygyny of the species, limited variance in lifetime reproductive success (Vanpé et al., 2009), and a relatively small proportion (<10%) of body mass lost during the rut, indicating a low reliance on stored energetic capital during that period (Apollonio et al., 2020).

However, as in other relatively monomorphic species (Boutin & Larsen, 1993; Lane et al., 2009), roe deer exhibit a competitive mating system under which sexual selection favours males capable of defending and maintaining a territory for a long time (Lane et al., 2010). For this reason, males' effort to manage resource acquisition and energy allocation to reproduction may exceed those of females.

According to our data, the wear score of molars and all molariforms teeth best correlated with the body mass and body condition of adult males. Interestingly, the best proxy of weight loss is the same for females (Chirichella et al., 2021), showing the importance of the entire chewing surface in shaping the ability of the animal to acquire energy throughout food comminution, chewing, and digestion in both sexes.

However, as a consequence of increasing tooth wear, body mass loss in males occurs earlier than in females (Fig. 3), suggesting a stronger resilience to the reduction of chewing capacity in females, which can contribute to explaining the longer life expectancy in this sex (Gaillard et al., 1993). This suggests that assessment of the degree of tooth wear may actually be considered as a descriptor of population structure and yields important information about the level of tooth wear at which roe deer males and females start losing their body condition with respect to the mean population value (Chirichella et al., 2021). In addition, it should be noted that with the onset of tooth wear, a slight weight loss was found for females while for males there seems to be no weight reduction (see Fig. 3). However, the body mass loss for males could be masked by their prolonged growth period that can reach 3–4 years of age (Mysterud & Østbye, 2006; Nussey et al., 2011). This process could be further masked as the molars of roe deer and other ruminants have a ‘secondary functional occlusal relief’. This means that only after some initial wear (when the cuspal enamel has been worn away) the teeth become fully functional, with an occlusal surface consisting of enamel ridges along the crown flanks and the infundibulum and dentinal troughs between these ridges (Fortelius, 1985).

Finally, our study allowed us to compare the new tooth wear scoring scheme with the other widely used indices of wear (i.e., the height and the height/breadth ratio of M_1). Both of these measurements failed to synthesise the wear stages of the entire occlusal surface of the molars and were less correlated with variations in body condition. Moreover, in males, this correlation was much lower than that already shown for females (Chirichella et al., 2021). These findings could be related to the high degree of wear of the first molar in the last years of life for deer species, which does not allow for much differentiation among individuals, especially in males (De Marinis, 2015; De Marinis & Toso, 2013).

Better knowledge of population conditions and survival rates is an important goal in wildlife management, particularly because severe alterations of both sex ratio and age structure in hunted populations are important drivers of population dynamics (Mysterud, 2011; Mysterud et al., 2005). Future work should aim to connect the true age of an individual with the tooth wear classes in order to verify when ageing starts being evident in males and females both through increases in tooth wear and decreases in body condition. This approach applied to populations of the same species living in different environments may help to elucidate intraspecies variability in population dynamics, being tooth size less ‘evolvable’, in terms of time required for changes, than body size (Clauss et al., 2022). These data could address new, relevant questions for low dimorphic species like roe deer, elucidating sexual dimorphism in relation to feed intake efficiency among different populations.

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Competing interests

The authors declare that they have no competing interests.

Consent to participate

The study complies with all relevant national, regional, and provincial Italian laws, and with the ethical standards of scholarly research.

References

Andersen, R., Duncan, P., & Linnell, J. D. C. (1998). *The European roe deer: The biology of success*. Scandinavian University Press.

Apollonio, M., & Mattioli, L. (2006). *Il lupo in provincia di Arezzo*. Editrice Le Balze [in Italian].

Apollonio, M., Merli, E., Chirichella, R., Pokorny, B., Alagić, A., Flajšman, K., & Stephens, P. A. (2020). Capital-income breeding in male ungulates: Causes and consequences of strategy differences among species. *Frontiers in Ecology and Evolution*, **8**, a521767.

