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TIME-FOCUSED ANALYSES OF WILD BOAR ECOLOGY AND THEIR IMPLICATIONS FOR MANAGEMENT

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And as to the land which yet remains, nature would stilly by her own operation, cover it with thorns, if human strength did not prevent; which, for the sake of a living, is accustomed to groan under the stout mattock, and to cut the earth with ploughs urged through it.

Quel che resta dei campi, lo coprirebbe comunque di rovi la natura con la sua forza, se la forza dell'uomo non resistesse, abituata, per sopravvivere, a gemere sul robusto bidente e a solcare la terra premendo l'aratro.

Lucretius, *De rerum natura*, book V, 206-210

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Summary

Being cause of crop damages, vehicles collisions, and spreading disease, wild boar is a keystone species of human-wildlife conflicts in Europe. Investigating wild boar ecology with a proper consideration of its temporal dimension would allow to synthetize the relationships among resources acquisition, reproduction, and survival, and to provide useful implications for its management on a large spatial scale and in local contexts. In Chapters 1 and 2, I investigated wild boar breeding strategies and reproductive temporal patterns, considering environmental factors such as weather and food availability. In Chapters 3, 4, and 5 I evaluated the temporal patterns of the use of two protected areas of different size by the wild boar, its risk-induced resources selection, and its behavioral reactions to the stress due to capture, respectively. A comprehensive interpretation of results highlighted that wild boar ecology is based on the achievement of a short-term reproductive success, overruling both resources acquisition and medium-term survival. In the perspective of wild boar management on a large scale, the additive mortality induced by culling plans is thus likely to result ineffective in provoking durable reductions of wild population numbers. Moreover, the investigated aspects of wild boar ecology provided several implications for specific management contexts. Adult males adopted a capital breeding strategy, while subadult males were income breeders. Male reproductive efficiency is thus likely to prove highly resilient against the human harvest and ecological perturbations, suggesting the inconvenience of a male-biased culling to control wild boar populations. Resources availability strongly influenced female reproductive timing and synchrony, without really affecting the ratio of reproducing females (constantly close to the total of adult and subadult females): this evidence should be considered when arranging culling plans, in order to avoid underestimations of the reproductive potential of wild boar populations. The finding that the observed increment in the use of a large protected

area in autumn was likely driven by food resources distribution, rather than hunting avoidance, suggests that the common belief of wild boar moving into reserves to avoid hunting should be verified on a local scale. Culling individuals inside protected areas in such circumstances can thus prove ineffective to reduce human-wild boar conflicts in the bordering areas. Wild boar adopted two opposite risk-taking strategies, implying the need for a plastic and multifaceted management approach. Finally, capture and handling induced strong behavioral modifications in wild boar, suggesting the need for standardized protocols aimed at reducing stress and protecting the welfare of captured individuals. In conclusion, investigating wild boar reproductive and behavioral ecology with a proper consideration of the temporal dimension substantially advanced the available knowledge on this species, opening interesting perspectives for its management on both large and small scales.

INTRODUCTION

In last decades, there has been a general increase of large mammal distribution and demography across Europe. This was mainly due to macroscopic environmental changes, as the progressive abandon of marginal rural areas and the consequential recover of natural habitats (Apollonio et al. 2010). A further role was played by protected area growth in number and extension (Grignolio et al. 2014), and by the improvement of conservation and management techniques (Putman & Apollonio 2014). Despite it was mostly beneficial for the conservation of biodiversity, several conflicts between human activities and some animal species arose from this rapid recover. Showing a widespread distribution and potentially reaching high local densities (Massei et al. 2015), wild boar (*Sus scrofa*) represents the keystone species for understanding this emerging situation. This species negatively affects a wide spectrum of human activities, as it provokes huge damages on both crops (Herrero et al. 2006) and livestock (Herrero & De Luco 2003), in addition to treating public safety by occupying urban areas (Cahill et al. 2012), causing vehicle collisions (Colino–Rabanal et al. 2012), and spreading zoonosis (Vicente et al. 2019). Both throughout direct consumption and rooting activity, wild boar can also have a strong impact on ecosystems by reducing animal and plant abundance and richness (Massei & Genov 2004, Barrios-Garcia & Ballari 2012). This set of factors makes wild boar, among large mammals, one of the most detrimental species for European economy and ecosystems. Becoming a priority in several European countries, its management was considerably enhanced in last years by a plentiful scientific research concerning wild boar biology and ecology (e.g., Tolon et al. 2009, Thurfjell et al. 2014, Canu et al. 2015, Brivio et al. 2017, Gamelon et al. 2017, Touzot et al. 2020). Nevertheless, our knowledge of wild boar ecology remains both fragmentary and deficient on specific aspects, leaving the management of this species as a never-ending problem. It would result particularly useful the achievement of a synthetic view of the set of trade-offs among resources acquisition, survival, and reproduction that wild boar face during their life. This goal may be accomplished by integrating the classical research on wild boar ecology with more information on its temporal dimension and by increasing the attention devoted to it. Time may indeed be considered as a further axis characterizing the complex system of physiologic, environmental, and anthropic factors that shape wild boar ecological strategies.

Beside its importance for the achievement of a synthetic view on the ecological trade-offs of wild boar, the consideration of the temporal dimension may also help to discern some specific aspects of its ecology, with direct implications for management. For instance, a deeper comprehension of the temporal dimension of wild boar reproductive strategies and its relationship with environmental factors would allow a more reliable management planning. Similarly, understanding how wild boar behavioral patterns measured at different temporal scales are affected by the existing management practices and human disturb may lead to a substantial improvement of management effectiveness. Accordingly, in part 1 of this thesis I investigated two temporal aspects of wild boar reproductive ecology: the time separating resources acquisition from their investment in reproduction among different sex and age classes (Chapter 1) and the plasticity in reproductive timing and synchrony of females in respect to the environmental variability (Chapter 2). In part 2, I evaluated temporal patterns of wild boar movement ecology in response to management practices and human disturb, and in particular the institution of protected areas where hunting is forbidden (Chapter 3), the proximity with human infrastructures and other risk-related spatial components (Chapter 4), and the capture, manipulation, and release of individuals (Chapter 5).

In Chapter 1 I evaluated variations of wild boar body weight of different sex and age classes in respect to the temporal occurrence of their reproductive investment, considering the availability of food resources. In forested areas, male reproductive investment coincides with the most food-rich season (i.e., the autumnal mast production), providing a unique occasion to investigate the trade-off between resources acquisition and reproduction. During the rut, males of polygynous species may indeed exhibit a feeding reduction (Brivio et al. 2010, Corlatti & Bassano 2014), or even suppression (Miquelle 1990, Apollonio & Di Vittorio 2004), in order to allocate more time to compete with other males for mating opportunities. But, differently from species with a rut temporally separated from the most food-rich season, such strategy may entail unsustainable survival costs for wild boar males. By measuring the time separating resources acquisition from their investment in reproduction, this investigation may also assess the position of different sex and age classes of wild boar along the capital-income breeding continuum. Individuals can indeed fuel their reproduction effort with resources either previously acquired and stored in form of body reserves (capital breeding) or collected at the same time of reproduction (income breeding, Jönsson 1997, Stephens et al. 2009). While capital-income breeding strategies were firstly described in theoretical evolutionary ecology studies (Jönsson 1997), they have substantial consequences on animal sensitivity to environmental changes. Capital breeders tend indeed to be less sensitive to environmental variability (Stephens et al. 2009, Kerby & Post 2013), because their ability to acquire (and store) resources prior to reproduction gives them the possibility to count on a longer and adjustable time window in which resources to fuel reproduction have to be collected. Moreover, a large capital of stored energy allows a much higher reproductive investment (Apollonio et al. 2020). Nevertheless, a capital breeding strategy entails higher costs in terms of metabolic expenditures for body reserves maintenance and transportation (Stephens et al. 2009). With regard to the capital-income breeding in wild boar, most attention has so far been devoted to females, which resulted to adopt different strategies depending on the environmental context (Servanty et al. 2009, Frauendorf et al. 2016, Gamelon et al. 2017). Conversely, studies on male position along the capital-income breeding continuum have never been conducted. As explained above, an income breeding strategy would likely entail a higher sensitivity of male reproductive effectiveness to environmental perturbations. Harsh conditions during the rut may thus be expected to reduce the ratio of fertilized females and ultimately limit population growth, while if males are capital breeders this would be unlikely to happen. Since the same individuals can also adopt different strategies at different steps of their life cycles, it would also be important to assess the position of subadult versus adult males, provided that subadult male wild boar can actually take part in reproduction as previously suggested (Šprem et al. 2011). If younger classes of males can counterbalance an eventual scarceness of adult males, then culling plans targeting only or predominantly adult males would have no chance to reduce the reproductive potential of wild populations.

Differently from males, female wild boar have a passive role during the rut and do not sustain any substantial reproductive costs during the autumnal food optimum. Nevertheless, in this period they regulate their future reproductive investment trough ovulation. During poor years (e.g., low mast production) female wild boar may thus decide to either take part in reproduction, or, alternatively, to skip it, allocating the scarce resources to their own maintenance and survival. In this context, the potential to plasticly delete ovulation in respect to environmental conditions would give females more time to get the nutritional condition needed to reproduce. In Chapter 2 I investigated female reproductive timing and synchrony of a wild boar population and their relationship with environmental factors such as weather and mast seed availability (in this regard, see the Appendix for an innovative sampling scheme to measure mast productivity of beech, *Fagus sylvatica*). Ungulates inhabiting temperate regions typically use photoperiodism to trigger their reproduction (Zerbe et al. 2012). While this rigid cue allows them to reliably anticipate predictable phenomena like seasonal cycles, it also limits their ability to plasticly adjust their reproductive timing to fine-scale environmental variability such as weather and current resource availability. Nevertheless, better nutritional conditions have been reported to slightly anticipate the female individual reproductive timing of some temperate ungulates (McGinnes & Downing 1977, Hamilton & Blaxter 1980, Flydal & Reimers 2002), providing advantages in terms of offspring survival (Côté & Festa-Bianchet 2001). In wild boar this skill seems to be stronger than in other temperate species, as a high plasticity has been reported at both the individual (Canu et al. 2015) and population (Servanty et al. 2009) levels. Tropical ungulates can also plasticly adjust reproductive synchrony (i.e., breeding season length) depending on the environmental conditions (Ogutu et al. 2015), but this phenomenon has never been reported for wild boar nor for any other photoperiodic ungulate of temperate latitudes. The potential of wild boar populations to adjust both timing and duration of breeding season lengths would have major implications for specific aspects of management. First, some environmental conditions would produce more synchronous ovulations and, thus, births. By means of the saturation of predators' efficiency (Darling 1938), this would be likely to enhance offspring survival and population growth. Second, in agricultural landscapes a shorter birth season would translate in a concentrated (and, at some extent, predictable) peak of potential damages on agricultural crops. Third, this further aspect of reproductive plasticity would allow to a higher proportion of females to take part in reproduction (if, for instance, those which are in suboptimal body conditions can delay ovulation and still achieve maternity), which should be considered when arranging culling plans.

In addition to provide valuable information supporting a robust management planning, scientific research can also enhance effectiveness of existing practices by investigating how they affect animal behavioral ecology. Since in many areas hunting represents the main cause of mortality (e.g., Merli et al. 2017), it is not surprisingly that hunting practices, particularly drive hunt, can induce major modifications of wild boar movement ecology (Keuling et al. 2008, Tolon et al. 2009, Thurfjell et al. 2013). In Europe this practice is typically performed in autumn: its temporal overlap with the food-rich season offers a good opportunity to investigate behavioral strategies adopted by wild boar in order to avoid human predation while simultaneously taking advantage of the food optimum. It is worth noting that drive hunt is only allowed during the day while wild boar feed at night (Brivio et al. 2017), so these two requirements are actually separated at the fine temporal scale. Protected areas may represent a perfect case of study for this issue, since they generally offer a complete shelter from hunting and often include heterogeneous conditions of habitats and food availability in respect to the surrounding, unprotected lands. Global surface included in protected areas substantially increased during the last decades, giving a fundamental contribution to the in-situ conservation of biodiversity (UNEP-WCMC IUCN 2018). Protected areas where hunting is strictly forbidden were indeed one of the most important factors which facilitated the rapid recover of large mammal populations in Europe but, nowadays, they can represent an obstacle for the effectiveness of large-scale control plans of pest species based on recreational hunting. The so-called “reserve effect” may be partially lessened by culling individuals inside protected areas, but this practice entails a few practical cons: disturb to plant and animal communities, costs to pay specialized operators, and blame from the public opinion. Tolon et al. (2009) showed wild boar to use a protected area as shelter during the hunting season, but their investigation was based on data with a poor spatial resolution (i.e., traditional telemetry) and a description of spatial behavior based on broad spatial scale (i.e., home ranges). Reliability of such results thus remains uncertain, as well as their generalizability to other wild boar populations or environmental contexts. Moreover, those authors did not consider the role played by protected area size, nor the potential influence of resources spatial availability in shaping wild boar movements. In more general terms, information on this pest movements in respect to protected areas’ borders should be locally collected to establish whether there is an objective need for applying control plans inside protected areas. Indeed, if the almost totality of wild boar move inside protected areas during the hunting season, then well-designed culling plans should be performed inside protected areas. Conversely, the above-mentioned disadvantages of culling individuals inside protected areas would not be scientifically justified. In Chapter 3 I evaluated the daily temporal patterns of wild boar use of protected areas, to determine if these conservation tools can represent refuges for this pest species, preventing or hindering its management.

Further than refuging inside protected areas, wild boar may exhibit other behaviors to reduce the real or perceived human-induced mortality risk. For instance, wild boar proximity with human infrastructures was associated with an increased risk perception (Greco et al. 2021), and the selection of covered habitats and a low mobility may reduce the likelihood to be culled by hunters (Merli et al. 2017). A similar effect may be expected for the selection of familiar sites, which was shown to enhance the ability to escape predators in another ungulate (roe deer, *Capreolus capreolus*, Gehr et al. 2020). With respect to resources availability, the safest places likely entail higher intra-specific competition for resources (e.g., food), while in more dangerous situation (e.g., human settlements) more feeding opportunities may be available at the cost of an increased risk. The trade-off between risk avoidance and food intake may thus shape individual strategies of risk-taking, but it is unknown whether the individual willingness to take risks is consistent across different behaviors (e.g., predator avoidance, selection for safe and familiar sites, average mobility). In Chapter 4, I modelled a risk-induced resources selection of wild boar belonging to two different populations experiencing drastically divergent environmental conditions. I considered the temporal dimension on a very fine scale (2 hours) in order to detect wild boar individual choices. By computing four risk-taking traits at the individual level, I aimed to assess if repeated measures of the individual willingness to take risks across different contexts provide a good proxy of individual homogeneous strategies of risk-tacking. Behavioral syndromes theory (Sih et al. 2004) predicts different risk-taking traits to be rigidly correlated, with individuals being totally risk-taker or, alternatively, totally risk-avoiders. While this phenomenon was demonstrated under controlled experimental conditions (e.g.,Van Oers et al. 2003, Vetter et al. 2016, Thys et al. 2017), it remains unclear whether these extreme strategies, entailing a strong constraint for animal adaptiveness, are exhibited by wild, free-ranging animals and if they remain valid at a fine temporal scale. From a management point of view, if those wild boars approaching human infrastructures are also avoiding safe sites (e.g., covered and familiar habitats), and are relatively fast-moving, then ordinary control plans would naturally tend to their removal, ultimately ensuring a strong reduction of human-wild boar contacts. If, conversely, wild boar compensate the risks induced by the proximity with humans by exhibiting prudent behaviors in respect to the likelihood to be culled, then specific management strategies should be designed to remove those individuals from wild populations.

Hunting and indirect disturb are not the only ways by which humans can influence wild boar behavior. Beside representing a common management strategy, capture and manipulation of individuals can indeed provoke major behavioral responses once wild boar have been released. In Chapter 5 I measured the lasting time of the effects that a capture and release event can have on wild boar behavior, comparing activity and movement patterns. For wild large mammals, capture and manipulation by humans is likely to represent one of the most stressful event to be experienced in their lives (Kock et al. 1987). Once released in the wild, this huge amount of human-induced stress typically forces animals to allocate more time to rest, for a variable period. During this time interval, in order to cope with their basic needs (e.g., resources acquisition, survival), animals may exhibit divergent activity and movement patterns. For instance, Alpine ibex (*Capra ibex*) reduced their activity rate after the release but maintained a regular movement rate, despite the capture induced a very mild, short-lasting effect on this species behavior (Brivio et al.

2015). Conversely, animals may be expected to react to a stronger capture stress with a fast recover of their activity rate but maintaining inhibited spatial movements for longer periods. In so doing, they would minimize the risk to encounter predators while ensuring a sufficient food intake, at the cost of feeding in suboptimal areas. Being one of the most important pest species to be managed in Europe, wild boar represents one of the most captured species and still no study investigated the effects that capture can induce on its post-release behavior. If, similarly to other species, wild boar exhibits depressed activity and movements for a certain period following the capture event, standardized release protocols should be designed in order to assure animal wellness and prevent risks for people to be injured by animals unable to flee. Moreover, identifying drug mixtures producing lighter side effects on wild boar post-release behavior would allow to arrange more rigorous, science-oriented, capture and handling protocols.

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Chapter 1

Capital-income breeding in wild boar: a comparison between two sexes

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Capital-income breeding in wild boar: a comparison between two sexes

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Organisms differ in the strategy adopted to fuel reproduction by using resources either previously acquired and stored in body reserves (capital breeding) or, conversely, acquired during their reproductive activity (income breeding). The choice of one or the other strategy is related to several internal and external factors which are counteractive in wild boar. Based on a large dataset of culled wild boar, we investigated individual body weight variability throughout the period of 1st September–31st January, which included the main part of the mating season, among different sex and age classes to determine their position along the capital-income breeding continuum. Though food resources were abundant during the rut, adult males lost body weight suggesting they adopted a predominantly capital breeding strategy, likely owing to the high intra-sexual competition entailed by the peculiar mating system of the species. On the contrary, subadult males seemed to behave as income breeders, likely enhancing the reproductive flexibility of wild boar populations. During the rut, females stored reserves, thus suggesting that they substantially relied on them to cover future reproductive costs.

The life history of an animal is comprised of sets of trade-offs among growth, survival, and reproduction that organisms face during their life[1]. A major aspect of life history diversity among animal species is that the resources allocated to reproduction are obtained either from stored reserves within the body or the current intake, resulting in the division between capital and income breeders (e.g.,[2,3]). This concept is of utmost importance in theoretical evolutionary ecology as it influences both the body condition-reproductive success relationship and the time lag of organisms-environmental resources linkage[3], but it can also be profitably applied to conservation and management as it affects a species' sensitivity to environmental changes[3,4]. Furthermore, given the wide exploitation of ungulates in hunting and their growing involvement in wildlife-human conflicts, their life histories are raising a strong interest among researchers.

Stephens and colleagues[3] reported that the degree of capital and income breeding of organisms is related to a variety of ecological, morphological, and physiological factors. In particular, high food availability during the breeding season typically promotes income breeding strategies[2], while temporal mismatches between resource supply and reproductive demand promote capital breeding[5]. Larger body size can facilitate capital breeding on account of a lower relative cost for reserve transportation and a higher metabolism efficiency[3]. The mating system and, specifically, the degree of polygyny may act as a further push-factor in positioning male ungulates along the capital-income continuum[6]. Indeed, higher levels of intra-sexual male competition for mating opportunities are likely to enhance the reproductive demands of polygynous males and, concomitantly, their tendency to adopt feeding reduction[7,8] and suppression[9,10] during the rut, inevitably forcing them to rely on a stored capital of reserves. Moreover, in order to maximize their lifetime reproductive success, individuals can occupy different positions along the capital-income breeding continuum throughout their life cycles[5]. Indeed, adult male ungulates typically show high body weight loss during the rut (i.e., high reliance on stored reserves) compared to younger males which, conversely, give priority to growth. As a consequence, young males show a limited or null body weight loss (for a review, see Mysterud et al.[11]), although they can still be fully or partially involved in reproduction[12,13]. When evaluating life history strategies, it is therefore essential to first characterise sex and age classes, as groups of individuals at different stages of the growth-reproduction trade-off are likely to adopt different strategies for the acquisition of resources to invest in reproduction.

One of the major constraints for studies on capital-income breeding lies in the difficulty to objectively circumscribe the time period over which the reproductive costs should be measured[3]. As female investment into reproduction usually includes a variety of activities linked to a single reproductive event (for mammals: mating,

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foetuses growth, giving birth, and lactation), studies on females are particularly concerned with the difficulties in circumscribing the period of such reproductive costs. Conversely, since most male ungulates have no further reproductive cost after conception[11], their reproductive effort is entirely included in the rutting season. Nevertheless, finding an objective way to circumscribe this period on a local level entails several practical complications. Previous studies on male ungulate life histories arbitrarily delimited the rut[14], obtained it from other studies[15], or roughly derived it from field behavioural observations[16].

As a rare example of highly polygynous species[17], exhibiting similar early-life growth rates in both sexes (e.g.,[18]) and a short generation time compared to other ungulates (e.g.,[19,20]), wild boar (*Sus scrofa*) is a particularly interesting species for studying life history strategies. The position of wild boar along the capital-income breeding continuum has previously only been determined for females and was found to be different according to the area and the study[19,21,22], with litter size being the only measure of reproductive effort considered. Conversely, males' reliance on the stored capital or the available resource income for reproduction has never been investigated, though this sex presents a unique combination of contrasting factors pushing simultaneously towards the two opposite strategies. On the one hand, the mating period occurs when food resources are relatively abundant. This should prevent the need of previously stored energy and facilitate income breeding. Oak (*Quercus* spp.), chestnut (*Castanea sativa*), and beech (*Fagus sylvatica*) seed production, which accounts for most of European wild boar's diet (at least in natural and semi-natural situations in which agricultural crops are scarce and supplementary feeding is not provided[23,24]), is typically concentrated in late autumn, when mating usually occurs[25]. On the other hand, wild boar morphology and reproductive biology should push males to adopt capital breeding, by reducing costs associated with this strategy and accounting for feeding suppression, respectively. As a matter of fact, large size and a thermally efficient body shape (sensu Allen[26]) should enhance wild boar metabolism efficiency, thus reducing costs of capital storing, transportation, and maintenance. The relatively high degree of polygyny of this species[17] entails high competition among males for mating opportunities. This may be expected to increase both the need and the potential reproductive value of relying on stored reserves and thus promote capital breeding[6]. In addition, the gregarious habits of females[27] and the high litter size[28] make male reproductive effort even more beneficial in terms of potential number of descendants, thus exacerbating intra-male competition.

Age can also be expected to heavily determine individual strategies to fuel reproduction, as younger wild boar still need to allocate part of the resources to growth. Consequently, they have lower body reserves to invest[29]. As mentioned above, it becomes essential to discuss individuals' reproductive reliance on stored reserves in the context of their growth stages, typically represented by age classes. Nevertheless, the available growth curves on wild boar are provided by studies limited by the use of either a small sample size[30,31], or descriptive statistics alone[18], or both[32,33].

When relatively high, hunting pressure can also play a role in shaping wild boar reproductive strategies, as an unbalanced removal of adult individuals can influence the first reproduction of both subadult males[34] and females[19]. If the harvest is adult male-biased (not the case of our study area[35]), hunting can also cause a shortage of adult males and, therefore, lower the levels of sexual competition[36], thus potentially reducing the reproductive effort and ultimately the need of capital breeding. Nevertheless, an opposite effect (i.e., increased male reproductive costs) was described by Mysterud et al.[14] in female-skewed moose (*Alces alces*) populations, likely because males had to enhance their displacements in order to take advantage of the higher number of available female groups.

Based on a large dataset of culled wild boar, we first modelled male and female body growth curves and identified age classes in order to properly compare breeding strategies among homogeneous groups of individuals. To independently determine the period over which male reproductive effort is sustained, we assessed female oestrus distribution throughout the year and used it as a proxy of the rutting season. We then compared body weight variability throughout autumn–winter in different sex and age classes in order to evaluate potential changes in male weight with respect to other classes owing to their reproductive effort. In so doing, we aimed to ascertain their position along the capital-income breeding continuum.

Results

Sex and age class identification.

Gompertz growth models' estimated parameters, summarised in Supplementary Table S1, were all statistically significant. Sexual size dimorphism appeared around 1 year of age. Males had to reach 3 years of age to exceed 90% of their asymptotical weight (85 kg), while the age for females was 2 years (female asymptotical weight = 61 kg, Fig. 1). On this basis, the following sex and age classes were identified: male and female piglets (individuals younger than 1 year), subadult males (males older than 1 year but younger than 3 years), subadult females (females older than 1 year but younger than 2 years), adult males (males older than 3 years), and adult females (females older than 2 years). Sample distribution among sex and age classes is reported in Supplementary Table S2.

Rutting season identification.

The intra-annual distribution of conception dates started in October, peaked in January and lasted until April, with most events concentrated in the period December-March (Fig. 2). The portion of conception events occurring during the sampling period (153 days starting from 1st September) was 59.68 ± 5.00% (mean ± SE) of the total.

Seasonal variability of individual body weight in different classes.

All selected best models (identified following the minimum Akaike's Information Criterion, AIC, see Methods for more details) significantly explained body weight variability (p-values of all included predictor are reported in Supplementary Tab. S3). Adult males' best model included sampling day, individual age, previous winter rain precipitation, and spring temperature as predictor variables ($R^2_{adj} = 0.100$). Throughout the sampling period, adult males showed a non-

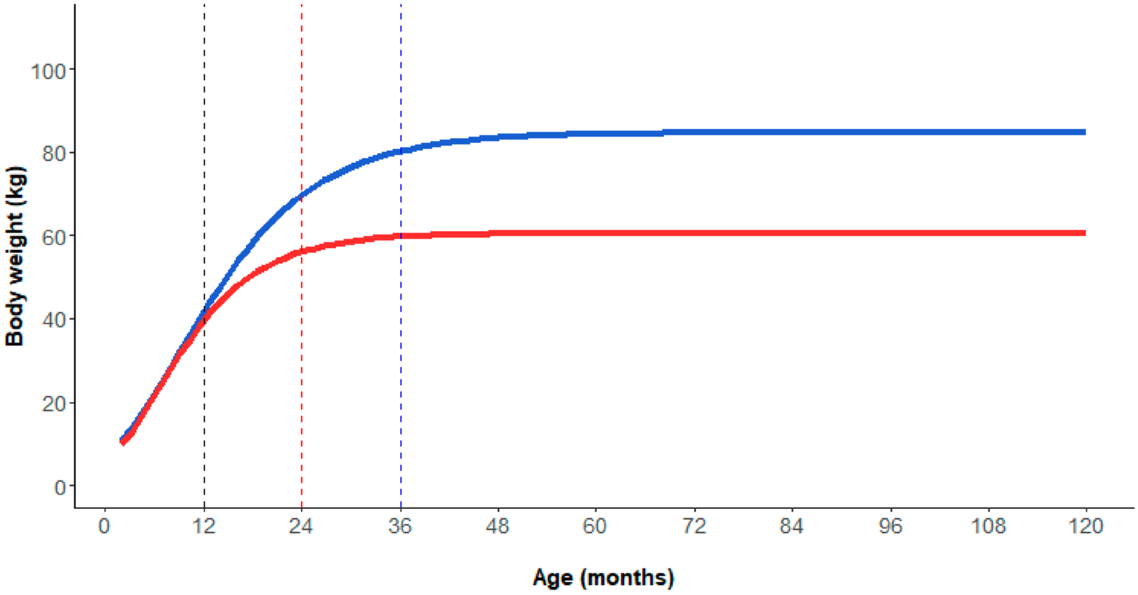

Figure 1. Body weight variation of males (blue line) and females (red line) at growing ages. Values were predicted by the Gompertz growth models separately for males and females (see the text for more details). Vertical dashed lines represent the limits between piglets-subadults (both sexes, black line), subadult-adult females (red line), subadult-adult males (blue line).

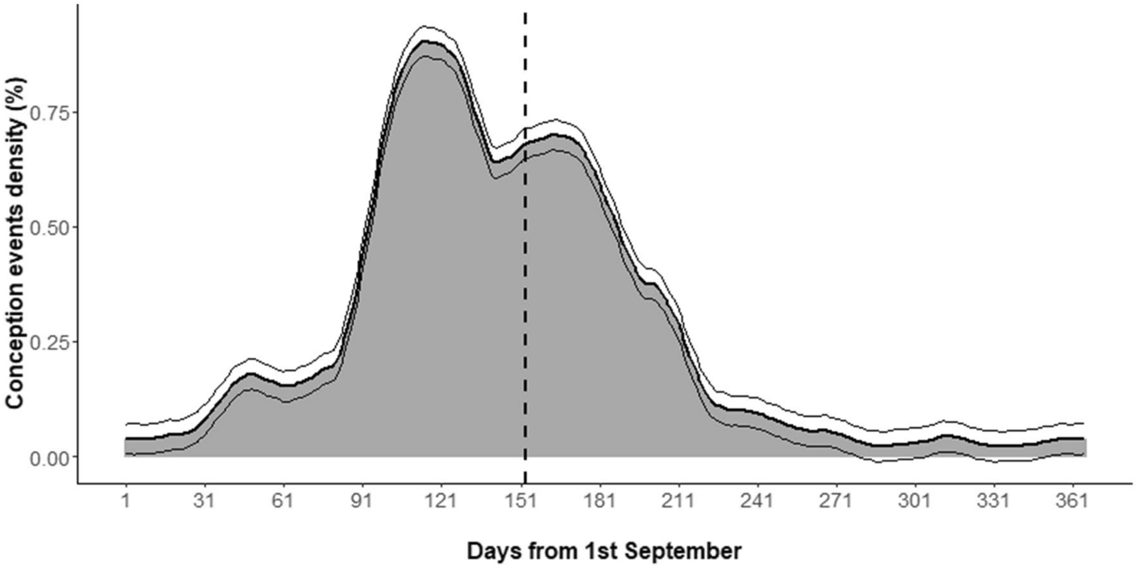

Figure 2. Conception event smoothed distribution throughout the year assessed from individual age of piglets and subadult individuals, culling date, and gestation period (see the text for more details). Upper and lower thin lines represent the distribution of mean + SE and mean − SE, respectively. Date is expressed as days from 1st September and equivalent to the sampling day. The dashed line represents the end of the sampling period (153 days, from 1st September to 31st January).

linear pattern of body weight variability, with a slight increase during the first part of the sampling period (lasting approximately 50 days) and a subsequent steady loss. Predicted weights ranged from a maximum of about 91 kg (around the 50th day of the sampling period) to 82 kg (at the end of the sampling period, Fig. 3a), thus showing a weight loss of 9.89%. As they grew older, adult males showed only a slight, constant weight gain. Adult male weights increased with increasing spring average temperature, until reaching a maximum peak with an average temperature of 8.0 °C, then slightly decreased above this optimal value, and finally stabilised above 9.5 °C. A slightly positive effect of previous winter rain precipitation was detected (see Supplementary Fig. S1).

The best model explaining adult female body weight variability included sampling day, individual age, and previous year chestnut productivity as predictor variables ($R^2_{adj} = 0.214$). Adult females gained body weight with a steady pattern throughout the sampling period, starting with an average weight of 55 kg and reaching up to 68 kg at the end of the period (Fig. 3b), with a total gain of 23.64% of the initial weight. In accordance with the

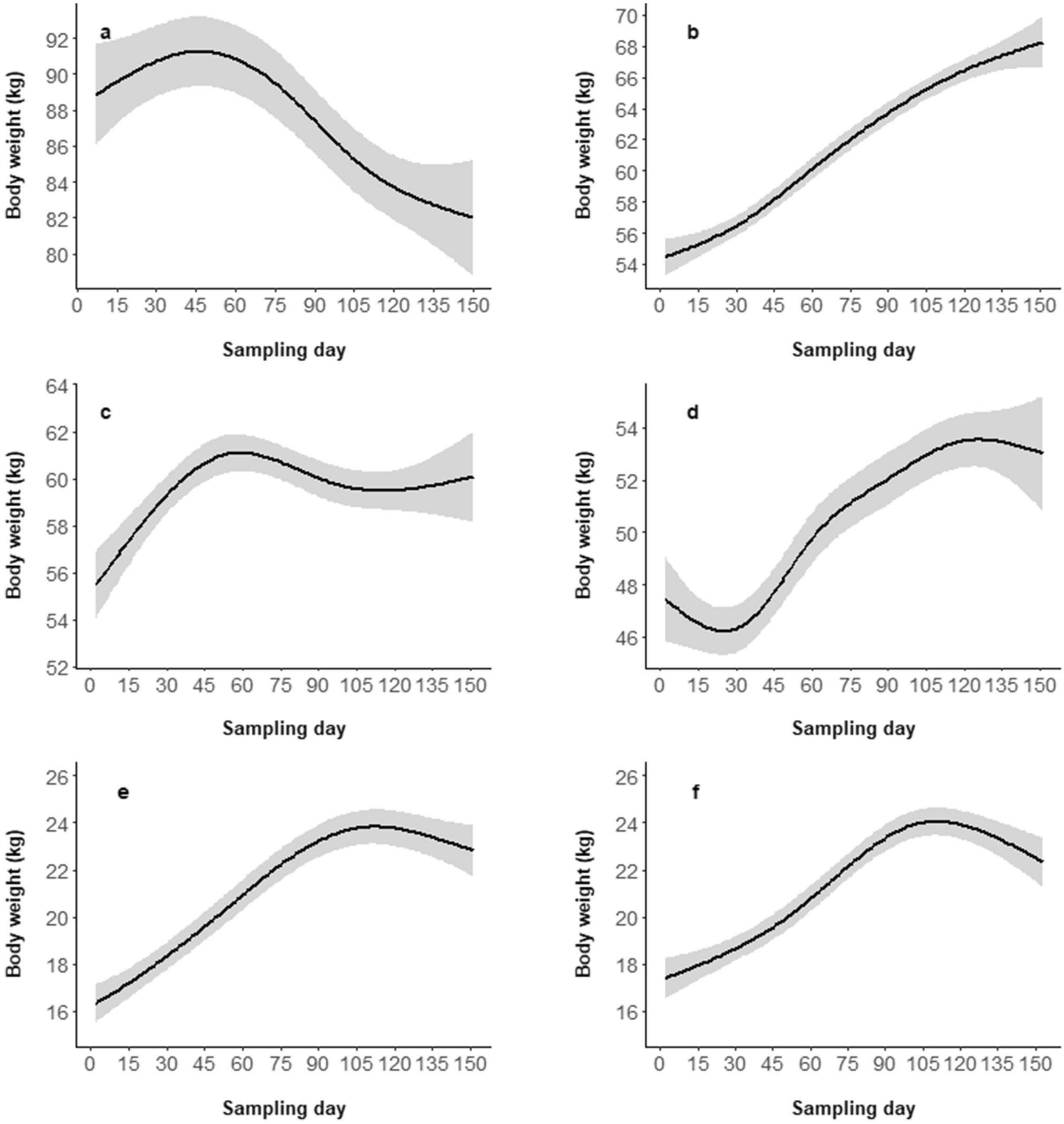

Figure 3. Body weight variation of adult males (**a**), adult females (**b**), subadult males (**c**), subadult females (**d**), male piglets (**e**), and female piglets (**f**) throughout the sampling period. The first sampling day corresponds to 1st September. Values were predicted by the best models separately for each class. Grey-shaded areas represent the estimated standard errors. The predictions are given according to the mean of all other covariates in the models.

results of the Gompertz body growth model, adult females showed substantially stable weights at growing ages. Though statistically significant, previous year chestnut productivity had a positive but biologically negligible effect on adult female body weight (Supplementary Fig. S2).

The best model predicting subadult male body weight variability included the following set of predictor variables: sampling day, individual age, previous year chestnut productivity, previous winter, spring, and summer average temperatures ($R^2_{adj} = 0.238$). Their body weight showed only small variations throughout the sampling period (predicted values: 55–61 kg), with an initial slight increase lasting about 50 days, followed by a horizontal pattern lasting for the rest of the season (Fig. 3c). Individual age had a clear, positive effect on the predicted body weight, while previous year chestnut productivity accounted for slightly higher body weight. Finally, the average temperature of the summer and spring months preceding the hunting season negatively affected subadult male body weight, while that of the previous winter months did not show any relevant effect (Supplementary Fig. S3).

Subadult female body weight variability was explained by the best model including sampling day, individual age, previous summer average temperature, previous winter rain precipitation, and current autumn rain precipitation as predictor variables ($R^2_{adj} = 0.233$). Females of this age class showed a steady increase of their body weight throughout the sampling period, a result which is similar to that of adult females, though with wider confidence intervals (Fig. 3d). Moreover, the relation with age was positive. As with subadult males, the best model predicted a substantial negative relation between body weight and previous summer average temperature. Subadult females reached their maximum body weight with mean values of rain precipitation during the previous winter (around 4 mm/day), while higher values of current autumn rain (above 5.0 mm/day) accounted for heavier body weights (Supplementary Fig. S4).

The best model explaining the variability of male piglet body weight included the predictors: sampling day, individual age, current year global productivity index, mean rain precipitation of previous summer, and average temperature of previous spring ($R^2_{adj} = 0.370$). In this class, body weight increased with a steady pattern throughout the sampling period until the 110th sampling day and slightly decreased during last 40 days of hunting (Fig. 3e). Individual age had a positive effect on the response variable, with older male piglets being constantly heavier than younger ones. The relation between male piglet body weight and current year global productivity index was linear and positive, whereas other predictor variables had a significant but biologically negligible effect (Supplementary Fig. S5).

As for female piglets, the best model included sampling day, individual age, and previous year Turkey oak (*Quercus cerris*) productivity as predictor variables ($R^2_{adj} = 0.331$). Their predicted body weight increased throughout the sampling period, with a pattern essentially identical to that of male piglets (Fig. 3f). Likewise, a positive effect of individual age was assessed. Finally, female piglet body weight was higher when previous year Turkey oak productivity was around 0.4 Mg/ha (Supplementary Fig. S6).

Discussion

We investigated wild boar capital-income breeding strategies by using a large dataset of culled individuals. We objectively characterised age classes and quantitatively assessed the timing of the rut with a large sample of conception dates and a comprehensive account of uncertainty. Our results suggest that adult males relied on a stored capital of reserves to cope with their reproductive requirements, although weight gains of other classes confirmed the expectation that food resources were particularly abundant during the rut.

Our sex and age classification on the basis of growth stages is consistent with that used in previous studies with regards to piglets of both sexes and females in general[19,25,36]. On the contrary, the subdivision between subadult and adult males was placed at 3 years, unlike other studies (2 years in[33,34,36]). As males were clearly still growing between 2 and 3 years of age, they could not afford a full investment in reproduction, despite being already sexually mature[37], which is the typical condition of subadults. In this respect, we would argue that our classification better generalised male growth stages. This enabled us to properly compare body weight variation patterns and breeding strategies among homogenous groups of individuals.

Only adult males showed an absolute weight loss during the sampling period (1st September–31st January), whereas all other classes gained body weight, though with different extents and patterns (Fig. 3). Food resources were particularly abundant during that time of the year, as confirmed by weight gains of other classes as well as by data referring to wild boar spatial behaviour within the same area[38]. Since hunting disturbance is known to have a minimal impact on wild boar behaviour[39,40] and the rich-food habitats (forest) are also the safest refuges from hunting risk in our study area[35], we can exclude the possibility that hunting affected the weight loss observed in adult males. Reproductive efforts were more likely to be the main cause of this negative trend, as supported by the temporal match between the start of adult male body weight decrease (around the 50th sampling day) and the start of the conception event distribution. We may directly estimate a relative loss of about 9.89% of the pre-reproductive adult male body weight (50th sampling day), though the total weight loss related to reproduction was likely much higher. Indeed, our sampling period was constrained by hunting season limits and covered only a part of the rutting season, including 59.68 ± 5.00% of all conception events (Fig. 2). If the relation between body weight loss and conception event distribution had remained the same as it was observed during the sampling period, we can estimate that adult males would have lost 16.57 ± 1.39% of their pre-reproductive body weight by the end of the rut. Adult male wild boar relative weight loss estimated by our analysis can be compared with that of male Alpine chamois (*Rupicapra rupicapra rupicapra*, 17–19% in Mason et al.[16] and 16.0% in Apollonio et al.[6]) and male red deer (*Cervus elaphus*, 19.5% in Apollonio et al.[6]), which are usually considered capital breeders[6,14]. Accordingly, our results position adult male wild boar towards the capital end of the capital-income breeding continuum.

Both the reproduction effort itself and feeding reduction or suppression during the rutting season possibly accounted for the reproduction-induced weight loss of adult males. Though information on male wild boar reproductive behaviour is still lacking, during the rut they are thought to roam widely in search for groups of receptive females, actively competing to monopolise and finally mate with them[41,42]. This behavioural pattern is likely to enhance the energetic expenditure of males during the rut. Even though hunting pressure may partially weaken the direct competition to monopolise female groups by unbalancing the population structure toward females[36], a female-skewed population has been shown to increase male reproductive cost in other species (e.g., in moose[14]). This is likely due to a higher energy expenditure in spatial movements, as each male would have the opportunity to mate with several scattered female groups. Nevertheless, in such a food-rich season, the massive weight loss observed can hardly be explained by energy expenditure alone. However, the almost total feeding suppression which characterises a number of male polygynous ungulates (see Miquelle[9] for moose; Apollonio and Di

Vittorio[10] for fallow deer, *Dama dama*) would be unaffordable for wild boar, given the long-lasting rut. Indeed, it was never detected in studies involving the analysis of wild boar stomach content[23,43]. We can therefore presume that adult male wild boar may adopt milder forms of feeding reduction during the rut, similarly to male Alpine ibex (*Capra ibex*)[7] and Alpine chamois[8]. This explanation is supported by the decrease of the insulin-like growth factor 1 concentrations (IGF-I, whose secretion is linked with energy supply) observed in males during autumn and winter by Treyer et al.[43]. This may have also contributed to weaken the effect of food abundance during the rut in determining the adoption of an income breeding strategy, by preventing individuals to fully exploit it.

Similarly to adult males, subadult males increased their body weight during the first part of the sampling period but then showed substantially stable values, with an almost flat slope (Fig. 3c). As they are still growing, subadult males may not have considerable stored reserves available for reproduction. The temporary 2–3 month growth break observed may indicate that subadult males took part in reproduction (as previously suggested by Šprem et al.[34]), though investing only resources from the current intake and thus behaving as income breeders. Since income breeding can only support a small reproductive investment and a direct competition with adults would be totally ineffective for them[44], we can argue that subadult males relied on alternative mating tactics to achieve at least some paternities[12,13]. Wild boar social organisation may have also contributed to the missed weight gain observed in this class. Indeed, during the rut adult males display agonistic behaviours against subadult males joining females groups[27], potentially moving them away from food-rich areas, which are typically occupied by females. Thus, we can argue that subadult males' reproductive contribution is inversely dependent on the availability of adult males in the population. This may therefore potentially reduce the negative effect of a male-biased culling on the reproductive outcomes.

Both adult and subadult females gained body weight almost steadily during the whole sampling period (Fig. 3b,d). However, this result did not allow us to directly determine their position along the capital-income breeding continuum. Indeed, female reproductive investment can be considered negligible during the mating season, then becoming substantial during the subsequent phases of foetuses formation, birth, and weaning, which essentially occupy the rest of the year. While subadult females were still growing and therefore may have allocated the resources acquired during autumn–winter to body growth, adult females have already completed their body development and reasonably invested the resources stored during this period in the subsequent reproduction phases. This suggests that adult females substantially relied on reserves stored in autumn–winter to cover future reproductive costs and, thus, adopted a capital breeding strategy.

We used a long-lasting dataset sampled during 14 consecutive hunting seasons but limited to 5 months per year. This prevented us from properly evaluating females' reproductive reliance on stored reserves and observing the last portion of the rutting season. However, we managed to predict the total reproductive cost carried by adult males by means of a quantitative and independent assessment of rut timing. Our large sample size provided a robust insight into wild boar life history at a population level, which would have been unfeasible with longitudinal studies as they are typically limited to few monitored individuals (e.g.,[12,45]). Nevertheless, further well-designed longitudinal studies may be extremely useful to evaluate the heterogeneity of wild boar life history on an individual level.

In conclusion, we demonstrated that adult male wild boar adopted a predominantly capital breeding strategy, while subadult males likely behaved as income breeders and enhanced the reproductive flexibility of the populations. Though we were not able to directly assess females' strategy, we detected a strong resource storage during the mast period, which is likely to be invested in the subsequent reproduction effort. Being capital breeders generally less sensitive to environmental variability[3,4], we can argue that wild boar reproductive outcomes will be highly resilient to ecological perturbations.

Materials and methods

Study area. Our study was conducted in the Alpe di Catenaia mountainous area (Northern Apennines, Italy, 43° 48′ N, 11° 49′ E, Supplementary Fig. S7) which covers a total surface of 13,400 ha and includes a protected area (Oasi Alpe di Catenaia) of 2,700 ha. Altitude ranges from 330 to 1,414 m above the sea level. The temperate-continental climate shows marked seasonal variations, with hot and dry summers (mean temperature of 18.7 °C and daily precipitation of 1.73 mm) and cold and rainy winters (mean temperature of 1.2 °C and daily precipitation of 3.55 mm). Snowfalls occur only occasionally between October and April. The area is mainly covered with mixed deciduous woods (67% of the total surface), with Turkey oak, beech, and chestnut as the most abundant tree species, while conifer woods (7%), agricultural crops (16%), and mixed open-shrubs areas (10%) cover the rest of the surface. Wild boar unselective drive hunts (i.e. targeting all social classes) involved 25–50 hunters and were performed in the surroundings of the protected area three times a week from September–October to January (on average of 58.3 hunting days per year). Hunting pressure was high and relatively constant over the years, with an average of 6.4 wild boar/km^2 harvested every year[35].

Data collection. We collected data on 8,763 wild boar of all age and sex classes culled within our study area from 1st September to 31st January in the period 2002–2016, for a total of 14 consecutive hunting seasons. Undressed body weight and culling date were recorded for each wild boar. Since female reproductive traits were not fully available for measurements, we could not subtract foetus weight from pregnant female body weight, thus potentially overestimating their body condition. Nevertheless, foetus weight (calculated on a subsample of 415 pregnant females with measurable reproductive traits) accounted for a negligible portion of mother total body weight (on average 0.51 ± 0.95%, mean ± SD). On the basis of their tooth eruption and abrasion[46], all wild boar were assigned to one of the following age intervals: < 3 months, 3–4 months, 5–6 months, 7–9 months, 10–12 months, 13–14 months, 15–16 months, 17–18 months, 19–20 months, 20–22 months, 22–24 months, 24–36 months, 3–4 years, 5–7 years, 8–10 years or > 10 years. Given the intrinsic characteristics of the tooth-based aging method, we are aware that precision decreased as age increased. Notwithstanding, this was the only feasible approach to age a large number of culled individuals.

Yearly seed productivity of beech, chestnut, and Turkey oak was acquired from an online database reporting local data collected in our study area[47]. Weather data were recorded daily in a weather station located inside our study area (43° 42′ N, 11° 55′ E) and kindly provided by the Regional Hydrological Service of Tuscany.

Ethical declarations. Data collection did not involve any alive animal. All wild boar included in analysis were culled according to Italian national and regional hunting laws.

Data analysis. *Sex and age class identification.* As we aimed to assess patterns of body growth of both sexes during different age stages, we distinguished culled individuals into males and females, thus creating 2 sub-datasets out of our original dataset (males, n = 4398, and females, n = 4365). We then assigned individual ages as the median of the age interval identified by means of tooth analysis. For each sub-dataset, body growth was then described by fitting weight to age with the Gompertz growth equation[45,48,49] through a 3-parameter nonlinear model:

$$W = a * e^{-be^{-cx}}$$

in which W is body weight at age x, a is the asymptotic body weight, e is the exponential constant, b is the displacement on the x-axis, and c is growth rate. We estimated a, b and c by means of the *SSgompertz* function of the *stats* package in R 3.2.2[50]. Finally, we used the growth curves obtained to identify 2 breakpoints: (i) age of sexual size dimorphism appearing and (ii) age of body weight exceeding 90% of its asymptotic value (sex-specific), rounding them on a yearly basis to correctly distinguish cohorts. Depending on their individual age, male and female wild boar were separately grouped into 3 age classes: piglets (below first breakpoint), subadults (above the first and below the second breakpoint) and adults (above the second breakpoint).

Rutting season identification. In order to identify the rutting season for the studied population, we estimated the temporal distribution of conception events. Individual conception dates were estimated from the age of culled piglet and subadult wild boar, culling date and gestation period, following the formula:

$$CoD = CuD - IA - GP$$

with CoD being the conception date, CuD the culling date, IA the individual age expressed in days of the culled wild boar, and GP an average gestation period of 118 days (obtained as the mean between a gestation period of 115 days reported by Henry[51] and of 121 days reported by Vericad[52]). IA was estimated as the median of the age interval identified. Only wild boar aged 2 years or younger were included in analyses, as their age interval width was ≤ 3 months, for a total of 6604 individuals. In order to take into account both sources of uncertainty (gestation period and ageing process), we smoothened the number of conception events occurring per date by means of the *loess* function of the *stats* package in R. We used a 41-day span width, i.e., the average standard error of conception date attribution, which was calculated as 1/1.96 of the sum of the mean age interval width (74 days) and the 6-day difference between two conception periods. Finally, we quantified the portion of conception events which occurred during our sampling period.