Apollonio, M., Putman, R., & Andersen, R. (2010). *Ungulate management in Europe in the XXI century*. Cambridge University Press.

Bassi, E., Willis, S. G., Passilongo, D., Mattioli, L., & Apollonio, M. (2015). Predicting the spatial distribution of wolf (*Canis lupus*) breeding areas in a mountainous region of Central Italy. *PLoS One*, **10**(6), e0124698.

Blant, M., & Gaillard, J. M. (2004). Use of biometric body variables as indicators of roe deer (*Capreolus capreolus*) population density changes. *Game and Wildlife Science*, **21**(1), 21–40.

Boutin, S., & Larsen, K. W. (1993). Does food availability affect growth and survival of males and females differently in a promiscuous small mammal, *Tamiasciurus hudsonicus*? *Journal of Animal Ecology*, **62**, 364–370.

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed.). Springer-Verlag.

Carranza, J., Alarcos, S., Sánchez-Prieto, C., Valencia, J., & Mateos, C. (2004). Disposable-soma senescence mediated by sexual selection in an ungulate. *Nature*, **432**, 215–218.

Carranza, J., & Pérez-Barbería, F. J. (2007). Sexual selection and senescence: Male size-dimorphic ungulates evolved relatively smaller molars than females. *The American Naturalist*, **170**(3), 370–380.

Chirichella, R., De Marinis, A. M., Pokorny, B., & Apollonio, M. (2021). Dentition and body condition: Tooth wear as a correlate of weight loss in roe deer. *Frontiers in Zoology*, **18** (1), 1–14.

Chirichella, R., Pokorny, B., Bottero, E., Flajšman, K., Mattioli, L., & Apollonio, M. (2019). Factors affecting implantation failure in roe deer. *The Journal of Wildlife Management*, **83** (3), 599–609.

Clauss, M., Heck, L., Veitschegger, K., & Geiger, M. (2022). Teeth out of proportion: Smaller horse and cattle breeds have comparatively larger teeth. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, **338**, 561-574.

Clutton-Brock, T. H. (1988). *Reproductive success: Studies of individual variation in contrasting breeding systems*. University of Chicago Press.

Clutton-Brock, T. H. (1991). *The evolution of parental care*. Princeton University Press.

De Felice, E., Pacioni, C., Tardella, F. M., Dall'Aglio, C., Palladino, A., & Scocco, P. A. (2020). Novel method for increasing the numerousness of biometrical parameters useful for wildlife management: Roe deer mandible as bone model. *Animals*, **10**, 465.

De Marinis, A. M. (2015). *Valutazione dell'età nei Cervidi tramite esame della dentatura. Guida pratica all'identificazione delle classi di età del Cervo. Manuali e Linee Guida n. 90.2*. ISPRA [in Italian].

De Marinis, A. M., Chirichella, R., Bottero, E., & Apollonio, M. (2019). Ecological conditions experienced by offspring during pregnancy and early post-natal life determine mandible size in roe deer. *PLoS One*, **14**(9), e0222150.

De Marinis, A. M., & Toso, S. (2013). *Valutazione dell'età nei Cervidi tramite esame della dentatura. Manuali e Linee Guida n. 90*. ISPRA [in Italian].

Debeffe, L., Focardi, S., Bonenfant, C., Hewison, A. M., Morellet, N., Vanpé, C., Heurich, M., Kjellander, P., Linnell, J. D., Mysterud, A., & Pellerin, M. (2014). A one night stand? Reproductive excursions of female roe deer as a breeding dispersal tactic. *Oecologia*, **176**(2), 431–443.

Ericsson, G., & Wallin, K. (2001). Age-specific moose (*Alces alces*) mortality in a predator-free environment: Evidence for senescence in females. *Ecoscience*, **8**, 157–163.

Fandos, P., Orueta, J. F., & Aranda, Y. (1993). Tooth wear and its relation to kind of food: The repercussion on age criteria in *Capra pyrenaica*. *Acta Theriologica*, **38**, 93–102.

Festa-Bianchet, M. (2007). Ecology, evolution, economics, and ungulate management. In T. E. Fulbright & D. G. Hewitt (Eds.), *Wildlife science: Linking ecological theory and management applications* (pp. 195–214). CRC Press.

Finch, C. E. (1994). *Longevity, senescence, and the genome*. University of Chicago Press.

Forsyth, D. M., Duncan, R. P., Tustin, K. G., & Gaillard, J. M. (2005). A substantial energetic cost to male reproduction in a sexually dimorphic ungulate. *Ecology*, **86**(8), 2154–2163.

Fortelius, M. (1985). Ungulate cheek teeth: Developmental, functional, and evolutionary interrelations. *Annales Zoologici Fennici*, **180**, 1–76.

Gaillard, J. M., Delorme, D., Jean-Marie, B., Van Laere, G., Boisaubert, B., & Pradel, R. (1993). Roe deer survival patterns: A comparative analysis of contrasting populations. *Journal of Animal Ecology*, **62**(4), 778–791.

Gaillard, J. M., Festa-Bianchet, M., Delorme, D., & Jorgenson, J. (2000). Body mass and individual fitness in female ungulates: Bigger is not always better. *Proceedings of the Royal Society of London*, **267**, 471–477.

Gaillard, J. M., Festa-Bianchet, M., & Yoccoz, N. G. (1998). Population dynamics of large herbivores: Variable recruitment with constant adult survival. *Trends in Ecology and Evolution*, **13**, 58–63.

Gaillard, J. M., Festa-Bianchet, M., & Yoccoz, N. G. (2001). Not all sheep are equal. *Science*, **292**, 1499–1500.

Gaillard, J. M., Festa-Bianchet, M., Yoccoz, N. G., Loison, A., & Toïgo, C. (2000). Temporal variation in fitness components and population dynamics of large herbivores. *Annual Review of Ecology and Systematics*, **31**(1), 367–393.

Hanzal, V., Janiszewski, P., Tajchman, K., & Košinová, K. (2017). The correlation between mandibular length versus body mass and age in the European roe deer (*Capreolus capreolus* L.). *Applied Ecology and Environmental Research*, **15**(4), 1623–1632.

Hewison, A. M., Vincent, J. P., Bideau, E., Angibault, J. M., & Putman, R. J. (1996). Variation in cohort mandible size as an index of roe deer (*Capreolus capreolus*) densities and population trends. *Journal of Zoology*, **239**(3), 573–581.

Janiszewski, P., Daszkiewicz, T., & Hanzal, V. (2009). Effect of environmental factors and time of shooting on carcass weight of European roe deer (*Capreolus capreolus* L). *Forest Research Papers*, **70**(2), 123–130.

Janiszewski, P., Gugołek, A., & Hanzal, V. (2011). Zoometric characteristics of the European roe deer (*Capreolus capreolus*) hunter-harvested in north-eastern Poland. *Annales UMCS, Zootechnica*, **29**(2), 20–31.

Key, C., & Ross, C. (1999). Sex differences in energy expenditure in non–human primates. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **266**(1437), 2479–2485.

Kirkwood, T. B. L., & Rose, M. R. (1991). Evolution of senescence: Late survival sacrificed for reproduction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **332**, 15–24.

Kojola, I., Helle, T., Huhta, E., & Niva, A. (1998). Foraging conditions, tooth wear and herbivore body reserves: A study of female reindeer. *Oecologia*, **117**, 26–30.

Kurt, F. (1991). *Das Reh in der Kulturlandschaft. Sozialverhalten und Ökologie eines Anpassers*. Verlag Paul Parey.

Lane, J. E., Boutin, S., Gunn, M. R., & Coltman, D. W. (2009). Sexually-selected behaviour: Red squirrel males search for reproductive success. *Journal of Animal Ecology*, **78**, 296–304.