Seasonal variability of individual body weight in different classes. In order to evaluate the variability of individual body weight throughout the sampling period and its relation with reproduction efforts, we divided our dataset into 6 sub-datasets corresponding to sex and age classes previously identified by means of body growth models (adult males, n = 752, adult females, n = 1376, subadult males, n = 1629, subadult females, n = 1318, male piglets, n = 2017, and female piglets, n = 1671). Individual body weight was modelled by means of Generalised Additive Models (GAMs) with a Gaussian distribution, which were implemented by means of the *mgcv* package in R, separately for each sub-dataset. Sampling day was standardised as the number of days from 1st September and used as predictor to observe the variability of individual body weight throughout the sampling period. In order to enhance the models' robustness, we also included individual age, previous and current year forest productivities, and weather variables as predictors. Individual age, expressed in months, was calculated as the median of the age interval identified by means of the tooth analysis and used to take into account the residual age-related source of variation in individual body weight. Current and previous year productivity of Turkey oak, beech, and chestnut, expressed as Mg/ha, were measured on a yearly basis and included in the models to consider inter-annual variability of food resource availability and its potential effect on individual body weight. Moreover, we included a global forest productivity index, which was calculated as the sum of the relative productivity of all three species, which were in turn obtained as the ratio of the productivity of a certain tree species in a given year over the mean productivity of the same species during the entire study period[38]. Finally, to account for the potential indirect effect of weather on individual body weight of wild boar, we included the seasonal average of temperature and rain precipitation in the models. Since all individuals were culled during the hunting season of year x, seasonal temperature and seasonal rain precipitation were calculated on a yearly basis with the following rule: weather variables were averaged from December of year x-1 to February of year x in winter, from March to May of year x in spring, from June to August of year x in summer, and from September to November of year x in autumn. Values of the 8 weather variables (average temperature and average daily rain precipitation for each of the four seasons) were then assigned to each individual according to the hunting season of culling. For each sub-dataset discretely, predictors were screened for collinearity (Pearson correlation matrix, rp) and multicollinearity (Variance Inflation Factor), with thresholds set to $r_p = \pm 0.7$ and VIF = 3, respectively[53]. Among the different sub-datasets, the most recurring groups of variables affected by collinearity included forest productivities of the same year, especially the chestnut-Turkey oak and beech-global index productivity pairs, and mean temperature and daily precipitation of the same season, particularly spring and autumn. To select the best candidate predictors among the collinear variables, we screened them by means of a machine learning method, the random forest calculation (*random.Forest* package), which ranked all predictor variables on the basis of their potential to explain body weight variability[54]. We dropped the worst predictor variable of each collinearity condition until no variable affected by multicollinearity remained.

The final step of analysis consisted of a model selection process for each sub-dataset. We built a full GAM which included all the predictor variables selected in the previous step, with the effect of all variables modelled as a natural cubic spline function. Subsequently, we used the dredge function of the *MuMln* package to run a set of models with all possible combinations of the full model predictor variables. The best models were then identified following the minimum AIC and the most parsimonious (in terms of number of predictor variables included) were selected in case of pairs and groups of models with $\Delta \text{AIC} < 2$[55]. We performed a validation of the models selected by visually inspecting their residuals to check for homoscedasticity, normality of errors, and independence[53].

Data availability

The dataset analysed during the current study is available from the corresponding author on reasonable request.

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Author contributions

M.A. originally formulated the idea. R.C. and E.B. conducted fieldwork. R.B., F.B. and E.M. collaborated in imaging and performing analysis. R.B. wrote the original draft of the manuscript. M.A., F.B., R.C. and E.M. provided editorial advice. M.A. provided materials tools and contributed to funding acquisition.

Competing interests

The authors declare no competing interests.

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Chapter 2

It’s time to mate: population-level plasticity of wild boar reproductive timing and synchrony in a changing environment

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Abstract

On a population level, individual plasticity in reproductive phenology can provoke either anticipations or delays in the average reproductive timing in response to environmental changes. However, a rigid reliance on photoperiodism can constraint such plastic responses in populations inhabiting temperate latitudes. The regulation of breeding season length may represent a further tool for populations facing changing environments. Nonetheless, this skill was reported only for equatorial, nonphotoperiodic populations. Our goal was to evaluate whether species living in temperate regions and relying on photoperiodism to trigger their reproduction may also be able to regulate breeding season length. During 10 years, we collected 2,500 female reproductive traits of a mammal model species (wild boar *Sus scrofa*) and applied a novel analytical approach to reproductive patterns in order to observe population-level variations of reproductive timing and synchrony under different weather and resources availability conditions. Under favorable conditions, breeding seasons were anticipated and population synchrony increased (i.e., shorter breeding seasons). Conversely, poor conditions induced delayed and less synchronous (i.e., longer) breeding seasons. The potential to regulate breeding season length depending on environmental conditions may entail a high resilience of the population reproductive patterns against environmental changes, as highlighted by the fact that almost all mature females were reproductive every year.

Key words: breeding season length, phenology, photoperiodism, population ecology, reproduction, wild boar.

Animals face changing environments throughout their whole life cycles. Individuals are adapted to the changes that are regular and predictable. The most common example is seasonality in temperate zones, for which photoperiod variation over the year represents a reliable and easily accessible predictor (Bradshaw and Holzapfel 2007). Other phenomena arise with irregular and usually unpredictable patterns, such as interannual weather variability and food or prey availability (e.g., fruit mast years) related to it (Nussbaumer et al. 2018). Whereas it is known that individuals and populations may react with plastic responses (e.g., Ruf et al. 2006; Ogutu et al. 2015), inter-individual phenotypic diversity may represent a further tool to deal with such irregular and unpredictable changes on a population level (Hertel et al. 2020).

A plastic reproductive phenology is a key ecological determinant of animal population sensitivity to changing environments as it represents the time dimension-linkage between reproduction and environment (Post et al. 2008; Ogutu et al. 2015). Such plasticity takes effect on several levels (ovulation, conception, and birth) on both individuals (Canu et al. 2015) and populations (Fernández et al. 2020). However, it is generally constrained by the reliance on rigid reproductive cues (i.e., photoperiod variations throughout the year, Bradshaw and Holzapfel 2007) that do not depend on the environmental conditions. Most ungulate populations, or at least those living at latitudes with clear day length variations throughout the year, typically show a tight reliance on photoperiod to trigger their reproduction (Zerbe et al. 2012). Nevertheless, evidence that favorable environmental and nutritional conditions facilitate a slightly earlier reproduction was frequently reported even in species whose predominant cue is photoperiodism (McGinnes and Downing 1977; Hamilton and Blaxter 1980; Flydal and Reimers 2002; Wolcott et al. 2015). Thus, a certain degree of plasticity in the reproductive timing (hereafter RT, always referred to the population level) seems to be quite spread among ungulate species and this can be expected to produce temporal displacements of breeding seasons among different years. In this context, the most plastic species have a reproductive output which is less constrained by environment, as they can respond to negative conditions by delaying the breeding season (Servanty et al. 2009).

The phenotypic diversity of reproductive phenology within a population (namely, "reproductive synchrony," Findlay and Cooke 1982, hereafter RS) directly affects breeding (and, consequently, birth) season length. Higher RS (i.e., shorter breeding seasons) was observed in ungulate species and populations living in more seasonal and constant environments (English et al. 2012; Zerbe et al. 2012), relying on more specialist foraging strategies (English et al. 2012), showing gregarious habits associated with precocial young (Sinclair et al. 2000) and an even, rather than female-biased, sex ratio of adults (Milner et al. 2007). In a number of equatorial savanna ungulates, a substantial interannual RS variability in response to environmental conditions was reported, with longer breeding seasons observed during drought years (Ogutu et al. 2010, 2014). This phenomenon comes as no surprise in species mainly relying on environmental cues (i.e., rainfall patterns) to time their reproduction through a nutritional status mediation (Ogutu et al. 2015). Conversely, environment-driven interannual RS variability in ungulates of temperate regions (i.e., relying on photoperiod variations, Zerbe et al. 2012) is not obvious and so far has never been reported. On the one hand, as photoperiodism follows genetic heritability (Bradshaw and Holzapfel 2007; Zerbe et al. 2012), we may expect RS degree to remain substantially constant under different environmental conditions, at least assuming that they homogeneously affect all individuals. In this respect, Zerbe et al. (2012) reported unaltered RS between wild ungulates and those kept in captive conditions with high resources availability. On the other hand, resource-poor years may provoke a higher inter-individual variability in the time needed to achieve the nutritional condition required to reproduce and ultimately reduce RS.

The simpler method to investigate the variability of both RT and RS on a population level is to compare the temporal occurrence and duration of an adequate number of breeding seasons with one or more environmental variables (Ogutu et al. 2010, 2014; Fernández et al. 2020). Unfortunately, this approach requires the condensation of large datasets into 1 observation per year, with a substantial loss of statistical power. To overcome this limitation, analytical strategies aimed at evaluating the temporal variability of the individual reproductive status with respect to certain environmental conditions should be applied. A further constraint for specific investigations of RS variability in response to environmental changes is the typically short breeding season of mammal populations inhabiting temperate regions (Garel et al. 2009; Mason et al. 2011). We thus chose wild boar (*Sus scrofa*) as a model species because it presents the rare condition of living in temperate regions (i.e., in highly seasonal environments) and, at the same time, showing relatively long breeding seasons (Santos et al. 2006; Canu et al. 2015). The reproductive output of this species was widely investigated thanks to the large amount of data regarding culled individuals provided by hunting activities (e.g., Servanty et al. 2009; Fonseca et al. 2011; Canu et al. 2015; Bergqvist et al. 2018; Touzot et al. 2020). A high degree of individual plasticity was reported for several reproductive parameters of wild boar females, including their reproductive phenology, which tends to be anticipated in response to good environmental conditions (e.g., Servanty et al. 2009; Canu et al. 2015). Nevertheless, so far, the relationship between environmental drivers and population RT and RS has never been evaluated.

In this study, we aimed to evaluate age-specific wild boar population responses to such environmental factors as weather and resources availability in terms of both RT (anticipated or delayed breeding seasons) and RS (longer or shorter breeding seasons). In so doing, we aimed to determine whether:

i. wild boar shows an interannual variability of both population RT and, though inhabiting temperate regions, RS;
ii. such interannual variability is the result of modifications of the overall individual likelihood of ovulating and getting pregnant, which in turn is affected by a number of environmental factors directly or indirectly related to resources availability; and
iii. such environmental factors influence the population RT and RS.

Materials and Methods

Study area

We collected data in a mountainous area of 13,400 ha in Central Italy (Northern Apennines, Italy, 43° 48′ N, 11° 49′ E), which includes 2,700 ha of protected area (Oasi Alpe di Catenaia). Lowest and highest altitudes reach 330 and 1,414 m above the sea level, respectively. The climate is temperate continental with a marked seasonality. A mean temperature of 18.7 °C and a daily precipitation of 1.73 mm are recorded in summer, whereas winters are cold (mean temperature of 1.2 °C) and rainy (daily precipitation of 3.55 mm). Snowfalls are sporadic in winter and can also occasionally occur in spring. Mixed deciduous woods are the prevailing habitat category (67% of the total surface) and are mainly composed of Turkey oak *Quercus cerris*, beech *Fagus sylvatica*, and chestnut *Castanea sativa*. Agricultural crops (16%), mixed open-shrubs areas (10%), and conifer woods (7%) cover the rest of the surface. In the surroundings of the protected area, wild boar is unselectively hunted in drive hunts by teams of 25–50 people. During the study period, drive hunting was generally permitted 3 times a week from September to January, with an average of 58.3 hunting days per year. As a yearly average of 6.4 wild boar/km^2 was harvested, the population underwent a high, but relatively constant, hunting pressure (Merli et al. 2017).

Data collection

We collected and examined reproductive traits of 2,500 female wild boars culled from 1 September to 31 January during 10 consecutive hunting seasons (2006–2016). Culling date and live body mass were recorded for each individual. In so doing, we included the reproductive trait mass, though it accounted only for a negligible percentage of female live body mass (Brogi et al. 2021). All females were aged on the basis of their tooth eruption and abrasion (Briedermann 1990) and assigned to one of the following age classes: juvenile (< 1 year), subadult (between 1 and 2 years), and adult (> 2 years).

In order to determine their reproductive status, we dissected ovaries and uterus of each female to check for the presence of corpora lutea and embryos/fetuses, respectively. Corpora lutea were used as a sign that ovulation occurred, whereas embryos and fetuses as a sign of ongoing pregnancy (e.g., Malmsten et al. 2017a). Over 823 culled juvenile females, only 30 ovulated and 3 pregnant individuals were identified. We thus decided to exclude the individuals belonging to this class from our analysis. The Regional Hydrological Service of Tuscany kindly provided weather data (average temperature and rain) daily recorded in a weather station located inside our study area (43° 42′ N, 11° 55′ E). We obtained local data on yearly seed productivity of beech, chestnut, and Turkey oak measured inside the Oasi Alpe di Catenaia from an online database (Chianucci et al. 2019) and used it as a measure of food availability.

Data analysis

Step 1: ovulation and pregnancy heterogeneity among years and classes

In order to assess interannual heterogeneity in ovulation and pregnancy patterns, we modeled both individual likelihood of ovulating and getting pregnant throughout the sampling period. We divided our dataset into 2 sub-datasets corresponding to subadult and adult females. By means of the glm() function of the *stats* package (R version 4.0.3, R Development Core Team, 2015) we ran a Generalized Linear Model (GLM) with a binomial distribution, with the individual reproductive states (ovulated or pregnant, alternatively) as binary-dependent variables, separately for the subadult and the adult female sub-datasets. The binary variable "ovulated" took the value 1 whenever at least 1 corpus luteum, embryo, or fetus was detected, and 0 otherwise; the binary variable "pregnant" took the value 1 whenever at least 1 embryo or fetus was detected, and 0 otherwise. In so doing, we built a total of 4 models, hereafter called "1S-ov" (model of ovulation in subadults), "1A-ov" (ovulation in adults), "1S-pr" (pregnancy in subadults), and "1A-pr" (pregnancy in adults). We included in all models the interaction term between the standardized culling date (expressed as days from 1 September) and the hunting season (categorical) as the only predictor to check for interannual variations in the effect of the standardized date. The hunting season was expressed as a sequential number from 1 (referring to the 2006–2007 hunting season) to 10 (2015–2016).

To check for statistical differences among age classes in ovulation RT and RS, we used the models 1S-ov and 1A-ov to predict yearly dates of onset, middle point, and end of ovulation on a population level for each monitored hunting season. The dates in which the proportion of ovulated females reached 0.025, 0.5, and 0.975 were used as onset, middle point (inflection point of the curve) and end date, respectively. In so doing, we included 95% of the predicted ovulation events between the onset and end dates. In order to test whether ovulation was significantly anticipated in a certain age class in respect to the other, we performed a paired *t*-test (t.test() function of the R package *stats*), which compared subadult and adult female middle point dates for each hunting season. Moreover, to check for inter-class differences in ovulation RS, we measured the duration of ovulation seasons (1 per year) as the number of days from the onset to the end dates in both subadult and adult females. We then calculated the average duration of the ovulation season and its associated variance, separately in subadult and adult females. Finally, we ran a 2 samples *t*-test for summary data implemented by the tsum.test() function (R package *BSDA*). The whole procedure was then exactly replicated on pregnancy RT and RS by using yearly predictions of the models 1S-pr and 1A-pr.

Step 2: factors influencing individual reproductive status

After the analysis to test potential differences among seasons within age classes, we aimed to identify internal and external factors which influenced ovulation and pregnancy ratios. We modeled the individual likelihood of ovulating and, alternatively, of getting pregnant by means of 4 GLMs with a binomial distribution (ovulation in subadults, ovulation in adults, pregnancy in subadults, and pregnancy in adults). The standardized culling date (days from 1 September) was used as predictor to consider photoperiod-mediated seasonal variations of the individual reproductive status. We also included such internal factors as individual age (months) and live body mass (kg) as predictors. Among external factors, 4 season average temperature and rain precipitation calculated on a yearly basis were used as predictors to account for the potential effect of weather. Because all individuals were culled between September of year x and January of year x + 1, winter weather variables were averaged from December of year x−1 to February of year x, spring ones from March to May of year x, summer ones from June to August of year x, and autumn ones from September to November of year x. Moreover, we used current year seed productivity of Turkey oak, beech, and chestnut (t/ha) measured on a yearly basis to check for potential effects of food availability on ovulation and pregnancy patterns. To summarize the influence of the 3 deciduous species in a single variable, we included a further global forest productivity index in the models, which we calculated following the protocol described by Bisi et al. (2018). Finally, we calculated the yearly average number of adult males per female as the number of culled adult males (>3 years; Brogi et al. 2021) divided by the total number of adult and subadult females. We added this yearly variable as a predictor within our models to take into account the potential effects of reproductive male relative abundance on female reproductive status (Milner et al. 2007). We recognize that, by measuring adult male availability on the basis of culling data, we may obtain an unreliable approximation of the real population structure. However, in this study, we were only interested in the variation of male availability throughout different years.

Separately for each sub-dataset, we screened all available predictors for collinearity and multicollinearity by means of a Pearson correlation matrix (r_p) and the variance inflation factor (VIF), setting thresholds to $r_p = \pm 0.7$ and VIF = 3, respectively (Zuur et al. 2009). Weather variables of the same season (particularly spring and autumn) were the most recurring pairs of collinear variables. We performed a random forest calculation (*random.Forest* package) to rank all predictors on the basis of their potential to explain the dependent variable (Breiman 2001). The worst predictor variable of each collinearity and multicollinearity condition was dropped until all r_p and VIF were below the corresponding thresholds. Finally, we included the remaining predictor variables in a full GLM and used the dredge() function (*MuMln* package) to run a set of models with all possible combinations of predictor variables. We followed the minimum Akaike's information criterion (AIC) and selected the most parsimonious in terms of number of predictors among groups of models with $\Delta AIC < 2$ (Symonds and Moussalli 2011), identifying the 4 best models: "2S-ov" (ovulation in subadults), "2A-ov" (ovulation in adults), "2S-pr" (pregnancy in subadults), and "2A-pr" (pregnancy in adults).

Step 3: effects of internal and external factors on RT and RS

In the last step of our analysis, we aimed to assess whether the factors affecting ovulating and pregnant female ratios (Step 2 of our analysis) may also provoke modifications in ovulation and pregnancy temporal patterns. We thus built 4 further GLMs, 1 for each combination of dependent variables and age classes (e.g., ovulation in subadult females). We included the set of predictor variables of the corresponding best model selected in Step 2 (e.g., 2S-ov) and added them all their interactions with the standardized date. Following the same protocol described in Step 2, we then screened this enlarged sets of predictor variables for collinearity and multicollinearity, ran full models, and processed them with dredge() function to finally select 4 new best GLMs including single and interaction terms: “3S-ov,” “3A-ov,” “3S-pr,” and “3A-pr.”

Results

Step 1: ovulation and pregnancy heterogeneity among years and classes

Interannual ovulation and pregnancy patterns predicted by 1S-ov, 1A-ov, 1S-pr, and 1A-pr are summarized in Figure 1. A marked interannual heterogeneity affected temporal patterns of both reproductive statuses considered, although the predicted portion of females achieving ovulation or pregnancy within the sampling period was always equal or close to 1 in both age classes. A number of reproductive seasons were relatively early and short (hunting seasons 2, 5, and 7), whereas others showed either a late onset (3, 6, and 10) or a longer duration (1 and 9). Likewise, the temporal distance between ovulation and pregnancy curves varied among the years, with the minimum value observed in hunting season 2 and the maximum in 5 and 8. Finally, subadult and adult females showed completely overlapped reproductive patterns in a number of hunting seasons (2, 6, and 7) and markedly divergent in other ones (3 and 4).

On average, the date when the proportion of ovulated females reached 0.5 corresponded to 82.46 (21 November) ± 14.67 (mean ± *SD*) and 83.77 (23 November) ± 13.60 days from 1 September in subadults and adults, respectively, without a statistically significant difference between the 2 age classes ($t = -0.55$, P-value $= 0.60$). A similar result was detected for pregnancy, as subadult females reached the middle point at 109.60 (19 December) ± 14.82 days from 1 September and adult females at 115.61 (25 December) ± 17.88 days from 1 September, with the paired t-test returning a non-significant difference ($t = -1.70$, $P = 0.12$). Conversely, the duration of the ovulation season (a measure of RS) was shorter in subadult (96.54 ± 9.46 days) than in adult females (114.00 ± 10.85 days) and this difference was statistically significant ($t = -3.84$, $P = 0.0012$). As 95% of subadult females got pregnant in 94.20 ± 10.65 days, whereas adult females in 121.13 ± 16.01 days from the onset, pregnancy season duration was significantly shorter in subadult females ($t = -4.43$, $P = 0.0004$).

Step 2: factors influencing individual reproductive status

Predictor variable sets included in the best model for the 4 GLMs explaining the individual likelihood of ovulating and getting pregnant are summarized in Table 1, whereas those selected for random forest analysis and dredge are summarized in Supplementary Table S1. Standardized date and average spring temperature were included in all 4 best GLMs and positively affected both ovulation and pregnancy rates in both age classes. Individual body mass only increased the likelihood of subadult females ovulating, whereas its positive effect on pregnancy ratio concerned both age classes. As for food availability, at least 1 predictor reflecting seed productivity was included in each best GLM. The relative abundance of adult males was not selected for any best GLM.

Step 3: effects of internal and external factors on RT and RS

The model subadult female ovulation (3S-ov) included individual body mass, spring average temperature, and autumn rain as single variables in addition to the 2 interaction terms composed of [global productivity index: date] and [spring temperature: date], all showing a positive effect on the dependent variable (Supplementary Table S2a). The increase of global productivity index did not cause a substantial displacement of the ovulation onset. However, it was related to a marked shortening of the ovulation season (higher RS) from ~110 days predicted for low productive years to ~70 days predicted for highly productive years (Figure 2A). Likewise, in years with higher average spring temperature, subadult female ovulation season was shorter, though with a markedly anticipated RT (Figure 2B).

For adult female ovulation patterns, model 3A-ov included spring average temperature, autumn rain, and chestnut productivity as single variables and [beech productivity: date] and [spring temperature: date] as interaction terms (Supplementary Table S2b). Beech productivity only accounted for a slight shortening of the ovulation season (higher RS), with no effect on the timing of its onset (Figure 2C). Conversely, warmer spring temperatures were associated to both anticipated RT and higher RS of ovulation seasons (Figure 2D).

The model 3S-pr, which explained subadult female pregnancy patterns, included individual body mass and chestnut productivity as single variables in addition to the same interaction terms selected for ovulation patterns of the same age class, that is, [global productivity index: date] and [spring temperature: date]. When seed productivity was higher, subadult female pregnancy showed an anticipated RT and a higher RS (Figure 2E). A similar pattern was observed for average spring temperature, though with a stronger effect in anticipating pregnancy RT (Figure 2F).

The model 3A-pr, which accounted for adult female pregnancy patterns, included individual body mass and chestnut productivity as single predictor variables in addition to the same interaction terms selected for ovulation patterns of the same age class, that is, [beech productivity: date] and [spring temperature: date]. Their effects on RT and RS were similar to those shown on adult female ovulation, though isolines showed an overall delay (Figures 2G,H).

Discussion

We showed that, in an ungulate species inhabiting temperate latitudes, breeding seasons can change in timing and duration, depending on environmental conditions. Both population RT and RS widely varied among different years and our analytical approach enabled to properly evaluate their dependence on the environment. These phenomena were essentially due to the individual tendency to reproduce even when a harsh environment made the investment risky in terms of offspring survival. Such population-level features likely entail a high resilience of the population reproductive patterns against ecological perturbations and environmental changes as confirmed by the extremely high average likelihood of females ovulating or getting pregnant by the end of the reproductive season in every sampling year.