Lane, J. E., Boutin, S., Speakman, J. R., & Humphries, M. M. (2010). Energetic costs of male reproduction in a scramble competition mating system. *Journal of Animal Ecology*, **79**, 27–34.

Lemaître, J. F., Ronget, V., & Gaillard, J. M. (2020). Female reproductive senescence across mammals: A high diversity of patterns modulated by life history and mating traits. *Mechanisms of Ageing and Development*, **192**, 111377.

Lieberg, O., Johansson, A., Andersen, R., & Linnell, J. (1998). Mating system, mating tactics and the function of male territoriality in roe deer. In R. Andersen, P. Duncan, & J.

Linnell (Eds.), *The European roe deer: The biology of success* (pp. 221–256). Scandinavian University Press.

Loe, L. E., Mysterud, A., Langvatn, R., & Stenseth, N. C. (2003). Decelerating and sex-dependent tooth wear in Norwegian red deer. *Oecologia*, **135**, 346–353.

Lucas, P. W. (2004). *Dental functional morphology*. Cambridge University Press.

McCullough, D. R. (1999). Density dependence and life-history strategies of ungulates. *Journal of Mammalogy*, **80**(4), 1130–1146.

Mysterud, A. (2011). Selective harvesting of large mammals: How often does it result in directional selection? *Journal of Applied Ecology*, **48**(4), 827–834.

Mysterud, A., Langvatn, R., & Stenseth, N. C. (2004). Patterns of reproductive effort in male ungulates. *Journal of Zoology*, **264**, 209–215.

Mysterud, A., & Østbye, E. (2006). Effect of climate and density on individual and population growth of roe deer *Capreolus capreolus* at northern latitudes: The Lier valley, Norway. *Wildlife Biology*, **12**(3), 321–329.

Mysterud, A., Solberg, E. J., & Yoccoz, N. G. (2005). Ageing and reproductive effort in male moose under variable levels of intrasexual competition. *Journal of Animal Ecology*, **74**(4), 742–754.

Nussey, D. H., Coulson, T., Delorme, D., Clutton-Brock, T. H., Pemberton, J. M., Festa-Bianchet, M., & Gaillard, J. M. (2011). Patterns of body mass senescence and selective disappearance differ among three species of free-living ungulates. *Ecology*, **92**(10), 1936–1947.

Nussey, D. H., Froy, H., Lemaître, J. F., Gaillard, J. M., & Austad, S. N. (2013). Senescence in natural populations of animals: Widespread evidence and its implications for biogerontology. *Ageing Research Reviews*, **12**(1), 214–225.

Ozaki, M., Kaji, K., Matsuda, N., Ochiai, K., Asada, M., Ohba, T., Hosoi, E., Tado, H., Koizumi, T., Suwa, G., & Takatsuki, S. (2010). The relationship between food habits, molar wear and life expectancy in wild sika deer populations. *Journal of Zoology*, **280**, 202–212.

Ozaki, M., Suwa, G., Kaji, K., Ohba, T., Hosoi, E., Koizumi, T., & Takatsuki, S. (2007). Correlations between feeding type and mandibular morphology in the sika deer. *Journal of Zoology*, **272**, 244–257.

Penn, D. J., & Smith, K. R. (2007). Differential fitness costs of reproduction between the sexes. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 553–558.

Petelis, K., & Brazaitis, G. (2003). Morphomoetric data on the field ecotype roe deer in southwest Lithuania. *Acta Zoologica Lituanica*, **3**(1), 61–64.

Pettorelli, N., Gaillard, J. M., Van Laere, G., Duncan, P., Kjellander, P., Liberg, O., Delorme, D., & Maillard, D. (2002). Variations in adult body mass in roe deer: The effects of population density at birth and of habitat quality. *Proceedings of the Royal Society B: Biological Sciences*, **269** (1492), 747–753.

Promislow, D. E. (1992). Costs of sexual selection in natural populations of mammals. *Proceedings of the Royal Society of London Series B: Biological Sciences*, **247**, 203–210.

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing https://www.R-project.org/

Sabalinkiene, G., Danusevicius, D., Manton, M., Brazaitis, G., & Simkevicius, K. (2017). Differentiation of European roe deer populations and ecotypes in Lithuania based on DNA markers, cranium and antler morphometry. *Silva Fennica*, **51** (3), 1743.

Skogland, T. (1988). Tooth wear by food limitation and its life history consequences in wild reindeer. *Oikos*, **51**, 238–242.

Stephens, P. A., Boyd, I. L., McNamara, J. M., & Houston, A. I. (2009). Capital breeding and income breeding: Their meaning, measurement, and worth. *Ecology*, **90**, 2057–2067.

Vanpé, C., Gaillard, J. M., Morellet, N., Kjellander, P., Liberg, O., Delorme, D., & Hewison, A. M. (2009). Age-specific variation in male breeding success of a territorial ungulate species, the European roe deer. *Journal of Mammalogy*, **90**(3), 661–665.

Veiberg, V., Mysterud, A., Gaillard, J. M., Delorme, D., Laere, G. V., & Klein, F. (2007). Bigger teeth for longer life? Longevity and molar height in two roe deer populations. *Biology Letters*, **3**(3), 268–270.

Wajdzik, M., Konieczny, G., Nasiadka, P., Szyjka, K., & Skubis, J. (2016). Impact of forest cover and the soil type on the quality of male roe deer in the Kielce region. *Sylwan*, **160**(5), 424–432.

Williams, G. C. (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution*, **11**(4), 398–411.

Zuur, A. F., Ieno, E. N., Walker, N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology & Evolution*, **1**, 3–14.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Study area. Map of the study area located in the Northern Apennines [Arezzo province (43°28′N, 11°53′E; black in the right panel), Tuscany (grey in the right panel), Central Italy]. The study area includes 22 hunting districts (left panel) where 531 adult roe deer (319 females, 212 males) were legally shot during the regular annual harvest (1 Jan–15 Mar for females; 15 Jun–15 Jul for males) between 2007 and 2017.

Figure S2. Sexual dimorphism in roe deer. Sexual dimorphism in 531 adult roe deer (319 females and 212 males) shot in the Northern Apennines (Arezzo province, Tuscany, Central Italy) during the regular annual harvest (1 Jan–15 Mar for females; 15 Jun–15 Jul for males) between 2007 and 2017: (1) body measurements (eviscerated body mass, BW; body condition index, BW/ML [calculated for individuals with tooth wear score $\leq$ 10]), (2) mandible measurements (mandible length, ML; diastema length, DL), and (3) dental measurements (height of M1, HM1 [measured only in individuals with M1 wear score = 0]; breadth of M1, BM1; height/breadth ratio of M1, HBRM1 [measured only in individuals with M1 wear score = 0]; cheek teeth row length, TRL).

Table S1. Chewing surface in roe deer. Differences in cheek teeth row length (TRL) between sexes, controlling for body size in 531 adult roe deer (319 females and 212 males) shot in the Northern Apennines (Arezzo province, Tuscany, Central Italy) during the regular annual harvest (1 Jan–15 Mar for females; 15 Jun–15 Jul for males) between 2007 and 2017. Model parameter estimates (β), standard errors (SE), *t*-values (*t*) and *P*-values of the model explaining differences in the cheek teeth row length (TRL), controlling for body size (i.e., a proxy of mandible length, ML), are presented.

Appendix S1. Dataset. Individual characteristics (ID, sex, and date of culling), tooth wear scores, eviscerated body weight, body condition index, mandible measurements (length of mandible, diastema and cheek teeth row), and dental measurements (M1 height, M1 breadth, and height/breadth ratio of M1) referred to 531 adult roe deer (319 females, 212 males) legally shot during the regular annual harvest (1 Jan–15 Mar for females; 15 Jun–15 Jul for males) in the Northern Apennines (Arezzo province, Tuscany, Central Italy) between 2007 and 2017.