We observed a high temporal heterogeneity among yearly reproductive patterns (Figure 1). However, in accordance with Servanty et al. (2009), the model described in Step 1 predicted an average individual likelihood of ovulating which reached values close to 1 before 31 January every year and in both age classes considered. Pregnancy followed similar patterns, thus proving that ovulation

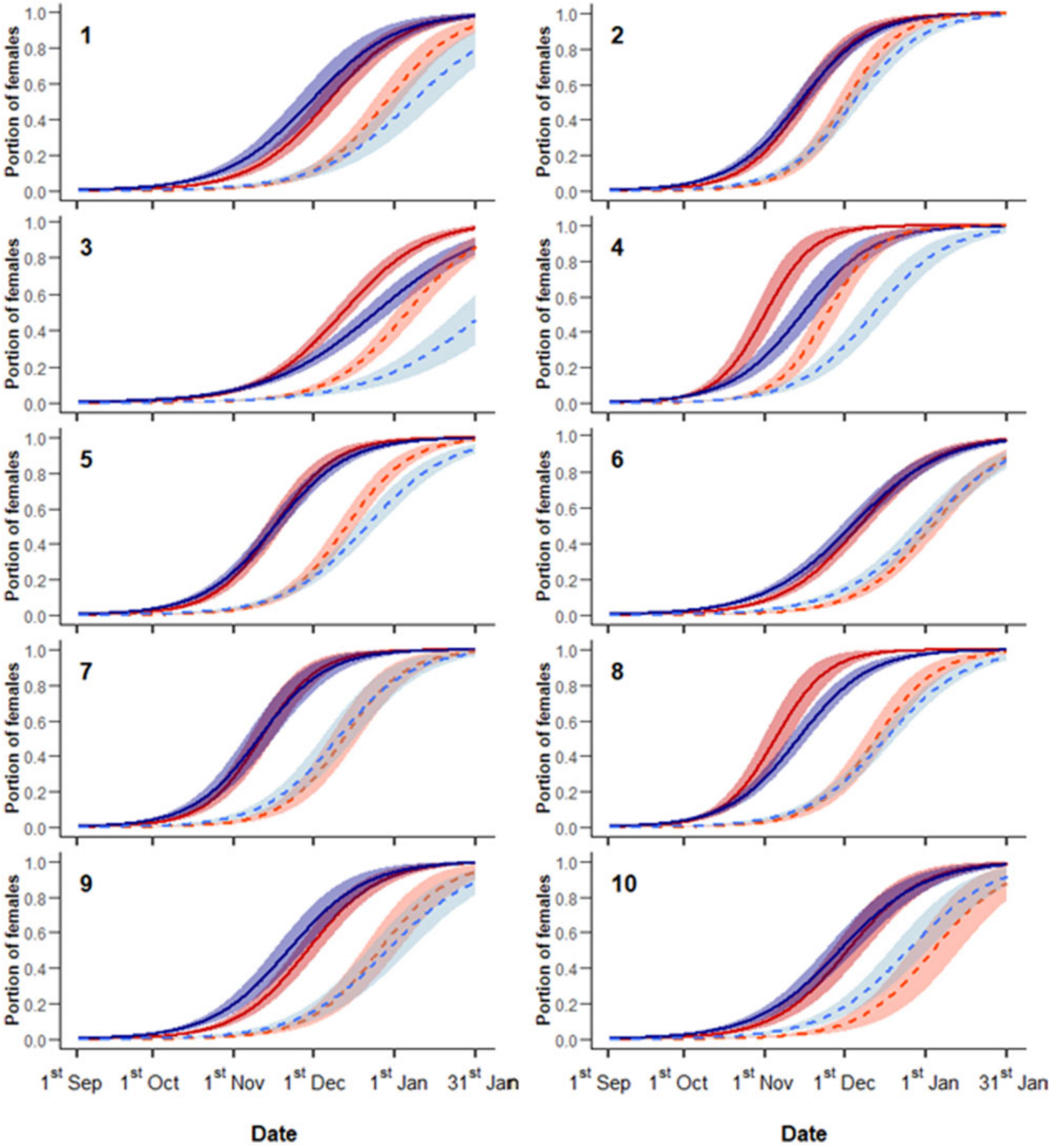

Figure 1. Ovulation (continuous lines) and pregnancy (dashed lines) patterns of subadult (red) and adult (blue) females throughout 10 hunting seasons in Northern Apennines, Italy. Values were predicted by 4 GLMs with the interaction between date and hunting season as the only predictor variable (see the text for more details). Color-shaded areas represent 95% confidence intervals.

rates represent a good wild boar pregnancy proxy. Interannual pregnancy delay variability in respect to ovulation was likely the effect of a variable proportion of ovulated females failing to get pregnant. However, thanks to their ability to repeat the estrus (Henry 1968; Barrett 1978; Macchi et al. 2010), all female wild boar (subadult and adult) were predicted to achieve pregnancy even in the years with the highest delays (e.g., hunting seasons 5 and 8). Although minor reproductive events may occur all year round in other wild boar populations (relying on artificial food, Macchi et al. 2010; Malmsten et al. 2017b; Bergqvist et al. 2018), our results showed that, for adult and subadult females, an actual breeding season existed and was included within our sampling period. The minimal number of reproductive juvenile females detected in our study (823 culled juvenile females, 30 ovulated, and 3 pregnant) may be a sign of their contribution to reproduction being negligible or the consequence of the 5 months sampling period duration being insufficient

Table 1. Sets of explanatory variables included in the best GLM on the individual likelihood of: subadult females ovulating (2S-ov); adult females ovulating (2A-ov); subadult females getting pregnant (2S-pr); and adult females getting pregnant (2A-pr).

Model	Sub-dataset	Reproductive state	Best model formula
2S-ov	Subadult females	Ovulation	Ovulated ~ standardized date + body mass + spring temperature + autumn rain + global productivity index
2A-ov	Adult females	Ovulation	Ovulated ~ standardized date + spring temperature + summer rain + autumn rain + chestnut productivity + beech productivity
2S-pr	Subadult females	Pregnancy	Pregnant ~ standardized date + body mass + spring temperature + summer rain + chestnut productivity + global productivity index
2A-pr	Adult females	Pregnancy	Pregnant ~ standardized date + body mass + spring temperature + chestnut productivity + beech productivity

Standardized culling date, culling date expressed as days from 1 September; body mass, individual body mass (kg); season *x* temperature, average environmental temperature recorded during the season *x*; season *x* rain, average daily rain precipitation recorded during the season *x*; productivity of species *y*, mast productivity of the tree species *y* during the current year expressed as t/ha; global productivity index, index summarizing all tree species productivity during the current year (see the text for more details).

to detect juvenile reproduction, which has been shown to occasionally occur in other wild boar populations (Šprem et al. 2016; Gamelon et al. 2017). Collecting data all year round (possible in cases of wild boar hunting being performed during the whole year) would be necessary to properly evaluate the reproductive contribution of different classes of females outside the core reproductive period, but it is worth noting that this was not the objective of this study.

Subadult females were significantly more synchronous than adults, likely on account of an overall higher homogeneity of their individual conditions. Differently from the older class, all subadult females belonged to the same cohort and most of them were at their first reproductive attempt (as confirmed by the almost null reproductive rate observed in juvenile females) and had not to cope with previous parental reproductive costs. Conversely, adult females had different ages and might have coped with different costs related to their previous reproduction (Hamel et al. 2010).

The fact that the average likelihood of ovulating and getting pregnant reached values close to 1 within our sampling period enabled an unambiguous interpretation of the Steps 2 and 3 analyses: the effects of the environmental factors identified only either anticipated or delayed changes of the reproductive status, without truly affecting the individual likelihood of ovulating and getting pregnant by the end of the reproductive season. This evidence helps to understand environmental influence on female wild boar reproductive status, which so far was widely investigated by focusing on the overall proportion of reproductive females (Fonseca et al. 2011; Bergqvist et al. 2018; Touzot et al. 2020) and seldom considering the temporal dimension (Servanty et al. 2009). In this context, a yearly proportion of reproductive females estimated without taking into account culling dates is prone to be substantially underestimated. In fact, females culled early in the hunting season with no sign of ongoing ovulation or pregnancy and considered "not reproductive" (Fonseca et al. 2011; Bergqvist et al. 2018; Touzot et al. 2020) should rather be considered "not reproductive yet."

The influence of the standardized date was included in all the best models selected in Steps 2 and 3 (as single predictor and in interaction with environmental variables, respectively). Thus, it is suggested that photoperiodism still constrained wild boar RT, though its influence was not so strong if compared with that exerted over most ungulates inhabiting temperate regions. This evidence places wild boar at an intermediate position along an ideal continuum between temperate ungulates (which rigidly rely on photoperiodism to time their reproduction, with minor environmental influence, Zerbe et al. 2012) and equatorial, seasonal breeding ungulates (whose reproductive phenology mainly relies on environmental cues, Ogutu et al. 2015).

The approach adopted to build Step 3 models enabled to evaluate ovulation and pregnancy temporal patterns of the population in respect to the environment, that is, to monitor the breeding season temporal onset, progress, and duration at varying environmental conditions. Ovulation and pregnancy RTs were substantially anticipated under good environmental conditions (i.e., higher resources availability and warmer spring temperatures) in both age classes (Figure 2), thus showing the high degree of ecological plasticity of wild boar reproductive phenology. The physiological phenomenon was likely mediated by individual nutritional conditions (McGinnes and Downing 1977; Hamilton and Blaxter 1980; Flydal and Reimers 2002; Wolcott et al. 2015), which were directly improved either by resource abundance or by the advanced vegetation growth due to high spring temperatures.

The possibility to either plastically anticipate or delay breeding seasons maximizes population reproductive outcomes under optimal conditions, whereas increasing its resilience against ecological perturbations. During favorable years, anticipated breeding seasons produce earlier births, which are known to increase offspring survival in ungulates (Côté and Festa-Bianchet 2001). In the case of wild boar, earlier births may directly reduce the young mortality caused by red fox (*Vulpes vulpes*) predation (Bassi et al. 2012) by producing a beneficial mismatch between the time when piglets are of vulnerable size and the time when fox food requirement is most intense (young raising, from May onwards in Southern Europe, Cavallini and Santini 1995). The potential to plastically anticipate breeding seasons may result extremely beneficial also when facing global change by softening or even preventing mismatches between births and the most favorable nutritional conditions for offspring. In this respect, wild boar may represent an exceptional case of a species "pre-adapted" to global change, as already suggested (Vetter et al. 2015; Touzot et al. 2020). Conversely, when less resources are available, a delayed breeding season gives individuals more time to get the nutritional condition needed to reproduce. In so doing, a higher proportion of mature individuals can achieve reproduction at the cost of an increased offspring mortality. The high hunting pressure may have increased the advantage of such a risky investment, as individuals counting on a short life expectancy have to exploit every reproductive opportunity to maximize their fitness (Festa-Bianchet 2003). We observed no relationship between the number of culled adult males per female and ovulation and pregnancy temporal

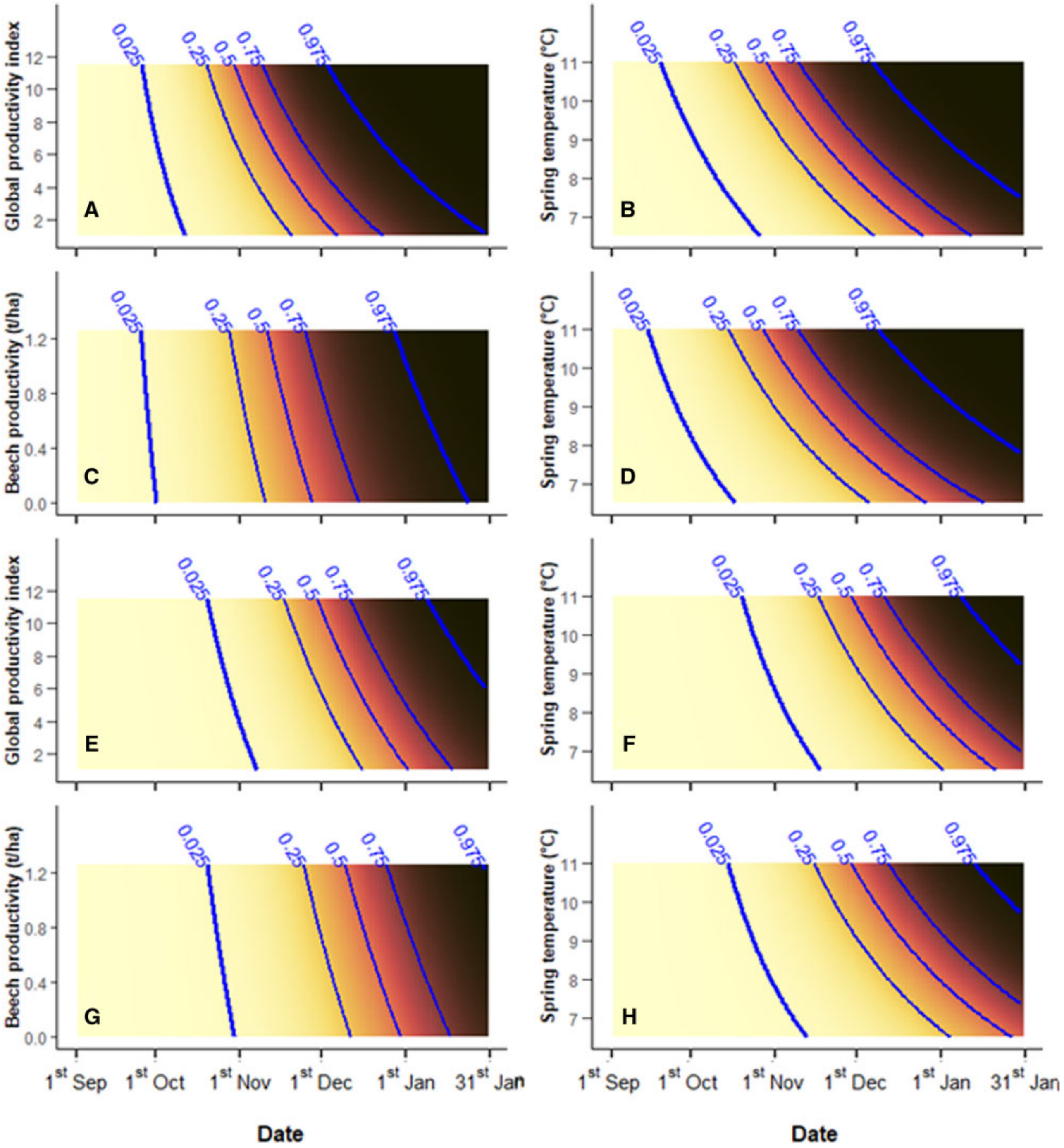

Figure 2. Predicted effect of the interaction between environmental variables and the standardized date on the proportion of: ovulating subadult females (A and B), ovulating adult females (C and D), pregnant subadult females (E and F), and pregnant adult females (G and H), expressed by the chromatic scale (white = low; black = high). Blue lines represent 0.025 (ovulation and pregnancy season onset), 0.25, 0.5, 0.75, and 0.975 (ovulation and pregnancy season end) isolines. Spring temperature: average air temperature of previous spring (°C); Global productivity index: mast tree global productivity index (see the text for more details); Beech productivity: beechnut productivity (t/ha).

patterns. This result is surprising in a heavily hunted population (i.e., subject to adult male scarcity, Fernandez-Llario and Mateos-Quesada 2003; Toïgo et al. 2008) and appears in contrast with the results obtained for other ungulate species (Milner et al. 2007). Nonetheless, it is consistent with the findings proposed by Diefenbach et al. (2019) on white tailed deer (*Odocoileus virginianus*) as well as with Brogi et al.'s (2021) hypothesis regarding the flexible reproductive involvement of subadult male wild boar. As we did not consider other population traits, such as density and structure, further investigations are needed to evaluate their potential effect on wild boar temporal reproductive patterns.

A number of environmental factors in interaction with the standardized date were included as predictors in Step 3 best models, thus showing that good environmental conditions (higher spring temperatures, higher food availability) enhanced RS and ultimately led to shorter breeding seasons (Figure 2). We thus showed that, as previously reported only for equatorial ungulates (Ogutu et al. 2010, 2014), photoperiodic species inhabiting temperate regions also have the potential to adjust breeding season length depending on environmental conditions. In the monitored population, RS was enhanced by higher spring temperatures in both age classes and by global seed and beechnut productivity in subadult and adult females, respectively. Breeding seasons following hot springs were ~40% shorter in respect to those following cold springs in both age classes. Global seed productivity had a similar impact (shortening of ~36%) on subadult female ovulation seasons, whereas years with a high beechnut productivity reduced adult female ovulation season length of ~20% in respect to less productive ones. These environmental factors likely induced a plastic anticipation of individual reproductive phenology but heterogeneously affected each individual. Conversely, only the average population RT would have been modified, with no effect on inter-individual differences and, therefore, on RS (as in the case of other environmental factors included as single predictors in Step 3 best models). We can suppose that, when the main food resources were more abundant, all females reached the threshold nutritional condition needed to reproduce early and achieved ovulation as soon as their internal photoperiodism enabled them to. This optimal nutritional condition induced a quite homogenous distribution of ovulation within the population. Conversely, in case of low resource availability, the pre-existing variability of individual conditions would be unaltered or even enhanced. For instance, foraging strategies would be more diversified, with a number of individuals either being able to outcompete the others for the scarce resources available or better exploiting secondary food items. The whole breeding season RT would be delayed (as observed, for example, when the global productivity index was low), though a number of individuals would be less affected than others by resource scarceness and still be able to pursue an early reproduction, thus inducing a substantial RS reduction. In this context, spring temperatures may have acted as a proxy of the vegetation growth season and regulated abundance and temporal occurrence of food resources other than mast seeds.

The possibility to regulate RS in respect to the environmental conditions may provide several advantages to the population reproductive outcomes. In particular, birthdates may be highly concentrated when, during the mating season, environmental conditions are good (and likely induced a high nutritional condition of females). When favored by resource availability, the advantageous (Côté and Festa-Bianchet 2001) phenotypic trait of early reproduction may thus be evenly distributed within the population. We can hypothesize that a higher birth synchrony may also reduce predation risk by saturating the number of newborns that predators (wolves, *Canis lupus*, and foxes in the monitored study area, Bassi et al. 2012) can catch per time unit (dilution effect, Darling 1938). Conversely, under suboptimal environmental conditions, the enhanced phenotypic diversity showed by the population reproductive phenology may produce more scattered birthdates. This may result in a more efficient resource partitioning among individuals that are raising young (Ims 1990). However, more scattered birthdates amount to a population trait and therefore may not be shaped directly by evolution and, as explained above, rather seems the consequence of the combination of individual adaptive features.

We provided the first evidence of breeding season length adjustment depending on environmental conditions in a species living in temperate regions and relying on photoperiodism to trigger its reproduction. This feature likely represents a key factor for wild boar renowned ecological plasticity and ultimately contributes to its high success and worldwide spread (Massei et al. 2015; Markov et al. 2019).

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Authors' Contributions

E.M., S.G., R.B., and M.A. originally formulated the idea. R.C. and E.B. conducted fieldwork. R.B., E.M., and S.G. collaborated in imaging and performing analysis. R.B. wrote the original draft of the manuscript. S.G., E.M., M.A., and R.C. provided editorial advice. M.A. provided materials tools and contributed to funding acquisition.

Supplementary Material

"Supplementary material can be found at https://academic.oup.com/cz."

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Chapter 3

Protected areas as refuges for pest species? The case of wild boar

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Protected areas as refuges for pest species? The case of wild boar

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ABSTRACT

Protected areas are often blamed for offering refuge to pest species populations, giving rise to the so-called "reserve effect". Nevertheless, this major conservation side effect has seldom been investigated or verified on a local scale. Along the borders of two protected areas of different size, we modelled wild boar individual likelihood of being either inside or outside the protected areas throughout the year, considering their activity rhythms and resource availability. No evidence of reserve effect was found in the small protected area, yet the percentage of wild boar moving across the border was smaller in the large one. Moreover, although wild boar use of the large protected area resulted to increase in autumn, we showed that this was not the consequence of hunting avoidance. Our results clearly highlighted the importance to verify reserve effect on a local scale with studies based on detailed information on animal spatial behaviour and environmental variables.

1. Introduction

In the last few decades protected areas showed a rapid growth in number and extension worldwide (UNEP-WCMC & IUCN, 2016). Their geographical expansion was associated with the increase of their functions. Indeed, nowadays protected areas are expected to serve their original purpose of conservation of landscapes, wildlife, and ecosystems in combination with further social and economic objectives (Watson et al., 2014). Nonetheless, although protected area effectiveness for in situ conservation is known and undoubted (Caro, 1999; Chu et al., 2018), their establishment can cause the rise of social conflicts with local human populations (Tisdell and Zhu, 1998; Brockington and Schmidt-Soltau, 2004). Protected areas are often blamed for offering refuge also to pest species, thus preventing the implementation of management activities and reducing the effectiveness of pest population control plans (Coffey and Johnston, 1997). This may result in a high population density of such species inside the reserves, either constantly or in limited time spans characterized by high levels of human disturbance in their surroundings.

For several species, the main source of human disturbance is hunting, which often causes displacements of individuals from unprotected to protected areas during the hunting season (Tolon et al., 2009; Grignolio et al., 2011). The so-called "reserve effect" can be a major concern for both protected area conservation purposes and human activities implemented nearby. On the one hand, the unnatural concentration of individuals inside protected areas can have a huge impact on their biodiversity (Côté et al., 2004; Bongi et al., 2017). On the other hand, individuals seeking refuge into reserves to avoid hunting are often blamed for causing damages to the nearby unprotected lands (Amici et al., 2012). Despite its crucial importance for protected area management, researchers seldom attempted to verify the occurrence of reserve effect on a local scale. The few

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authors who approached such study found evidence of no-reserve effect (white-tailed deer, *Odocoileus virginianus*, Root et al., 1988), reserve effect limited to a part of the population (wild boar, *Sus scrofa*, Tolon et al., 2009), or reserve effect varying according to sex and age classes (roe deer, *Capreolus capreolus*, Grignolio et al., 2011). Despite the limited number of studies and the variability of their results, reserve effect is generally considered a common issue for protected areas by both local human populations and a large part of wildlife technicians. In this light, further investigations are necessary to evaluate the real spread, impact, and patterns of this conservation side-effect.

Previous studies never considered the role potentially played by protected area extension in shaping reserve effect patterns. Several authors contributed to the SLOSS (Single Large Or Several Small) longstanding debate, by attempting to evaluate the relationship between size and conservation effectiveness of protected areas (Lomolino, 1994; Ovaskainen, 2002; McCarthy et al., 2005). It has been shown that protected area effectiveness strictly depends on the comparison between its size and the average home range size of the target species (Bertocci et al., 2017; Di Franco et al., 2018). Thus, one may expect that the larger the protected area, the higher the portion of the population which can take advantage of its protection. As for reserve effect, this would imply that only protected areas which are large enough can offer an even temporary refuge to pest populations. On the other hand, large protected areas may be able to permanently host more individuals inside their boundaries, thus reducing negative impacts of pest species on the surrounding unprotected areas.

Other aspects should be taken into consideration when examining reserve effect, including the overall resource availability, their temporal variations, and their selection by animal species as well as the level of protection from hunting activities the area can offer. The importance of resource availability is underlined by its high potential to shape animal movements (e.g. Morelle and Lejeune, 2015) and to affect reserve effect patterns (Adam et al., 2016). Indeed, animals require a minimum availability of food resources even in case of temporary occupation of protected areas. When resource availability of protected areas is low, animals may be forced to choose between safety and food abundance. Conversely, protected areas offering abundant pulsed resources may be expected to temporarily attract animals with patterns very similar to those of individuals avoiding hunting.

A further fundamental issue to take into account regards the activity rhythms of the focal population. In a number of studies, researchers failed to distinguish the use of refuge areas during daily active and inactive phases. This information, combined with high-resolution movement data, may allow to identify daily patterns of reserve effect and their relationship with animal activities. This can be particularly useful when disturbance in unprotected lands is limited to a certain part of the day (for example, hunting tends to be performed only during daytime, Thurfjell et al., 2013; Tolon et al., 2009). In these cases, to fully understand reserve effect, it is necessary to know whether animals are moving or resting when disturbed and how they change their use of protected areas accordingly.

In the present study, we investigated the use of reserves as a potential strategy to avoid human disturbance, also taking into account ecological variables and food resource availability, by using high resolution spatial data obtained by means of GPS tracking. To do this, we studied the behaviour of wild boar, one of the most important mammal pest species in Europe. Given its major impact on both biodiversity (Massei & Genov 2004; Barrios-Garcia and Ballari, 2012; Bongi et al., 2017) and human activities (Frackowiak et al., 2013), along with the high hunting pressure it commonly experiences (Massei et al., 2015; Merli et al., 2017; Keuling et al., 2016), wild boar has the highest potential to be affected by reserve effect, with several negative consequences for its management. Nevertheless, despite the relatively high attention paid to hunting influence on wild boar spatial behaviour (Keuling et al., 2008; Scillitani et al., 2010; Saïd et al., 2012; Sodeikat and Pohlmeyer, 2002; Thurfjell et al., 2013), only Tolon et al. (2009) investigated reserve effect in wild boar, by focusing on VHF telemetry-based home range distribution in respect to the boundaries of a single protected area. This study showed that reserve effect consisted in a concentration of home ranges inside the protected area during the hunting season. Such effect only regarded the individuals with pre-hunting home ranges “in contact” with the protected area.

In this framework, we selected a study area hosting two protected areas of different size in order to analyse the role their extension plays in shaping reserve effect patterns. For each location of wild boar, we modelled the likelihood of being either inside or outside the protected area and developed the following predictions:

1) Although both protected areas provided total shelter from hunting, the large protected area was expected to have a higher potential to cope with other needs of wild boar (e.g., food resources, safe resting sites). Thus, wild boar were predicted to show a strong reserve effect in the large protected area and a weak or null reserve effect in the small protected area.
2) Given the strictly nocturnal habits of wild boar in our study area (Brivio et al., 2017) and the fact that hunting is permitted only during daytime, diurnal locations were expected to be influenced exclusively by the need for shelter while nocturnal ones mainly by the spatial distribution of food resources. Thus, we predicted a stronger reserve effect during daytime.

2. Materials and methods

2.1. Study area

The study area was in the Casentino valley, in the Tuscan Apennine (Province of Arezzo, central Italy, 43°48′N, 11°49′E, Fig. 1). Climate is temperate-continental, with hot and dry summers and cold and wet winters. Occasional snowfalls occur between October and April. Temperature reaches its highest and lowest values in July and January, respectively. A rich ungulate community inhabits the study area, with wild boar showing a homogeneous distribution and a high population density. Roe deer, red deer (*Cervus elaphus*), and fallow deer (*Dama dama*) are also present with heterogeneous distribution and density throughout the study area. The area is characterised by a high density of wolf (*Canis lupus*), with 1.21 ± 0.27 packs/100 km^2 estimated during the data collection period (Mattioli et al., 2018). Wild boar resulted to be the main component of wolf diet (Mattioli et al., 2011; Bassi et al., 2012). The study area hosts two protect areas of different sizes. The large one, Foreste Casentinesi National Park (FCNP), covers a total surface of 362 Km^2, with a perimeter of about 187 Km (surface/perimeter ratio = 1.936). The study was conducted around its southern border, with elevation ranging from 500 to 1289 m a.s.l.. Inside this part of the FCNP, the habitats are composed of 55% of highly seed-productive deciduous forests (oaks, *Quercus* spp., chestnuts, *Castanea sativa*, and beeches, *Fagus sylvatica*, both as high stand or coppice), 25% of coniferous forests (silver fir, *Abies alba*, black pine, *Pinus nigra*, and Douglas fir, *Pseudotsuga menziesii*), 5% of mixed forests of all the above mentioned species, 3% of shrubs, and 12% of agricultural lands and pastures. Outside the protected area borders, landscape composition shifts to 40% of deciduous forests, 3% and 2% of coniferous and mixed forests, respectively, 3% of shrubs, and 52% of agricultural lands and pastures. The small protected area, Oasi Alpe di Catenaia (OAC), covers a total surface of 27 km^2, with a perimeter of 43 Km (surface/perimeter ratio = 0.628). The area including the reserve and the hunting districts in proximity of its boundaries has an elevation range of 300-1414 m a.s.l.. Deciduous forests account for 68% of the OAC protected surface, with coppices of oaks and chestnuts and high stands of beech; 18% is composed of coniferous forests of black pine and Douglas fir, 3% of mixed deciduous-coniferous forests, 6% of shrubs, and the remaining 5% agricultural lands and pastures. Outside the protected area, hunting lands have a similar habitat composition, with 71% of deciduous forests, 8% of coniferous forests, 3% of mixed forests, 6% of shrubs, and 12% of agricultural lands and pastures.

Any form of hunting is strictly forbidden inside both protected areas, while wild boar hunting reaches high intensity levels in their surroundings. It is performed with drive hunts involving 25—50 hunters and tens of dogs, three times a week from October to December only during daytime (see Grignolio et al., 2011 for further details).

2.2. Data collection and analysis

We captured wild boar by means of baited traps and vertical dropping nets from June 2013 to October 2017, following the protocol detailed by Brogi et al. (2019). Captured individuals were first immobilized and sedated with a mixture of zolazepam — tiletamine or zolazepam — tiletamine — xylazine, alternatively. Each wild boar was weighted, aged based on teeth eruption and abrasion (Heck and Raschke, 1980), and finally equipped with a GPS collar (GPS PRO Light collar, Vectronic Aerospace). Such devices were configured to record 12 GPS locations/day, with a regular 2-h interval. To obtain a uniform sampling of protected area potential use, all captures were performed in a buffer of 1700 m from the reserve boundary inside both FCNP

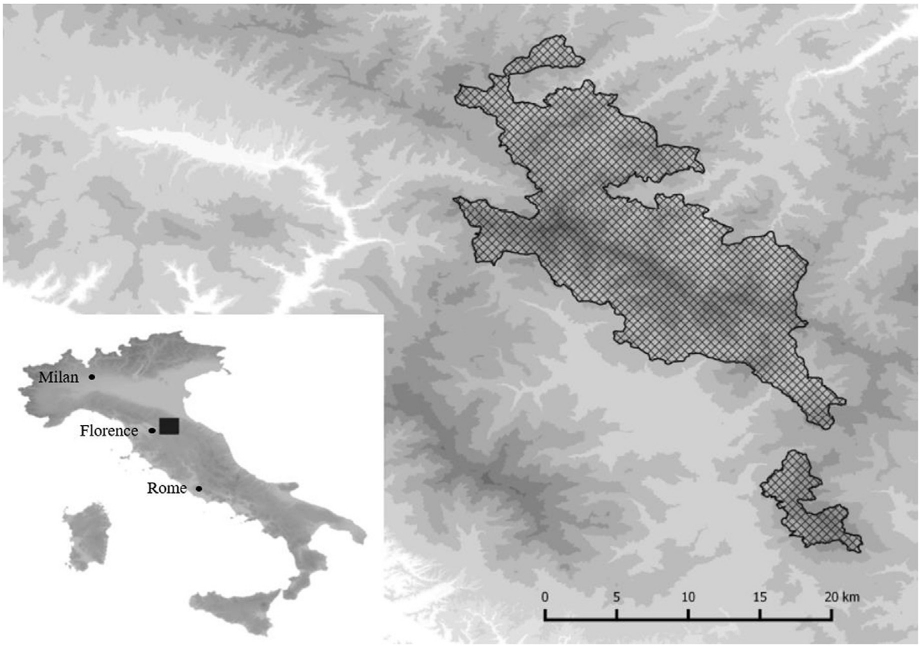

Fig. 1. Map of Italy (down-left), with the black rectangle showing the location of the study area and its relative enlargement in which the northern and southern reticulated areas represent FCNP (large) and OAC (small) protected areas, respectively. The greyscale represents the altitudinal gradient (dark: higher altitudes; light: lower altitudes).

and OAC. The distance between the capture site and the nearest reserve boundary averaged a mean of 409 ± 410 m (mean ± SD) in FCNP and 455 ± 433 m in OAC. In order to avoid capture site related bias, we checked for correlation between such distance and the external/total location proportion at an individual level by means of a Pearson correlation coefficient calculation.

Regional Hydrological Service of Tuscany kindly provided weather data (mean air temperature, mean air humidity, and rain precipitation), hourly recorded in the weather station of Poppi (Arezzo province, 43°44′09″ N, 11°45′42″ E). We used CORINE Land Cover database (2013) to assess study area habitat composition.

2.2.1. *Identification of commuters and residents*

Unverified locations, i.e., with dilution of precision (DOP) higher than 10 or obtained with less than 4 satellites, were discarded from our analysis. Each of the remaining locations was assigned an “In-Out” value based on its relative position in respect to reserve boundaries, which was 0 for locations inside the protected areas and 1 for locations outside of them.

Based on the individual average of “In-Out”, we subdivided wild boar into “residents” (less than 5% of locations outside the reserve) and “commuters” (more than 5% of locations outside the reserve). We chose a relatively low threshold value, consistent with the conservative approach we aimed to adopt, in order to also take into account external locations of individuals which seldom left the protected areas. In fact, even few locations outside the reserve can produce a high impact on agricultural crops, especially when they are concentrated in a certain period of the year and time of the day. By using a 5% threshold, we avoided underestimating any potentially important use of unprotected lands by discarding individuals which rarely left the reserve from the commuter group. Thereafter, since hunting land use was negligible for the resident group, we restricted advanced statistical analysis only to the commuter group for both protected areas.

We assigned each location to either daylight or nightlight by comparing recording date and time with local sunrise and sunset times.

2.2.2. *Models on protected area use by commuter wild boar*

In order to assess the relation between reserve use and environmental predictors in each protected area, we divided our dataset into 4 sub-datasets with a spatial (FCNP vs OAC) and temporal (daylight vs nightlight) criterion. For each sub-dataset, the “In-Out” variable was modelled by means of Generalised Additive Mixed Models (GAMMs) with a binomial distribution, which were implemented by means of the *mgcv* package in R 3.2.2 (R Core Team, 2015). To account for the nested nature of data, we used individual wild boar identity as random factor. Such biological factors as sex, age, and body weight were added as predictors. Julian date was included in our models as explanatory variable to check for potential intra-annual patterns of variation in reserve use. We also inserted the environmental variables which resulted to significantly affect wild boar behavioural ecology (Brivio et al., 2017), i.e., mean air temperature, mean air humidity, and total rain precipitation, calculated on the 2-h interval preceding each location. Finally, to take into account the potential effect of resource availability, we assessed the home range (Minimum Convex Polygon – MCP - 90%) for each month/wild boar and calculated the relative percentage of the 3 most important habitat types (forest, shrubs, and open areas) by using Corine Land Cover (2013) database and the QGis 2.12.2 software (QGIS Development Team, 2016). These percentage values were assigned to all locations according to the month of recording and used as predictors in our models.

For each sub-dataset discretely, we performed a variable selection process to choose only explanatory variables unaffected by collinearity, following Zuur et al. (2009). Firstly, we calculated Pearson correlation coefficients among all possible predictor variable pairs and then ran a multicollinearity test by using the corvif function (*AED* package). In case of variables affected by not-negligible multicollinearity (VIF≥3), we performed a random forest calculation (*random.Forest* package) and excluded the worst variable of each collinearity condition affecting two or more variables. In the FCNP sub-datasets, we detected a not-negligible collinearity between age, weight, and sex. In accordance with the random forest results, we excluded weight from the daylight sub-dataset and both age and sex from the nightlight sub-dataset. Habitat composition variables contained a couple of predictors affected by collinearity in all four sub-datasets (forest-open areas and forest-shrubs for FCNP and OAC sub-datasets, respectively): as we preferred not to exclude any habitat composition predictor in this phase, we built alternative models with one or the other collinear variable.

In the final step, for each sub-dataset, we built a GAMM with a full model structure including the explanatory variables selected during the previous steps (Table 1). The effect of Julian date was modelled as a cyclic cubic regression spline in order to consider its circularity, while the effect of all the other variables was modelled as natural cubic spline functions. For each sub-dataset, we subsequently ran a set of models with all possible combinations of the predictor variables included in the full model (Table 1) by means of the dredge function (*MuMln* package). We selected the 4 best models following the minimum AIC criterion (Symonds and Moussalli, 2011). In case of models with ΔAIC<2, we selected the most parsimonious in terms of number of predictor variables included (Symonds and Moussalli, 2011). If two or more models had ΔAIC<2 and the same number of predictors, we considered the minimum AIC model as the best model, accepting any ΔAIC value.

3. Results

We captured and monitored 18 and 8 wild boar in FCNP and OAC, respectively. The distance between the capture site and the nearest reserve boundary resulted to be poorly correlated with the external/total location proportion at an individual level in both FCNP and OAC (FCNP: r = 0.102, P = 0.038; OAC: r = 0.041, P = 0.001): individuals whose capture site was located

Table 1
Summary of explanatory variable sets used for the four sub-datasets in random forest, full model, and best model, respectively.

		age	sex	weight	J date	temp	humidity	rain	forest	open areas	shrubs
FCNP day	Random forest	x	x	x	x	x	x	x	x	x	x
	Full model	x	x		x	x	x	x	x	x	x
	Best model				x	x	x		x		x
FCNP night	Random forest	x	x	x	x	x	x	x	x	x	x
	Full model			x	x	x	x	x	x	x	x
	Best model				x	x	x	x	x		x
OAC day	Random forest	x	x	x	x	x	x	x	x	x	x
	Full model	x	x	x	x	x	x	x	x	x	x
	Best model	x			x	x	x		x	x	
OAC night	Random forest	x	x	x	x	x	x	x	x	x	x
	Full model	x	x	x	x	x	x	x	x	x	x
	Best model	x			x	x	x		x	x	

x = the explanatory variable was included; empty cell = the explanatory variable was not included; age = individual age expressed in years; weight = individual body weight; J date = Julian date; temp = mean air temperature; humidity = mean air humidity; rain = total rain precipitation; forest = forest cover availability; open areas = open area availability; shrubs = shrub cover availability; FCNP = Foreste Casentinesi National Park; OAC = Oasi Alpe di Catenaia; Random forest = explanatory variables selection process; Full model = GAMM including all the explanatory variables selected; Best model = best alternative model selected following the minimum AIC criterion.

further in the protected areas showed slightly higher proportions of external locations. We identified 9 commuters and 9 residents in FCNP and 6 commuters and 2 residents in OAC. In FCNP, commuters had an external/total location proportion of 0.52 ± 0.15 (mean ± SE) during daylight and 0.51 ± 0.12 during nightlight, while in OAC the proportion was 0.29 ± 0.06 and 0.35 ± 0.05 during daylight and nightlight, respectively.

3.1. Models on protected area use by commuter wild boar

3.1.1. Large protected area (FCNP)

The two best models explaining the likelihood of being outside FCNP included the predictor variable sets summarized in Table 1 (daylight $R^2_{adj} = 0.442$; nightlight $R^2_{adj} = 0.381$). For the diurnal sub-dataset, Julian date had a significant but weak effect with a slightly lower proportion of external locations falling from the 250th (7th September) to the 350th day of the year (16th December, Fig. 2a). Conversely, the effect of Julian date on the likelihood of being outside during the night was strong and significant, with wild boar being predominantly outside for most of the year and then increasing their stay inside the protected area boundaries from around the 250th day of the year (7th September) until the 334th day (30th November), with a sharp minimum peak around the 290th day of the year (17th October) and a gradual increase until the 30th day of the subsequent year (30th January, Fig. 2b). Mean air temperature affected the response variable with a positive but almost flat pattern during the day (Fig. A1a). On the other hand, it had a positive, not-negligible effect on the likelihood of being outside the protected area during the night, with wild boar external locations raising at higher environmental temperatures (Fig. A1b).

During both daylight and nightlight, wild boar resulted to have generally more external locations when the shrub cover availability was lower: the maximum likelihood of being outside was recorded with shrubs covering about 5% of their monthly home range, then progressively decreasing as shrub cover increased (Fig A2a and A2b). Monthly forest cover availability affected both diurnal and nocturnal likelihood of being outside in a similar way: the likelihood was high with high forest cover availability and sharply decreased with forest cover below 68% and 65% for daylight and nightlight, respectively (Fig. A3a and A3b). Although statistically significant, the other predictor variables (mean air humidity and rain precipitation) had biologically negligible effects on the response variable (Fig. A4 and A5).

3.1.2. Small protected area (OAC)

Best models explaining the variation of the likelihood of wild boar being outside OAC included the same set of explanatory variables for diurnal and nocturnal sub-datasets, as summarized in Table 1 (daylight: $R^2_{adj} = 0.529$; nightlight: $R^2_{adj} = 0.366$). Older wild boar tended to locate outside the reserve more frequently than younger individuals during both the day and the night (Fig. A6a and A6b). Julian date affected the diurnal likelihood of being outside the reserve with a complex pattern, which fluctuated during the first half of the year and became quite stable in the second half (Fig. 2c). Wild boar use of the reserve during the night did not vary markedly during the year, showing three weak positive peaks of the likelihood of being outside the protected area around the 80th, 190th and 330th day of the year (21st March, 9th July, and 26th November, respectively) characterized by wide confidence intervals (Fig. 2d). The likelihood of being outside resulted to be lower with higher air temperature, although this effect was strong and significant for the diurnal sub-dataset but relatively weak for the nocturnal one (Fig. A7a and A7b). During the day, the relationship between forest cover and the likelihood of being outside estimated by the best model was complex and its biological significance difficult to disentangle (Fig A8a). During the night, the likelihood of being outside was high when monthly forest cover availability was higher than 70% and decreased when it was smaller (Fig

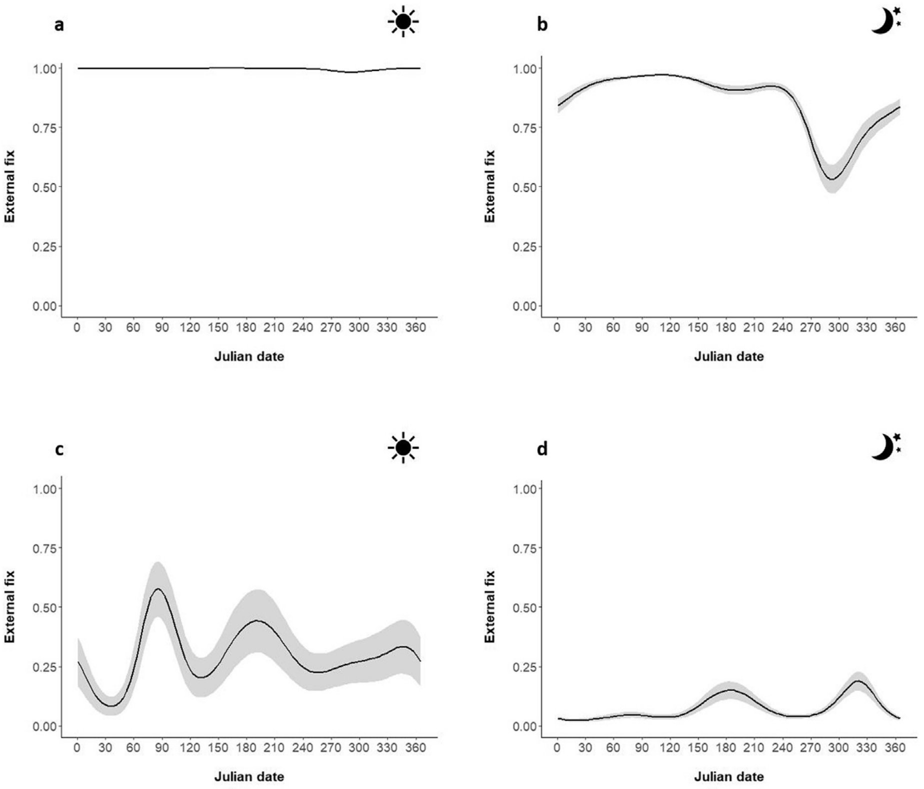

Fig. 2. Effect of Julian date on the likelihood of wild boar being outside the FCNP protected area during daylight (a) and nightlight (b) and the OAC protected area during daylight (c) and nightlight (d), respectively. The values reported were predicted by the best generalised additive mixed model, separately for each sub-dataset (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

A8b). As in the case of FCNP models, the other explanatory variables included in best models (mean air humidity and open area cover) had a biologically negligible effect on the likelihood of being outside OAC (Fig. A9 and A10).

4. Discussion

Our study investigated reserve effect patterns in two contiguous protected areas of different size. The results highlighted the importance of using detailed information on both animal behaviour and environmental resources in evaluating reserve effect. Although biologically negligible, the positive correlation between the distance separating the capture site from the nearest reserve boundary and the proportion of external locations clearly excluded the possibility that our samples were biased by the opportunistic choice of capture sites. As expected, a relevant number of individuals had a negligible use of unprotected lands. We showed that this portion of “resident” wild boar was two times higher in the large protected area (FCNP) in respect to the small one (OAC), as a wider surface is likely to sustain a higher number of individuals all-year round. The use of the protected lands was neither limited to nor concentrated in a specific part of the day, thus pointing out a lack of use of both the large and the small protected areas as refuge during hunting activities.

We did not detect variations in the likelihood of wild boar being outside the small protected area during both daylight and nightlight, whereas a clear decrease was observed in the large protected area in autumn, though surprisingly only during nightlight. This finding completely refutes our second prediction, since the likelihood of wild boar being inside the protected area in autumn did not increase when hunting activities were actually performed (that is, during daylight). Conversely, it increased during nightlight, when any kind of human disturbance was either minimal or absent. In fact, though statistically significant, the intra-annual variation of the likelihood of being outside the large protected area during daylight was biologically negligible (Fig. 2a). In the light of a recent research in the same study area showing that wild boar is strictly nocturnal (Brivio et al., 2017), we can argue that wild boar homogeneously used resting areas located outside the protected area throughout the year. Conversely, in the same area, the likelihood of wild boar being outside during the night sharply decreased from 7th September until 30th November, with a negative peak around 17th October and a gradual increase until 30th January (Fig. 2b). It is worth noting that any form of hunting is strictly forbidden during the night and that any other source of human disturbance is also expected to be minimal if compared with daylight. Moreover, the temporary decrease of the likelihood of being outside did not exactly correspond to the hunting season (lasting from around 1st October until 31st December), which began and ended after such decrease. In conclusion, we can speculate that wild boar increased their use of the large protected area in autumn to implement their foraging activities and not to find refuge from hunting. This explanation is supported by the fact that chestnuts are known to be a key food resource for wild boar in our study area during autumn (Cutini et al., 2013; Bisi et al., 2018) and chestnut forests are abundant inside the large protected area boundaries and quite scarce outside. The lack of reaction to hunting we observed is consistent with previous studies showing that human activities had minimal impacts on the behaviour of this species (Sodeikat & Pohlmeyer 2002, 2004; Keuling et al., 2008; Brivio et al., 2017; Melletti and Meijaard, 2017). Moreover, it indicates that, on account of their behavioural plasticity, wild boar can use environmental resources regardless of human disturbance. Although we are unable to provide any biological explanation of the complex variation pattern of the likelihood of wild boar being outside the small protected area throughout the year, we clearly did not detect any increase in its use throughout the hunting season during both daylight and nightlight (Fig. 2c and d). Though offering total shelter from hunting disturbance, we can argue that the small reserve lacked in satisfying other requirements because of its limited extension, thus making stays for medium-long periods unfeasible for wild boar. Thurfjell et al. (2013) reported that wild boar, fleeing into refuge areas owing to hunting disturbance, were negatively affected by the intra-specific competition with resident individuals. Similarly, in our case-study, competition with resident wild boar for the limited resources of the small protected area may have played a major role in discouraging commuter individuals to seek refuge inside the reserve during the hunting season. This effect was likely to concern wild boar diurnal resting as well as their nocturnal foraging activities, as both secure resting sites and food availability may act as limiting resources. It is worth nothing that, even though our results showed the absence of reserve effect, protected areas may still act as reservoir for wild boar in a sources-sinks system. Nevertheless, as commuter wild boar and individuals outside the protected area have the same likelihood to be culled during hunting, this phenomenon would only concern the resident group. Our findings are consistent with a previous study on white-tailed deer (Root et al., 1988), though they are in contrast with the results of Tolon et al. (2009) on another wild boar population and Grignolio et al. (2011) on roe deer. Root et al. (1988) showed that white-tailed deer did not move inside the protected area when intensive hunting started. Notably, their protected area covered only 7 Km^2. Conversely, Tolon et al. (2009) highlighted a marked displacement of wild boar home ranges from hunting lands to a protected area when hunting started, though this only affected the individuals whose home ranges were already “in contact” with the protected area. It is worth noting that our wild boar sample was entirely captured inside the protected areas. Thus, we could not have overestimated reserve effect by monitoring individuals which were not in contact with the protected areas. Finally, the study conducted by Grignolio et al. (2011) found that hunting with hounds (targeting wild boar and hares, *Lepus europaeus*) forced roe deer to select safe but sub-optimal areas. By comparing their results with ours, we can suggest that different species and populations may tend to prioritise either predation avoidance (i.e., roe deer) or resource supply (i.e., wild boar). Nevertheless, while the aforementioned studies focused on home ranges, our finer-scale approach (based on the likelihood of single locations being inside the protected areas and high resolution spatial data) is necessary to detect the occurrence of reserve effect on a local scale. Moreover, our study points out the need to consider the activity rhythms of species and the temporal distribution of anthropic sources of disturbance in order to fully understand the drivers affecting behavioural patterns.

Since both protected areas are located on the top of mountainous ridges, we expected a negative effect of mean air temperature on the likelihood of wild boar being outside both reserves, as individuals can compensate environmental temperature variations by moving across the altitudinal gradient (Lamberti et al., 2004; Mason et al., 2014; Brivio et al., 2019). Such supposition was verified in the small protected area (relevant only during daylight, Fig A7), while the relation between mean air temperature and the likelihood of being outside was positive in the large protected area (relevant only during nightlight, Fig A1). We can suppose that this unexpected result is another consequence of the peculiar resource distribution of this area, in which the main food resources are concentrated inside its boundaries (that is, at higher altitudes) in the cold seasons. However, it is to note that we included in our analysis this and other environmental and biological variables to take into consideration their influence on wild boar behaviour, i.e., to enhance the robustness of our results and not to properly investigate their effect on the use of the protected areas.

5. Conclusions

We showed that a relevant portion of wild boar living along the borders of the protected areas was composed of resident individuals permanently located inside the reserves and that this number was positively related to the size of the protected area. Therefore, pest species population control practices performed inside protected areas to limit damages on neighbouring unprotected lands may lack effectiveness, as their effort would be partially wasted on the control of harmless resident individuals. This consideration is especially valuable for the management of large protected areas. As we aimed to evaluate the intra-annual variation of individual likelihood to be located outside protected areas, we only included in our advanced analysis individuals showing a not-negligible use of both the protected and the unprotected areas, that is, the commuter group. Nevertheless, further investigations are needed to evaluate which factors affect the likelihood of individuals to act either as inside resident, commuter or outside resident.

We did not detect any increased use of protected areas during wild boar hunting period. Thus, the effectiveness of management practices ordinarily performed on unprotected surfaces is unlikely to be negatively affected by the presence of protected areas. Moreover, in our study area, abnormal concentrations of individuals inside the protected areas during the hunting season are unlikely to occur.

Variability of results of the few studies on reserve effect (Root et al., 1988; Tolon et al., 2009; Grignolio et al., 2011; this paper) suggests that this phenomenon is not as widespread as thought. Moreover, in the case of wild boar, its known behavioural variability can play a major role in producing even more variable reserve effect patterns. The case-dependence of reserve effect clearly highlights the necessity to verify its occurrence on a local scale. To better understand its complexity, researchers should base further investigations on fine-scale information on animal movements and activity rhythms, such as those obtained from GPS tracking.

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Ethical standards/permits

This study complies with all national and regional laws dealing with ethics and animal welfare. Capture and manipulation protocols were approved by Tuscany Regional Administration (no. 103/5936/152–13/03/2002). The research adhered to the ASAB/ABS Guidelines for the Use of Animals in Research.

Author contributions

MA, RB, SG originally formulated the idea. RB conducted fieldwork. RB, SG and FB collaborated in imaging analysis. RB, FB and SG performed statistical analyses. RB, SG and FB wrote the original draft of the manuscript. MA provided editorial advice. MA provided materials tools and contributed to funding acquisition.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix

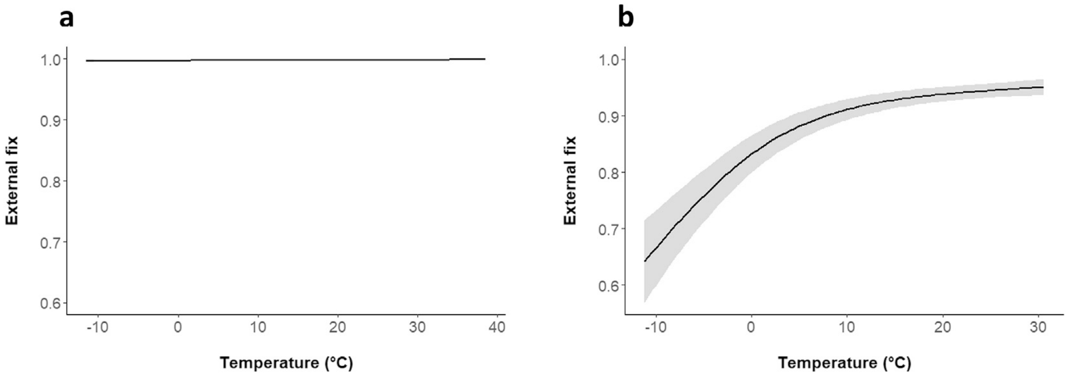

Fig. A1Effect of mean air temperature on the likelihood of wild boar being outside the FCNP protected area during daylight (a) and nightlight (b). The values reported were predicted by the best generalised additive mixed models (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

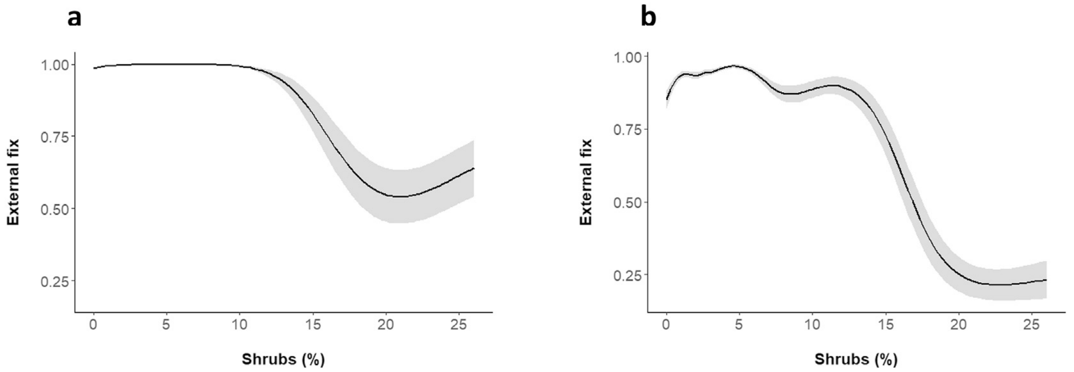

Fig. A2. Effect of shrub cover monthly availability on the likelihood of wild boar being outside the FCNP protected area during daylight (a) and nightlight (b). The values reported were predicted by the best generalised additive mixed models (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

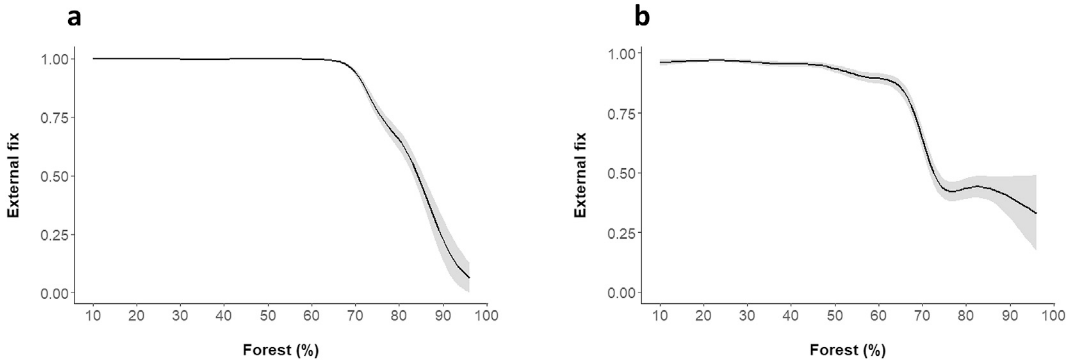

Fig. A3. Effect of forest cover monthly availability on the likelihood of wild boar being outside the FCNP protected area during daylight (a) and during nightlight (b). The values reported were predicted by the best generalised additive mixed models (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

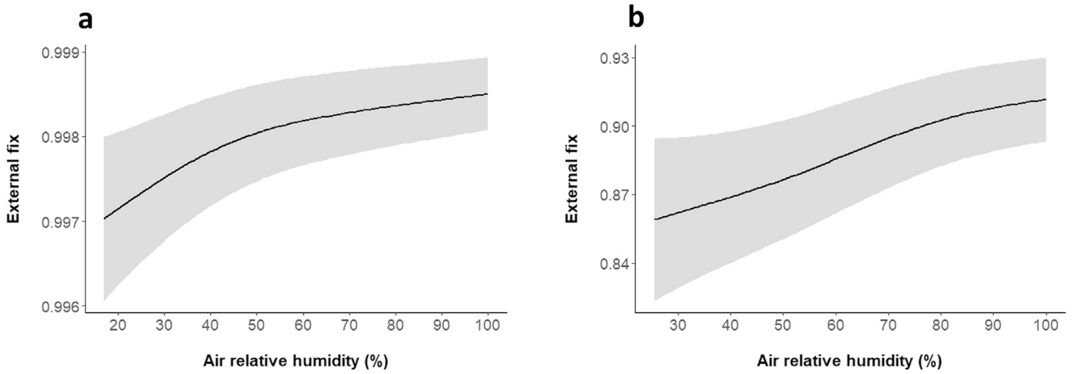

Fig. A4. Effect of air relative humidity on the likelihood of wild boar being outside the FCNP protected area during daylight (a) and nightlight (b). The values reported were predicted by the best generalised additive mixed models (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

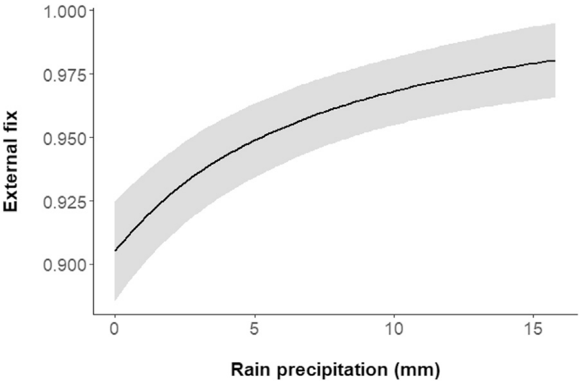

Fig. A5. Effect of rain precipitation on the likelihood of wild boar being outside the FCNP protected area during nightlight. The values reported were predicted by the best generalised additive mixed model (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

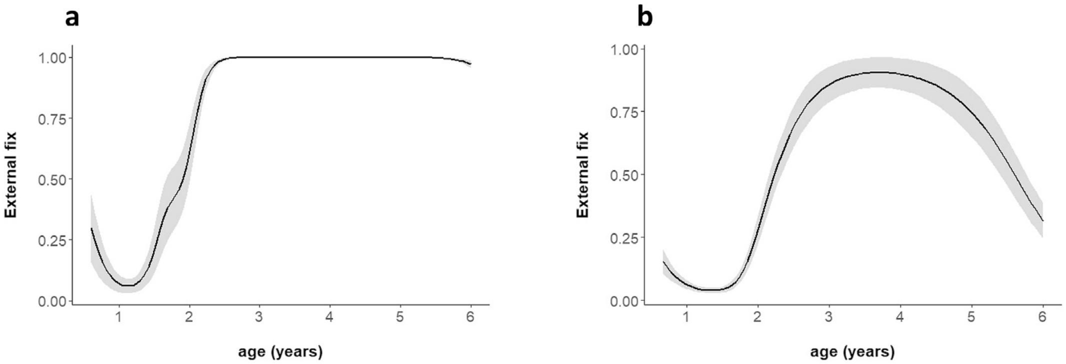

Fig. A6. Effect of individual age on the likelihood of wild boar being outside the OAC protected area during daylight (a) and nightlight (b). The values reported were predicted by the best generalised additive mixed models (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

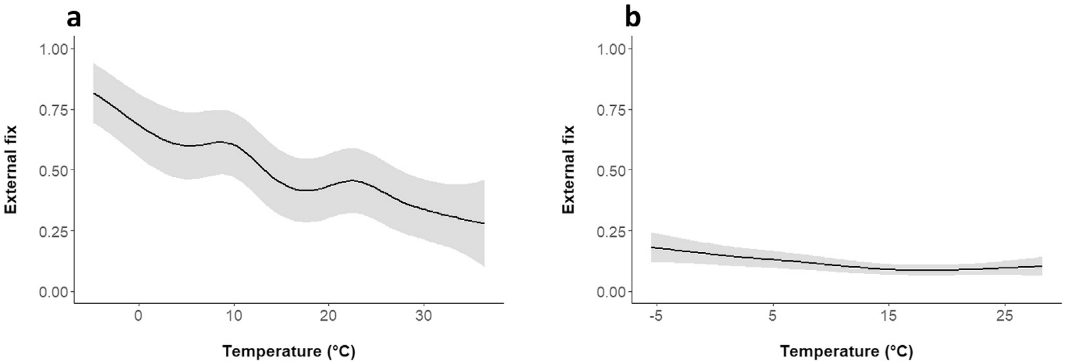

Fig. A7. Effect of mean air temperature on the likelihood of wild boar being outside the OAC protected area during daylight (a) and nightlight (b). The values reported were predicted by the best generalised additive mixed models (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

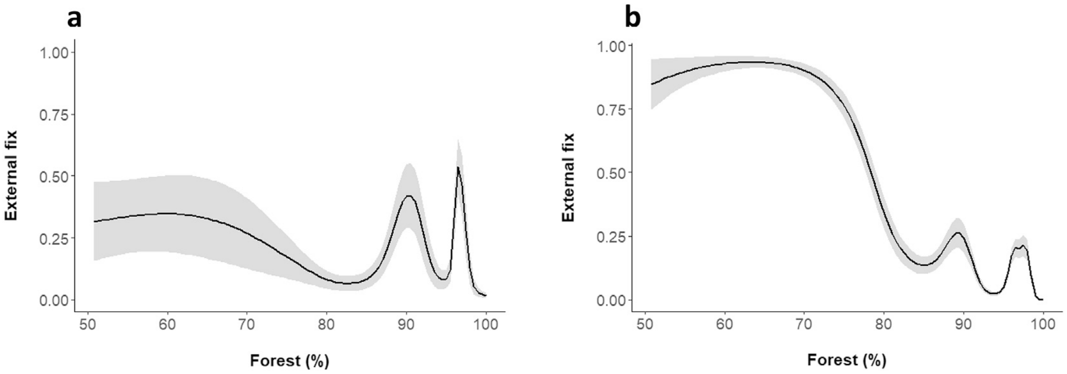

Fig. A8. Effect of forest cover monthly availability on the likelihood of wild boar being outside the OAC protected area during daylight (a) and nightlight (b). The values reported were predicted by the best generalised additive mixed models (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

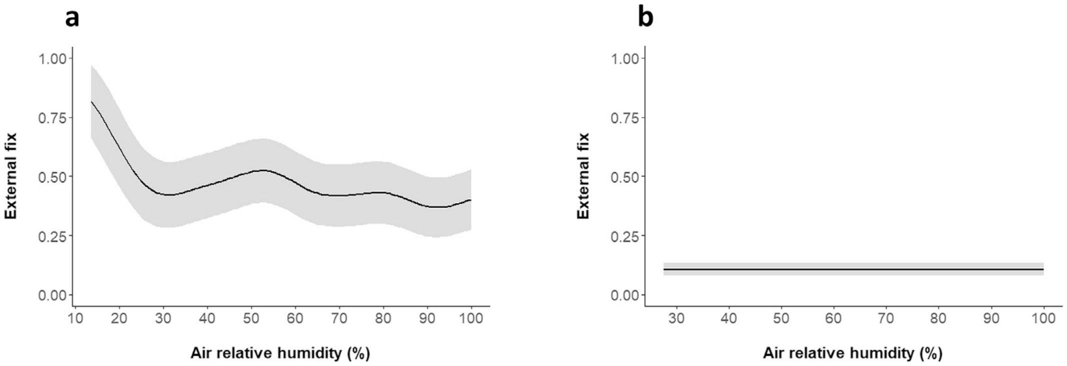

Fig. A9. Effect of air relative humidity on the likelihood of wild boar being outside the OAC protected area daylight (a) and nightlight (b). The values reported were predicted by the best generalised additive mixed models (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

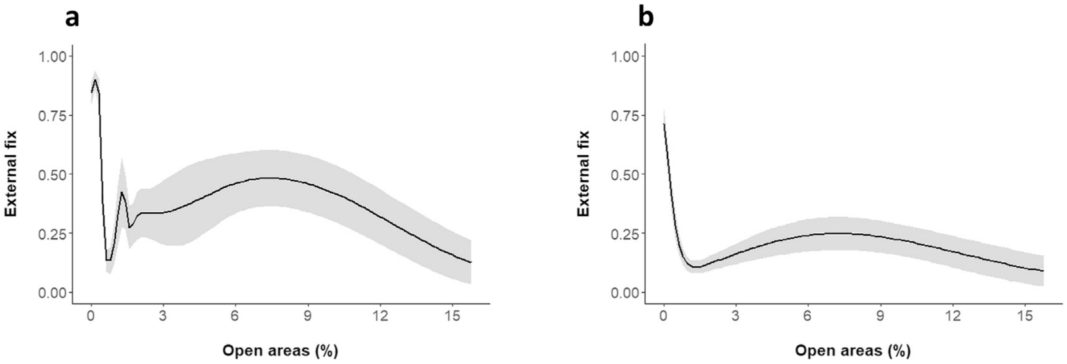

Fig. A10. Effect of open area monthly availability on the likelihood of wild boar being outside the OAC protected area during daylight (a) and nightlight (b). The values reported were predicted by the best generalised additive mixed models (see the text for more details). The predictions are given according to the mean of all other covariates in the model. In the graphs the gray-shaded areas represent the estimated standard errors.

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Chapter 4

Behavioral syndromes going wild: individual risk-taking strategies of free-ranging wild boar

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Behavioral syndromes going wild: individual risk-taking strategies of free-ranging wild boar

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Abstract

To investigate risk-taking behaviors on free-ranging animals, large datasets of animal relocations may represent a high potential alternative to classical behavioral tests. Analyzing a risk-induced habitat selection would allow to simultaneously detect multiple risk-taking traits, as repeated measures of the individual willingness to take risks across different contexts. Investigations under controlled conditions showed multiple personality traits to be correlated, with populations typically consisting of risk-taker and risk-avoider individuals. Nevertheless, it remains unclear whether these extreme strategies are exhibited by wild, free-ranging animals, as well as the role played by environmental conditions. We modelled the risk-induced habitat selection of 43 free-ranging wild boar, from two populations living in drastically different environmental conditions. We extracted four different risk-taking traits at both the population and the individual level in order to investigate the tendency of individuals to be gathered in groups sharing homogeneous sets of risk-taking traits. We detected a significant risk-induced habitat selection, showing that animal relocation data may be profitably used to investigate risk-taking strategies on free-ranging animals. Within both populations, individuals clustered in two groups sharing homogeneous risk-taking strategies, but we detected compensation, rather than correlation, among single risk-taking traits. We demonstrated that risk-taking strategies observed under experimental conditions are not comparable with those exhibited by free-ranging animals in nature, which are likely advantaged by strategies characterized by inconsistent risk-taking traits. The similarities of risk-taking strategies among the two monitored populations showed that our results were not driven by the peculiarity of a single population or of local conditions. Nonetheless, we could speculate that phenotypic plasticity regulated the expression of the individual willingness to take risks across different contexts.

1. Introduction

Investigations on risk-taking of free-ranging animals is a major challenge for behavioral ecologists, due to difficulties in controlling experimental conditions in the wild. A reliable measure of the individual willingness to take risks should reflect a consistently repeated choice (Carter et al. 2013): researchers may implement classical behavioral tests in the wild, but it is typically hard to obtain a sufficient number of repeated measures on the same individual (e.g., Miranda et al. 2013, Breck et al. 2019). In this context, the analysis of large datasets of animal relocations represents an alternative and high potential approach for investigating individual risk-taking on wild animals (Ciuti et al. 2012, Hertel et al. 2020). To get a robust evaluation of repetitiveness of risk prone behaviors across different situations, researchers may take advantage of analytical tools able to extrapolate individual choices from animal relocations. This goal can be achieved by means of Step Selection Functions (SSFs, Fieberg et al. 2021) aimed at modelling a risk-induced habitat selection. Further than providing many measures of a single risk-taking trait (e.g., the individual mobility, which increases the likelihood of encounter predators, Hertel et al. 2020), animal relocations would allow to simultaneously extract multiple risk-taking traits. For instance, Ciuti et al. (2012) measured both the average mobility and the individual selection for safe habitats in free-ranging red deer (*Cervus elaphus*), analyzing their influence on mortality. Nevertheless, the potential of large datasets of animal relocations to investigate multiple risk-taking traits and their distribution across individuals remains largely unexploited. The individual tendency to avoid predators (at least those that are spatially predictable, like humans) and the preference for familiar locations (which are known to reduce predation risks, Gehr et al. 2020) may represent further risk-taking traits to be measured on free-ranging animals by modelling their risk-induced habitat selection.

Behavioral syndromes theory predicts different personality traits to be correlated, with bold individuals being also more aggressive, active, and explorative in respect to shy individuals (Sih et al. 2004). In this context, the willingness to take risks is typically referred to boldness (e.g., against predators, Turner et al. 2020) but can relate to other personality traits as well, such as exploratory behavior and activity. More explorative northern elephant seals (*Mirounga angustirostris*) indeed underwent higher individual risks (Abrahms et al. 2018), as well more active salamander larvae (*Ambystoma barbourin*, Sih et al. 2003). According to behavioral syndromes theory, different risk-taking traits should shape two main multi-trait strategies, identifiable in “pure” risk-taker and risk-avoider individuals. This may be accounted to different behavioral traits being genetically correlated, implying a genetic constraint for the individual diversity across different contexts (Sih et al. 2004). Correlations of different risk-taking traits were actually verified on several species in controlled conditions (e.g.,Van Oers et al. 2003, Vetter et al. 2016, Thys et al. 2017), while their occurrence on free-ranging animals is supported by weaker evidences. Miranda et al. (2013) and Breck et al. (2019) reported individuals to exhibit correlated risk-taking behaviors across two experimental contexts, but the number of observed behavioral traits and repetitiveness of measures were constrained by the above mentioned difficulties in manipulating experimental conditions. Conversely, free-ranging spotted hyenas (*Crocuta crocuta*) exhibited consistent willingness to take risks when artificially exposed to threatening objects and conspecifics, but not when facing the natural occurrence of predators (Turner et al. 2020). A robust evaluation of the relationships occurring among multiple risk-taking traits, exhibited across different contexts by free-ranging animals, is still to be done. On the one hand, the genetically determined components of behavior (i.e., personality, Van Oers et al. 2003) should drive a rigid willingness to take risks in any experimental condition. Free-ranging individuals should thus exhibit the same patterns observed in controlled conditions (i.e., correlated risk-taking traits, Vetter et al. 2016, Thys et al. 2017). On the other hand, risk-taking traits measured in wild and uncontrolled conditions are likely the result of experience (Stamps and Groothuis 2010) and environment (Dingemanse et al. 2010) interacting with the “real”, heritable, personality, rather than a pure expression of the personality alone. Whether this complex network of factors simply hinder the detectability of behavioral syndromes in wild animals, or conversely facilitate adaptive variations of the individual consistency in the willingness to take risks across different contexts, remains unknown. Indeed, in wild conditions animals are forced to contemporary face many and articulated sources of risk: a compensation among different risk-taking traits may result more advantageous than the extreme strategies exhibited by pure risk-taker and pure risk-avoider individuals.

Field behavioral studies conducted on free-ranging animals are thus necessary to have a realistic insight of individual risk-taking strategies and their distribution within wild populations. Such investigations should take into account that environmental and other population-level drivers may influence the expression of risk-taking traits and even their mutual relationships, ultimately altering behavioral syndromes (Bókony et al. 2012). For this reason, comparing risk-taking strategies of wild populations experiencing drastically divergent selective pressures may improve results' robustness and biological meaning.

Wild boar (*Sus scrofa*) represents a good model species to investigate risk-taking as the tradeoff between foraging and mortality avoidance, as it has high energetic requirements (Russo et al. 1997, Morelle et al. 2014) and typically suffers high mortality, which is mostly caused by humans (Keuling et al. 2013, Merli et al. 2017), both directly (i.e., hunting) and indirectly (i.e., car accidents). We tracked 43 free-ranging wild boar belonging to two different European populations living in drastically different environmental conditions by means of satellite telemetry. We used this large and high-resolution spatial dataset to perform SSFs and model wild boar risk-induced habitat selection. We extracted four distinct risk-taking traits at both the population and the individual level: site-fidelity, human avoidance, selection for covered habitats, and average mobility. We then observed the distribution of individuals in this four-axes environment and evaluated their tendency to be gathered in groups of individuals sharing similar multi-trait risk-taking strategies. We developed the following predictions:

i) a significant risk-induced habitat selection will be detected in both populations;

ii) according to the behavioral syndromes theory (Sih et al. 2004), two groups of individuals sharing homogeneous multi-trait risk-taking strategies will emerge within each monitored population;

iii) these two groups will correspond to risk-taker (weak site-fidelity, low human avoidance, weak or null selection for covered habitats, and high mobility) versus risk-avoider (strong site-fidelity, high human avoidance, strong selection for covered habitats, and low mobility) individuals.

2. Methods

2.1 Study areas

Animal spatial data were collected from two distinct wild boar populations, located in Central Italy (Northern Apennines, 43.7961 N, 11.7845 E) and in north-western Sardinia (40.6992 N, 8.1917 E), a large island in the western coast of Italy (Fig. S1).

Central Italy study area is mostly mountainous (altitudes range 330 - 1,400 m above the sea level) while north-western Sardinia is characterized by a typical Mediterranean environment (altitudes range 0 - 424 m a.s.l.). In Central Italy the climate is temperate continental, with hot, dry summers and cold, rainy, and occasionally snowy, winters. Monthly average temperatures varied between 4.7°C of January to 21.9°C of July, with an annual average pluviometry of 779 mm. Conversely, the climate of north-western Sardinia is Mediterranean, with very hot and dry summers, and windy and cold winters. Minimum and maximum monthly temperature of 9.8°Cand 24.3°Cwas reached in January and August, respectively. Rain precipitations of this area are much lower than those of Central Italy, with an average of 495 mm recorded annually.

Central Italy study area was covered by 74% of forests, 5% of shrubs, and 18% of open areas (pastures and agricultural landscapes). Human infrastructures (mainly single houses, small villages, and roads) were sparse and occupied about 3% of the entire study area. Conversely, in north-west Sardinia forests, shrubs, and open areas occupied 25%, 26%, and 46% of the study area, respectively. Human infrastructures covered the remaining 3% but were less dispersed than in Central Italy, mainly consisting of medium-sized villages and roads.

In Central Italy, wolves (*Canis lupus*) were present at a high density (Mattioli et al. 2018), and wild boar represented its main prey (Bassi et al. 2012). Conversely, no natural predators were present in Sardinia. Wild boar drive hunts were performed in both study areas during 5 months per year (September-January). One large (Foreste Casentinesi National Park, 362 km^2) and one small (Oasi

Alpe di Catenaia, 27 km^2) protected areas were present in Central Italy, while the Sardinian study area included a single protected area of 54 km^2 (Porto Conte Regional Park). Two recent studies conducted in Central Italy showed hunting not to directly influence wild boar activity and use of protected areas (Brivio et al. 2017, Brogi et al. 2020).

2.2 Wild boar spatial data

Wild boar were captured from 2013 until 2020 in Central Italy (n=28, 17 females and 11 males) and Sardinia (n=15, 8 females and 7 males), by means of baited traps and vertical dropping nets, following the protocol detailed by Brogi et al. (2019). They were sedated by means of different drug mixtures (zolazepam – tiletamine or zolazepam – tiletamine – xylazine, alternatively) and then equipped with a GPS collar (PRO Light collar and Vertex Lite collar, Vectronic Aerospace, in Central Italy and Sardinia, respectively). Such devices were configured with a 2-hours relocation schedule, thus recording 12 GPS relocations/day. A total of 82,282 and 61,150 valid wild boar relocations were recorded in Central Italy and Sardinia, respectively, and used for subsequent analyses. All collars were also configured to measure activity by means of a two-axis accelerometer, recording activity data along a continuous range (0-255) with a four-minutes schedule (for more details, see Brivio et al. 2017).

2.3 Step Selection Functions

In order to select only those steps (movements between two consecutive relocations) which could reflect the individual choice to get in a certain place, we removed steps corresponding to inactive periods (i.e., resting) of wild boar. First, we transformed the raw activity data recorded by the collars into binary activity statuses (0 inactive, 1 active), following the protocol described by Brivio et al. (2021) in order to take into account inter-individual differences in the activity measuring process. Second, we assigned to each step the activity statuses corresponding to the period between the recording times of its start and end relocations. Being relocations recorded every two hours and activity every four minutes, 30 activity statuses were assigned to each step. Third, we selected only those steps with at least three active records (i.e., at least 12 minutes spent moving).

We used the `amt` R package (Signer et al. 2019) to run a Step Selection Function on each individual wild boar. We preliminary estimated movement parameters for each individual, such as step length (average linear distance between consecutive relocations, expressed in meters) and turning angle (average angle between consecutive steps, expressed in radiant). We then generated a time-dependent availability distribution by simulating potential movements (steps) from the previously observed relocation (i.e., a model of animal movements in absence of habitat selection). In particular, 10 random steps were generated for each observed one, using the range of variation of the movement parameters (step length and turning angle) previously estimated for each individual. A new binary variable "used" was added to distinguish random (used=0) from observed (used=1) steps and used as response variable of subsequent models (Fieberg et al. 2021).

By means of a rasterized CORINE Land Cover database (resolution of 10 meters), we assigned the following spatial covariates to all steps (either random or observed), on the basis of their end relocation: 1) "familiar", a binary variable assuming 1 when the step end relocation fell on a land cover patch containing at least an observed relocation of the individual wild boar during the previous week; 2) "distance", a continuous variable measuring the linear distance (m) from the nearest human infrastructure (house, urban areas, factory, paved road, railway); 3) "covered", a binary variable referring to the vegetation coverage and assuming 1 for step end relocations falling within forests and shrubs and 0 for those falling within pastures and agricultural fields. Finally, we used a digital surface model online database (EU-DEM v1.0) to assign to all step end relocations three further covariates describing the terrain morphology: altitude (m a.s.l.), slope index

(0=vertical terrain, 250=horizontal terrain), and surface orientation (sine of North degrees, 0=East and West, 1=North, -1=South).

2.4 Extrapolation of risk-taking traits at the population level

With the aim to establish whether the risk-related spatial covariates (“familiar”, “distance”, and “covered”) actually influenced wild boar movements, we modelled the average habitat selection of wild boar of Central Italy and Sardinia, separately. We performed binomial logistic regressions on “used” (i.e., on the likelihood of each step end relocation to be selected by wild boar). As we aimed to evaluate wild boar selection of familiar locations, we included “familiar” as predictor. “Distance” was included to assess the preference for staying away from human infrastructures, while “covered” was used to evaluate wild boar preference for moving to location with dense vegetation. Altitude, slope index, and surface orientation were added as control predictors to account for their potential influence on wild boar movements. We also included step length and its log as further predictors to reduce bias of the habitat selection parameters (Forester et al. 2009). All predictors were screened for collinearity (Pearson coefficient $|r_p| < 0.7$) and multicollinearity (Variance Inflation Factor, VIF < 3, Zuur et al. 2009).

Separately for each population, we first created a conditional GLM (Generalized Linear Model, with strata formed by combining each observed step with the random steps generated from its start relocation) with a full model structure, including all the predictors described above. We then chose a best population model by applying a manual step AIC procedure, removing the predictors that contributed to increase the model AIC. We used the coefficients obtained for “familiar”, “distance”, and “covered” as average population measures of site-fidelity, human avoidance, and selection for covered habitats, respectively (Table 1).

2.4 Extrapolation of individual risk-taking traits

We followed the protocol described by Fieberg et al. (2021) to asses individual traits of habitat selection. We ran the best population model separately on each individual wild boar (e.g., the best population model selected for Central Italy was used for all wild boar monitored in that study area), in order to get comparable individual parameters of habitat selection among wild boar of the same population (Fieberg et al. 2021). Coefficients obtained for “familiar”, “distance”, and “covered” were then used as individual measures of site-fidelity, human avoidance, and selection for covered habitats, respectively, while individual average step lengths were used as measures of mobility (Table 1). As we were interested in the whole mobility (and not in the average speed observed only during active periods), the individual average step length was here calculated including inactive steps.

Table 1. Risk-taking traits extrapolated form wild boar movements.

Trait	SSFs coefficient or parameter	Relationship with actual or perceived risk	Supporting evidence
Site-fidelity	coefficient for “familiar”	inverse: individual site-fidelity was associated to lower risks in roe deer (*Capreolus capreolus*) and pinnipeds	Abrahms et al. (2018); Gehr et al. (2020)
Human avoidance	coefficient for “distance”	inverse: humans were the most important predator of wild boar in the monitored populations; wild boar proximity with humans was associated with an increased risk perception	Stillfried et al. (2017); Bassi et al. (2020); Greco et al. (2021)
Selection for covered habitats	coefficient for “covered”	inverse: individuals using covered habitats suffered lower human-induced mortality in wild boar and red deer	Ciuti et al. (2012); Merli et al. (2017)
Mobility	step length	direct: more mobile individuals are more likely to encounter humans; more mobile wild boar and red deer are more likely to be culled by hunters	Ciuti et al. (2012); Merli et al. (2017); Hertel et al. (2020)

SSFs coefficient = coefficient for a certain predictor variable included within the binomial conditional logistic regressions of SSFs (Step Selection Functions); SSFs parameter = movement parameter preliminary estimated by SSFs; “familiar”, “distance”, and “covered” = predictor variables included within the binomial conditional logistic regressions (see Methods section for more details); step length = individual average linear distance (m) between consecutive relocations.

2.5 Identification of multi-trait strategies

By observing the distribution of individuals along the four-axes corresponding to the four risk-taking traits, we aimed to establish whether groups of individuals sharing similar risk-taking multi-trait strategies were identifiable. Separately for each monitored population, we first assessed the clustering tendency of individual traits by means of `factoextra` R package (Kassambara and Mundt 2017). Second, in case of detection of a significative clustering tendency, we used the `NbClust` R package to determine the best clustering scheme of the population by comparing all combinations of number of clusters, distance measures, and clustering methods (Charrad et al. 2014).

Results

All risk-related spatial covariates (“familiar”, “distance”, and “covered”) significatively influenced wild boar movements in both study areas (Tables S1 and S2). Both populations showed an average preference for sites visited in the previous week (positive coefficients for “familiar”) and for proximity with human infrastructures (negative coefficients for “distance”). Conversely, wild boar population of Central Italy tended to avoid covered habitats, while an average selection for them was detected for the Sardinian population.

The four considered risk-taking traits widely varied across individuals (Fig. S2). “Covered” coefficients showed a range of variation including both negative and positive values, with a number of individuals selecting relocations with covered habitats and others avoiding them, within both populations. The same happened with “distance”: while a number of wild boar avoided humans, others tended to select relocations in proximity to human infrastructures. Despite all individuals positively selected relocations included in land cover patches which had already been visited during the previous week, the strongness of this selection varied among individuals, especially in the population of Sardinia (Fig. S2). Individuals markedly differed in their mobility, with average step lengths spanning from 67 to 249 m/2 hours (mean 162 m/2 hours), and from 105 to 310 m/2 hours (mean 188 m/2 hours), for populations of Central Italy and Sardinia, respectively.

Individuals of both populations showed a significative clustering tendency along the four considered risk-taking traits, with the best clustering scheme consisting in two clusters of individuals for each population (Fig. 1). The identified clusters were similar among populations of Central Italy and Sardinia, in terms of both the proportional number and the average risk-taking traits of the individuals included. A first, smaller, cluster included individuals sharing a multi-trait risk-taking strategy characterized by a relatively strong selection for covered habitats and relatively low mobility, human avoidance, and site-fidelity (Fig.2). This first cluster included 6 individuals (all females) out of 28 (21 %) and 4 individuals (one female and three males) out of 15 (27 %), for populations of Central Italy and Sardinia, respectively. A second, larger, cluster consisted of wild boar with a relatively low selection for covered habitats, a high mobility, a marked tendency to avoid humans, and a strong site-fidelity (Fig. 2). The second cluster included the remaining 22 individuals (11 females and 11 males, 79% of the monitored wild boar) and 11 individuals (7 females and 4 males, 73% of the monitored wild boar), within populations of Central Italy and Sardinia, respectively.

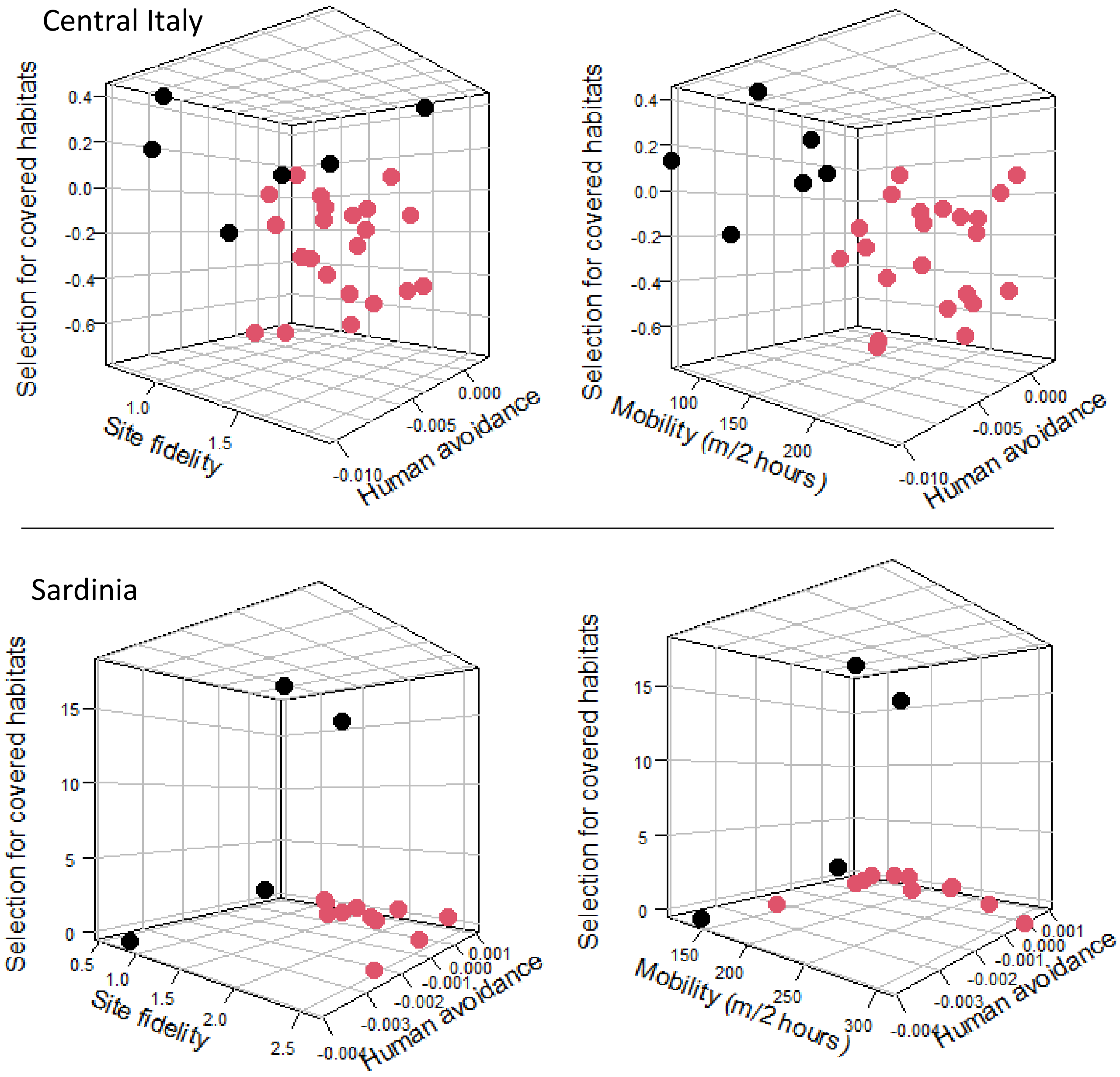

Figure1. Identified clusters of individual wild boar on the basis of four risk-taking traits, in Central Italy (top) and Sardinia (down) populations, respectively. Each point represents an individual, with the color showing the belonging cluster (black: cluster 1, red: cluster 2). Site fidelity, selection for covered habitats, and human avoidance refer to the coefficients of the respective predictors inserted in the individual Generalize Linear Models (see the text for more details); mobility refers to the individual step length. To allow a comprehensive visualization of results, either site fidelity (left) or mobility (right) was shown on the x-axis.

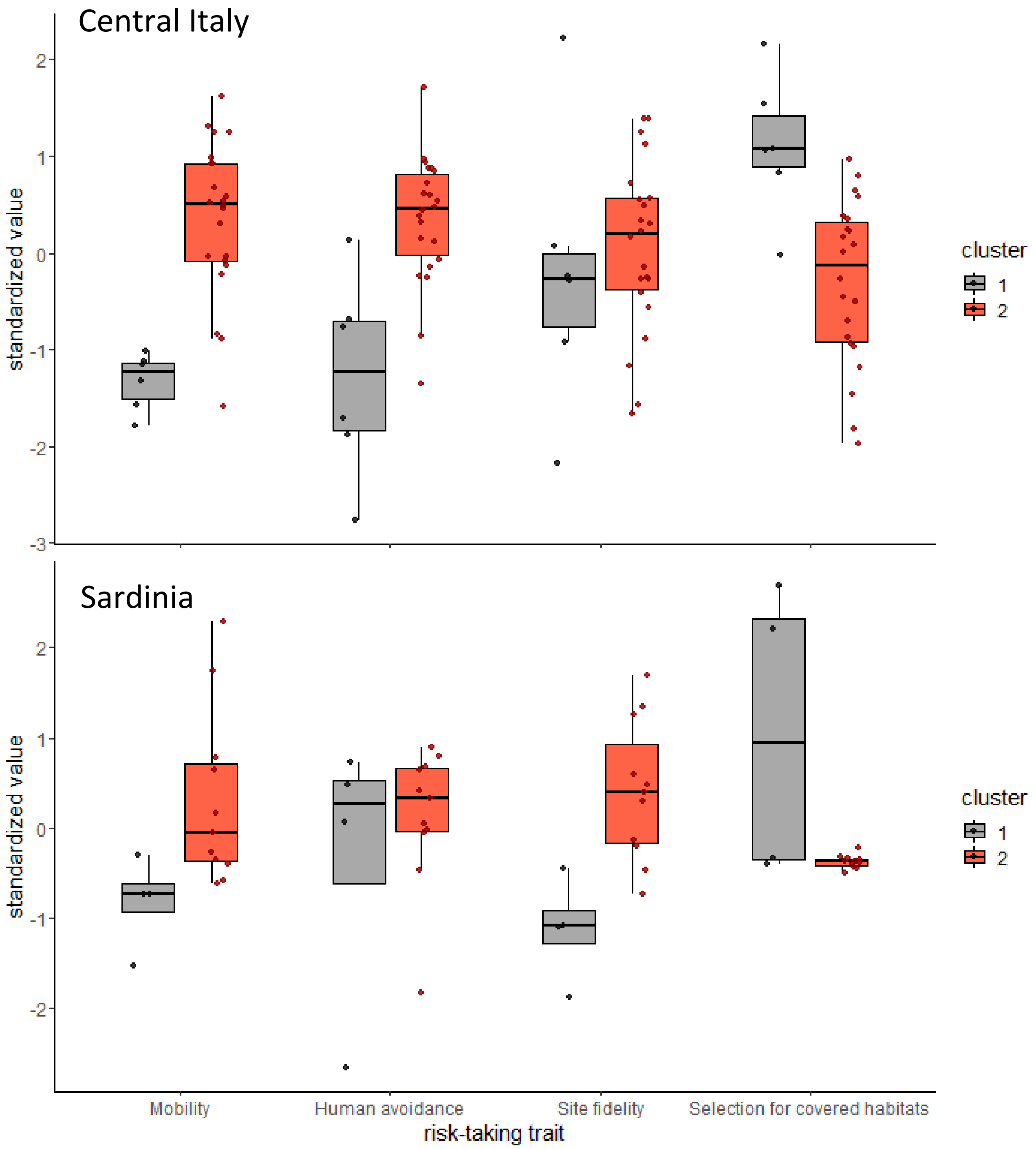

Figure 2. Distribution of individual risk-taking traits across the two identified clusters, for wild boar populations of Central Italy (top) and Sardinia (down). Boxplots show median (bold black line) and quartiles. Points represent individuals (the horizontal noise was added in order to avoid overlaps). Colors of boxplots and points refer to the belonging cluster (1 grey, 2 red).

Discussion

We showed that relocations of free-ranging animals can be successfully used to model their risk-induced habitat selection and to extract individual risk-taking traits. Individuals adopted two alternative multi-trait risk-taking strategies, which were similar within both monitored populations despite they lived within drastically divergent environmental contexts. Nevertheless, these strategies were characterized by compensation, rather than consistency, across single traits.

The extraction of risk-taking traits from a large dataset of animal relocations provided robust behavioral measurements based on a much higher number of observations in respect to those obtained by classical behavioral tests, in both captive (e.g., Vetter et al. 2016, Thys et al. 2017) and wild conditions (e.g., Miranda et al. 2013, Breck et al. 2019). In accordance with our first prediction, all three predictors associated to an actual or perceived risk (Tab. 1) significatively affected wild boar average selection of relocations, both in Central Italy and in Sardinia study areas (Tab. S1 and S2). Our analytical approach indeed revealed SSFs as an effective tool to measure risk-taking traits on free-ranging animals, at both the population and the individual level. We are aware that our sampling approach may have been biased toward bold individuals, which may be more likely to enter baited traps. Nevertheless, the high inter-individual variability observed for all the measured risk-taking traits shows that our sample provided a wide and comprehensive overview of population behavioral diversity. Risk-taking traits of most individuals substantially diverged from the population average. For instance, the majority of individual wild boar avoided human infrastructures both in Central Italy and Sardinia (Fig. 2), while an average preference for human proximity was detected within their respective populations (Tab S1 and S2). This evidence showed that behavioral ecology studies focusing on only the population level can oversimplify, and ultimately mislead, our comprehension of behavioral patterns of wild populations. This seems particularly important for species characterized by a high behavioral diversity, such as wild boar (Keuling et al. 2009, Brogi et al. 2020), suggesting that caution is needed when measuring behavioral responses to perceived risks as a population average in this species (Stillfried et al. 2017).

We detected two alternative multi-trait risk-taking strategies in both the monitored populations, in accordance with our second prediction. Nonetheless, they consisted of compensating, rather than converging, risk-taking traits. For instance, individuals proving to be risk-avoiders on account to their preference for familiar places (high site-fidelity) and for avoiding humans, turned out risk-prone in terms of their low tendency to select safe habitats (low selection for covered habitats) and of their relatively high mobility, facilitating encounters with predators. While this was the strategy exhibited by the majority of wild boar in both populations, the remaining individuals showed an opposite, perfectly symmetrical strategy, which was still characterized by risk compensation across single traits. This evidence was in contrast with our third prediction and to the expectation that the willingness to take risks would have been correlated across different context, as predicted by the behavioral syndromes theory (Sih et al. 2004) and typically reported within studies conducted in controlled experimental conditions (e.g., Van Oers et al. 2003, Thys et al. 2017, and, on wild boar, Vetter et al. 2016). Conversely, we showed that a rigid correlation across multiple risk-taking traits may be not exhibited by free-ranging animals in wild conditions. Our findings are in accordance with those reported by Turner et al. (2020) on free-ranging spotted hyenas, in which the correlated risk-taking behaviors exhibited in experimental conditions were inconsistent with those naturally occurring in proximity of predators. Behavioral responses displayed in experimental conditions against a single, simplified, and artificial source of risk seem thus to be not comparable to those exhibited in wild and uncontrolled conditions, where animals must deal with multiple, articulated, natural sources of risks occurring simultaneously. Indeed, the potential of animals to compensate the risk entailed by different situations is likely to result adaptive in the wild. For instance, wild boar choosing to get close to human infrastructures to take advantage of anthropogenic resources could compensate the risk due to their proximity with their main predator by moving less and selecting covered habitats during active periods, ultimately reducing their detectability. Conversely, if different risk-taking traits were rigidly correlated, pure risk-taker wild boar would have undergone unsustainable costs in terms of survival when attempting to exploit anthropogenic resources.

We obtained similar results from two different populations (Fig. 1 and 2), experiencing extremely divergent environmental conditions, showing that environment played only a minor role, if any, in shaping individual risk-taking strategies. For the same reason, we can exclude that the observed patterns were driven by the peculiarity of a single population. Rather, it is likely that the two detected risk-taking strategies were the most adaptive to balance foraging requirements and mortality avoidance in landscapes with a moderate human presence, although characterized by very different environmental conditions. On the one hand, evolution may have shaped similar risk-taking strategies among the two populations, either by means of a remote event occurred before the evolutive isolation of the two populations or of a converging microevolution. Nevertheless, including the detected strategies inconsistent risk-taking traits, evolutive events would have entailed the need to uncouple genetically correlated behavioral traits (Sih et al. 2004). We can thus speculate that the exhibition of optimal risk-taking strategies by wild boar was more likely the consequence of phenotypic plasticity regulating the expression of underlying genetic determinants of the individual willingness to take risks (Stamps and Groothuis 2010).

We provided a robust and comprehensive evaluation of individual risk-taking traits of free-ranging wild boar, showing that individual risk-taking strategies are characterized by compensation, rather than consistency, across single traits, likely increasing the individual fitness in the wild. Our results contributed in advancing the knowledge of risk-taking behavior of free-ranging animals and particularly of wild boar, with previous studies on this species being limited to captive conditions (Vetter et al. 2016) or focusing on the population average behavior instead of individual strategies (Stillfried et al. 2017). The fact that two, opposite strategies characterized by compensating risk-taking traits coexisted in wild populations has useful implications for the management of a pest species such as wild boar. First, it should be considered that those individuals preferably getting close to human infrastructures reduce their detectability by means of a low mobility and a preference for covered habitats. Managers should particularly focus on these individuals to reduce human-wild boar contacts, by means of specific removing practices. Second, control plans should be diversified when a broad reduction of the population size is required, for instance to reduce the spread of zoonosis (e.g., capturing or culling individuals only in open habitats would likely result ineffective for a substantial part of wild boar populations).

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Chapter 5

Capture effects in wild boar: a multifaceted behavioural investigation

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Although the proliferation of the wild boar in Europe makes capturing and handling necessary for both management and research, the behavioural responses of this species to capture are still unknown. We evaluated how capture affects wild boar behaviour during the first 30 days after the release, focusing on the animals' total activity, mobility and activity rhythms and their variation in response to different drug mixtures used for sedation. Low levels of activity and mobility characterized the first 10 post-capture days. After this period, a gradual restoring of stable levels occurred. Wild boar captured by using different drug mixtures exhibited slightly different patterns of activity depression. We also showed capture to produce a partial effect on wild boar behavioural rhythmicity. Our findings highlight the case study variability of the capture effect and offer useful insights into several conservation and management implications.

Keywords: activity rhythms, chemical immobilisation, spatial behaviour, *Sus scrofa*

The capture of individuals is a key tool for pest species management, both as a direct management option and as a fundamental resource for research on their biology. In order to mitigate the impact on agriculture and ecosystems, it is often useful to remove individuals from the environment: capturing living animals allows for their displacement or confinement in areas where their presence is not in conflict with human activities. This practise is essential when culling is legally or ethically unfeasible, as in the case of many protected areas and in most urban or suburban contexts. Moreover, an efficient pest species management needs continuous updates of information on the species' biology, ecology and behaviour. While non-invasive procedures provide some useful research data (e.g. direct observation for behavioural studies and collection of faecal, hair, feather or carcass samples for molecular investigation), certain pieces of information can only be obtained by capturing and handling animals. This is the case of blood samples, repeated biometric measures, individual marking for identity recognition and the application of tools for biotelemetry studies.

In the last decades, wild boar *Sus scrofa* populations rapidly increased in Europe because of both human manipulation and environmental changes (Apollonio et al. 2010, Massei et al. 2015, Vetter et al. 2015). As this proliferation has caused conflicts with human activities (damages to crops, zoonoses transmission and vehicles collisions) and is a threat for local biodiversity conservation (Massei and Genov 2004), wild boar is considered a pest species in many European countries and the capture of individuals has become an increasingly common practice. Nonetheless, how and how long a capture event can affect wild boar behaviour remains yet unknown. This lack of information results in unpredictable potential disturb effects on behavioural research results when capture is involved.

Capture is probably one of the most stressful episodes which can occur in the life of large mammals (Koch et al. 2017) as it often overturns their behavioural patterns (Chi et al. 1998, Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014) and can even increase their mortality rate (Kock et al. 1987, Beringer et al. 1996, Arnemo et al. 2006, Jacques et al. 2009). Capture-related stress can affect animal behaviour in many ways. A general higher tendency to avoid humans after capture events was observed by

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Chi et al. (1998) in black bear *Ursus americanus*. Similarly, Morellet et al. (2009) found that captured roe deer *Capreolus capreolus* remained further from anthropic structures in the first 10 days after the capture event in comparison with the subsequent 40 days. A sharp reduction of activity and/or movements of the captured individual was also observed (Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014, Brivio et al. 2015). Activity gradually increases back to the normal base-line situation over a period that varies, depending on species and capture methods, from a maximum of 36 days reported for black bear movement rates (Cattet et al. 2008) to a minimum of two days for restoration of normal activity levels of Alpine ibex *Capra ibex* (Brivio et al. 2015). On the other hand, a weak, but still notable, inverse effect has been observed on moose *Alces alces* by Neumann et al. (2011), who found greater spatial displacements for up to 4.5 days after capture.

Only recently, researchers have devoted their attention to investigate the circadian rhythms of wild mammals through a chronobiological approach, one which must include analyses of the periodicity of locomotor activity (Brivio et al. 2016, 2017, Grignolio et al. 2018). This kind of analysis is rare in research on wild fauna, partly because it demands detailed information on wild animals' activity that can only be provided by highly sophisticated technology, such as GPS-collars equipped with accelerometers. On the other hand, this approach would provide the opportunity to examine the potential alterations of behavioural circadian rhythms related to capture-stress, which have never been evaluated in large mammals.

The relation between stress and circadian system, however, has been thoroughly investigated in laboratory rodents. Stress is able to affect the circadian clock and stress responsiveness varies during the day (Koch et al. 2017). For instance, the expression of *Period1* and *Period2*, two cardinal components of the molecular circadian clock network, were found to be affected by acute or chronic stress (Takahashi et al. 2012, Al-Safadi et al. 2015). Animals have evolved to adapt to stress at both a physiological and a behavioural level by the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the release of glucocorticoids. The HPA axis and its hormonal components are under the direct control of the circadian timekeeping system (Oster et al. 2006, Nader et al. 2010). Indeed, glucocorticoids display marked diurnal rhythms, with the highest levels during the active phase, and their response elements are present in the promoter of *Period* genes (Kalsbeek et al. 2012, Dickmeis et al. 2013). Capture is bound to cause acute stress in animals, potentially inducing these modifications in their circadian rhythms.

Capture induced stress is caused mainly by manipulation (i.e. trapping, handling, eventual translocation and releasing). Accordingly, the method implemented to capture wild animals can differently affect the animals' health conditions, their long-term survival probability and their behavioural responses to capture (Kock et al. 1987, Beringer et al. 1996, Brivio et al. 2015). Large mammals can be captured with different methods, such as leg snares, vertical and horizontal dropping nets, net-guns, traps and teleanaesthesia. Although avoiding the use of drugs during the capture prevents any drug side effect, it entails higher injury risk for both animals and operators and an even higher potential stress effect, due to the fact that animals are handled while awake. For example, although roe deer were captured without sedation, they showed depressed activity levels and shifted space and habitat use for up to 10 post-capture days (Morellet et al. 2009). Moreover, in case of larger or potentially aggressive species (such as adult wild boar) the animals' body mass and strength make sedation a necessary choice to prevent risks for operators during handling. On the other hand, anaesthesia may trigger several side effects, including hyperthermia, hypoxemia and heart rate variation (Fahlman et al. 2011). Different in vivo and in vitro investigations showed that anaesthesia also strongly affects the circadian clock by altering the expression of its molecular components and by phase-shifting or disrupting behavioural rhythmicity (reviewed by Poulsen et al. 2018). Interestingly, the impact of anaesthesia on circadian rhythms appears to be stronger when drugs are administered during the animals' active phases and when the selected drug mimics the mechanism involved in the adaptation to photoperiodic variations (Cheeseman et al. 2012, Ludin et al. 2016).

Our aim was to investigate how and how long the protocols generally implemented by managers to capture wild boar can affect its behaviour, focusing on its behavioural circadian rhythms, activity and movements rates. Based on previous research on other species, we predicted that wild boar would exhibit a depression of activity and movements for a period of *n* days after capture and that achievement of stable levels would follow a gradual increase. Secondly, we predicted that different drug mixtures would affect post-capture behavioural patterns differently.

Material and methods

Study area

The study was conducted in two different study areas located in the Casentino valley, in the Tuscan Apennine (Province of Arezzo, central Italy, 43°48′N, 11°49′E, Fig. 1). In both study areas, the climate is temperate-continental, with hot and dry summers and cold and wet winters. The highest mean temperatures are reached in July and the lowest in January. Snowfalls are occasional and usually start in October and may continue through April.

The Oasi Alpe di Catenaia study area (OAC) covers a surface of about 120 km^2 and includes a forested protected area of 27 km^2. Elevation ranges from 300 to 1414 m a.s.l. Seventy-six percent of OAC is composed of mixed deciduous woods, dominated by copses of oaks *Quercus* spp. and chestnuts *Castanea sativa* as well as beeches *Fagus sylvatica* used as high stand; 17% of it consists of open areas and bushes and the remaining 7% of conifer woods (mainly composed of black pine *Pinus nigra* and Douglas fir *Pseudotsuga menziesii*; see Merli et al. (2017) for more details about OAC). The wild boar and the roe deer are the most abundant ungulate species, but red deer *Cervus elaphus* and fallow deer *Dama dama* have also been observed. In the OAC study area, the wild boar is the main prey for wolves *Canis lupus*, while the

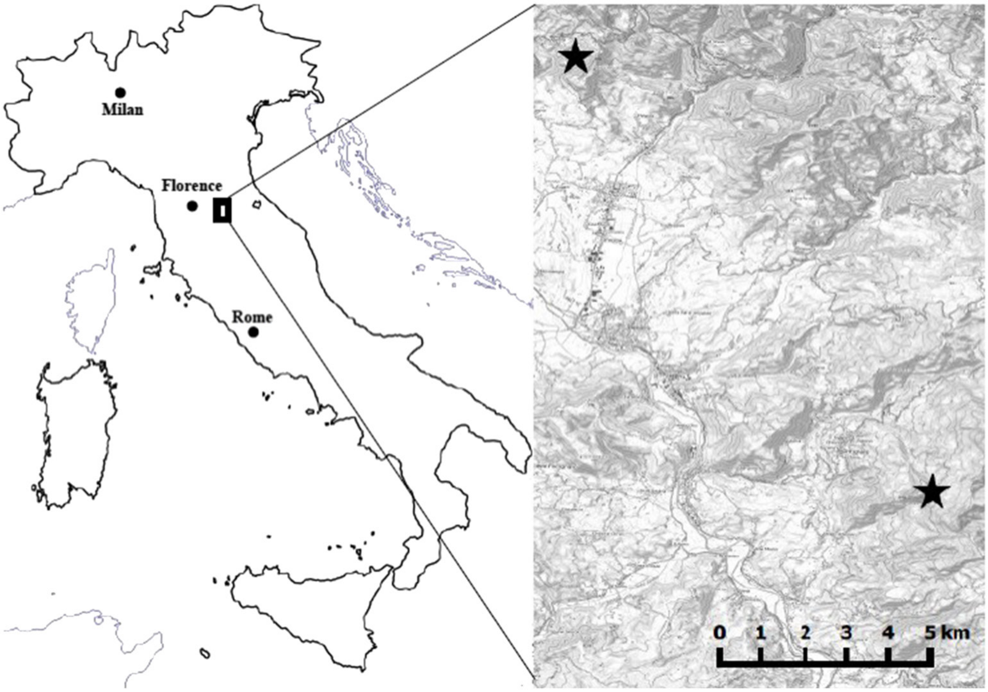

Figure 1. Map of Italy (left) showing the localisation of Casentino valley and an enlargement of the map with both study areas localisation (right). The southern and the northern stars represent the Oasi Alpe di Catenaia and the Foreste Casentinesi National Park study areas, respectively.

red fox *Vulpes vulpes* preys only on piglets (Bassi et al. 2012, Davis et al. 2012).

The second study area lies in the southern part of the Tuscan slope of Foreste Casentinesi National Park (FCNP). About 137 km^2 of its surface (150 km^2) is included in the protected area and elevation ranges from 500 to 1289 m a.s.l. About 85% of landscape is covered by woods (mainly composed by beech, oaks, chestnut, silver fir, *Abies alba*, Douglas fir and black pine), in large part used as high stands, while 15% is occupied by shrubs and pastures. The FCNP study area is inhabited by a rich ungulate community, with high densities of wild boar, red deer, fallow deer and roe deer. As in OAC, in FCNP the wolf preys mainly on wild boar (Mattioli et al. 2011).

In both protected areas any form of hunting is strictly forbidden, while outside wild boar hunting is permitted from about mid-September to the beginning of January (for more details see Grignolio et al. 2011).

Data collection

Wild boar were captured by means of traps baited with maize from June 2013 to January 2017 (Supplementary material Appendix 1 Table A1). Baited traps were set at night only, in order to minimize the physiological stress due to high temperatures during the hot season. Traps were activated at dusk and checked in the early morning to minimize the period of time any captured animal would spend in the trap. Each captured wild boar was first forced into a small cage that strongly limited its movements and then manually sedated. We sedated the captured animals in the early morning: thus, the temporal effect of drugs on their circadian system was minimized (Poulsen et al. 2018), as the wild boar resting period typically starts in the early morning (Brivio et al. 2017). Sedation was performed using a mixture of zolazepam and tiletamine (Zoletil 50 + 50 mg ml^{-1}), either alone or in combination with xylazine (Fournier et al. 1995, Casas-Díaz et al. 2015). At each capture, type and amount of the injected drugs and time of injection were recorded. The operators visually estimated the weight of the captured boar in order to define the dosages to inject. The actual mean of performed injections (i.e. drug dosage /animal body weight estimated by dynamometer) was: 4.00 ± 1.59 mg kg^{-1} of zolazepam–tiletamine mixture, when used alone, and 0.99 ± 0.18 mg kg^{-1} of zolazepam–tiletamine mixture when used together with xylazine (1.70 ± 0.47 mg kg^{-1}). Biometric measures (i.e. body weight, total length, neck and thorax circumference and age, estimated by teeth eruption and consumption) were taken for each individual. Finally, a GPS collar (GPS PRO Light collar) was applied. The handling of each captured animal took about 40 min. All collars were configured to record their GPS position every two hours. Moreover, collars were equipped with activity sensors (i.e. dual-axis accelerometers) so as to measure the acceleration experienced by the collar themselves (within the dynamic range –2G / +2G, with G = gravitational constant). Activity was measured four times/second as the acceleration variation between consecutive values on axis x (forward/backward direction) and y (sideward and rotary direction) independently. Activity data were averaged over a time interval of 4 min and recorded in the collar memory within the relative range between 0 (no activity) and 255 (–2G / +2G), with associated date and time. Only activity measured on x-axis has been analysed, as it was found to be highly correlated with y-axis activity (Heurich et al. 2014, Brivio et al. 2017).

The Regional Hydrological Service provided weather data (mean air temperature, mean air humidity and total rain), hourly recorded in the weather station of Poppi (Arezzo province, 43°44′09″ N, 11°45′42″ E).

Data analysis

Actograms were drawn with Activity Pattern software (ver. 1.3.1, Vectronic Aerospace GmbH). In each actogram the presence of activity rhythm was determined by χ^2 periodogram analysis (ActogramJ 1.0; Schmid et al. 2011). Periodogram analyses were performed on 10-day intervals on the whole actogram. Furthermore, we calculated the daily acrophase (ActogramJ 1.0) and determined the average acrophase on 10-day intervals by using vector addition. We then performed a Rayleigh test to determine whether the acrophases deviated from uniform dispersion around the clock and whether they were concentrated at a given time of the day ($p < 0.05$). A Mardia–Watson–Wheeler test was performed to look for differences among average acrophases of different periods ($p < 0.05$).

Our multivariate analysis focused on two patterns of wild boar behaviour: total activity and mobility, expressed within two variables named activity rate (AR) and mobility rate (MR). To assess whether and how they are affected by environmental and capture-related factors, AR and MR were used as dependent variables in two sets of generalised additive mixed models (GAMMs). Wild boar identity was used as a random factor given the nested nature of data. For each individual, only data (activity and GPS positions) recorded during the first 30 days after their capture were included in the analysis. We ran all analyses in R software (ver. 3.2.2, <www.r-project.org>).

Activity values were first transformed by dividing them by the maximum value recorded by the activity sensor (255), obtaining values varying within the relative range 0–1. Depending on the time when they were recorded, all activity records were assigned to twelve 2-h intervals. Then, an AR value was calculated for each interval for each date, as the arithmetic mean of all activity values included. To improve the models' normality of residuals, AR was arcsine square root-transformed and used as dependent variable.

Only ascertained localisations, recorded with at least four satellites and with dilution of precision (DOP) smaller than 10, were used in our analysis. MR was obtained by dividing the straight-line distance between two consecutive positions (m) by their time interval (h). As collars can fail some positioning attempts, the time interval between consecutive localisations could be greater than 2 h. Nevertheless, we excluded from our analysis all MR records with time intervals greater than 6 h. Finally, MR was natural logarithm-transformed and used as dependent variable in the models.

Following the information-theoretic approach (Dochtermann and Jenkins 2011), we started by building a set of alternative hypotheses explaining the possible relations between dependent and explanatory variables, based on the effect of environmental conditions on wild boar activity assessed by Brivio et al. (2017) and on previous research investigating the effect of capture on other species (Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014, Brivio et al. 2015). Each of the four resulting hypotheses was transformed into a statistical model (Table 1). Each competing model was run and the best one selected following the minimum AIC criterion (Symonds and Moussalli 2011), for AR and MR, respectively. Models with $\Delta AIC<2$ were assumed to be as good as the minimum AIC model. When models had equivalent goodness of fit (Symonds and Moussalli 2011), the simplest one was selected.

In order to account also for not capture-related sources of variation in wild boar behavioural patterns, we used variables that were known to shape this species' behaviour. Considering wild boar activity variation patterns observed by Brivio et al. (2017) on both seasonal and daily scales, the Julian date and the time of day were included as continuous predictor variables in the models. In the same study, a significant relation between activity and weather conditions was observed. Thus, we added mean air temperature (°C), mean air humidity (%) and rain precipitation (mm) as continuous variables in the models (mean values of temperature and humidity were calculated for each activity and mobility value, averaging all records within the corresponding time interval, while total rain precipitation values were obtained from the sum of all records found in the same interval). To investigate any detectable effect that a capture event could have had on wild boar behaviour, we added the time elapsed since the capture event (hours) as predictor variable in the models. The kind of drug used to sedate each individual (zolazepam–tiletamine versus zolazepam–tiletamine–xylazine mixture) was included within the interaction term with the time after the capture, as any drug effect was supposed to be related to the time elapsed since the drug was injected. Finally, the study area was used as a categorical variable in order to detect possible behavioural

Table 1. Set of alternative hypotheses predicting the variation of activity rate and mobility rate of wild boar in the Alpe di Catenaia and Foreste Casentinesi National Park (Tuscany, Italy).

No.	Model	Hypothesis	Supporting evidence
1	Base	Wild boar behaviour was only affected by seasonal and daily cycles and by weather conditions as temperature and rain precipitation, without any capture-related effects.	Brivio et al. 2017
2	Capture effect	In addition to day of the year, time of day and weather, wild boar activity and movements were affected by the capture event.	Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014, Brivio et al. 2015.
3	Capture and drug effect	Same as hypothesis no. 2, but with capture effect varying according to the kind of drug used to sedate the wild boars.	Cattet et al. 2003
4	Study area effect	Similar to hypothesis no. 1, but with wild boar behaviour markedly differing between individuals from different environmental conditions of the two study areas.	Brivio et al. 2017

differences between wild boar captured in different locations (OAC and FCNP).

To avoid collinearity, we checked for possible correlations between continuous predictor variables, calculating Pearson correlation coefficient within all possible predictor variables pairs (Zuur et al. 2009). We found a not negligible correlation only between mean air temperature and mean air humidity ($r = -0.7$). A random forest calculation (R package 'randomForest') showed that mean air temperature was the best predictor of variation for both AR and MR, therefore mean air humidity was dropped from the predictor variables sets.

Results

We captured and monitored six wild boar (four females and two males) in OAC and 12 (six females and six males) in the FCNP (Supplementary material Appendix 1 Table A1). We excluded two males (no. 8319 and no. 8749) of FCNP from the activity rhythms analysis and one male (no. 12288.2c) of OAC from the movement analysis, because of data failure. We thus used the data related to 16 wild boar for daily activity rhythms analysis and AR analysis, and data on 17 wild boar for the models fitting MR. We recorded an average of 354.88 ± 16.79 AR/wild boar and of 284.06 ± 89.58 MR/wild boar.

Daily activity rhythms

Capture did not alter the daily activity rhythm of most of the wild boar investigated: 10 out of 16, five males and five females (Fig. 2a–b, Supplementary material Appendix 1 Fig. A1a–h). Both males and females of this unaffected group showed a unimodal and nocturnal activity pattern synchronised to the onset of civil dusk. The mean daily acrophase occurred between 21:16 and 23:12 (Fig. 2a–b, Supplementary material Appendix 1 Fig. A1a–h; Rayleigh test, $p < 0.0001$) and did not significantly change throughout the period investigated (Mardia–Watson–Wheeler test; $p > 0.05$).

In six out of 16 wild boar, capture had a marked effect on activity rhythms. After the release, two wild boar (females: no. 16597 and no. 16599; Fig. 2c, Supplementary material Appendix 1 Fig. A1i) showed a diurnal pattern with acrophase in the late afternoon (between 15:00 and 17:00). In contrast, a male (no. 16603, Fig. 2d) showed an inversion of activity pattern from crepuscular to diurnal and the mean acrophase changed from 19:20 during twilight to 05:30–07:00 during diurnal activities (Mardia–Watson–Wheeler test, $p < 0.00001$). The inversion of activity pattern was also found in a female (no. 12292c, Supplementary material Appendix 1 Fig. A1j), though for a short period of four days only. Capture had a marked effect on the daily activity rhythms of two wild boar (females: no. 12290c and no. 16602; Fig. 2e–f). In one individual (no. 12290c; Fig. 2e), we observed arrhythmia in daily activity for a period of about a week. Subsequently, this wild boar showed a daily rhythm with a clear nocturnal activity with acrophase about at 21:00. Another female (no. 16602, Fig. 2f) showed a similar response to capture: during the subsequent three days, her activity was considerably reduced and spread across the 24 h. After these initial alterations, all wild boar showed a nocturnal pattern of activity with a peak during the early hours of the night.

Activity rate

The best model explaining the variation of AR included Julian date, time of day, mean air temperature, total rain precipitation and the interaction term between time after capture and drug type (i.e. model no. 3; R^2 (adj) = 0.423; Table 2A). AR did not show a significant relation with Julian date, while its daily pattern highlighted the importance of the predictor variable time of day, clearly showing the preference of wild boar for nocturnal activity (Supplementary material Appendix 1 Fig. A2a). Both air temperature and rain precipitation affected wild boar activity (Supplementary material Appendix 1 Fig. A2b–c), the first with a non-linear and unclear pattern, the latter with a positive relation with AR reaching a plateau with values of about 5 mm of rain precipitation, but with wide confidence intervals (especially with high precipitation values, Supplementary material Appendix 1 Fig. A2c). Results showed that the interaction between the time elapsed from the capture and the drug mixture treatment significantly contributed to explain the activity pattern of the captured individuals. Wild boar exhibited lowest AR values immediately after their capture and gradually increased their activity until the reaching of a plateau, about 10 days after their capture, with both kinds of drug mixture (Fig. 3). Results suggested that this reduction of activity was slightly more pronounced in the wild boar sedated with a mixture of zolazepam–tiletamine and xylazine compared with individuals treated with zolazepam–tiletamine only. Nonetheless, the estimated activity patterns for both sets of individuals had either partly or completely overlapping confidence intervals. Finally, wild boar sedated with zolazepam–tiletamine–xylazine had a more irregular activity pattern. The weak effect of the drug mixture caused a relatively low difference of R^2-values between the first and the second ranked models (Table 2). Moreover, as the time elapsed from the capture only influenced wild boar's behavioural patterns during 10 days out of 30, there was little difference in R^2 between the best models and the alternative models including or excluding this variable.

Mobility rate

The best model explaining the variation of MR included Julian date, time of day, mean air temperature, total rain precipitation and time after capture (model no. 2; R^2 (adj) = 0.307, Table 2). Julian date affected wild boar movements with a weakly significant relation and a non-linear pattern; a higher MR was observed around the 270th day of the year (Supplementary material Appendix 1 Fig. A3a). The effect of the time of day was very similar to that for AR, with wild boar moving longer distances at night (Supplementary material Appendix 1 Fig. A3b). The relation between mean air temperature and MR was almost steady for temperatures below 25°C but became positive when temperatures exceeded this threshold (Supplementary

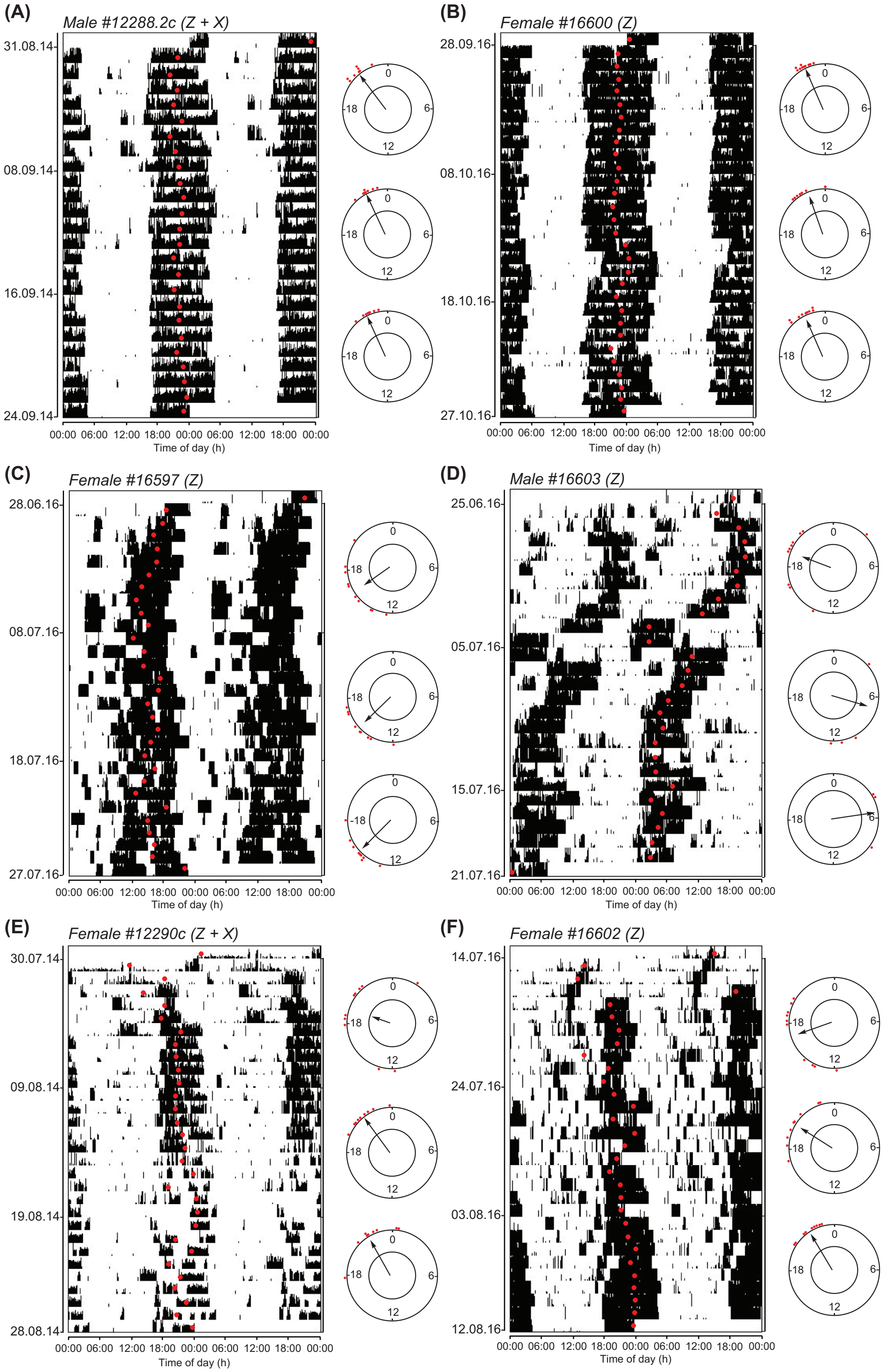

Figure 2. Representative actograms of daily activity of 6 radio-collared wild boar. Records are double plotted on a 48-h time scale to help the interpretation. Red dots on the actograms mark daily acrophases. On the right-hand of the actograms, circular diagrams showing acrophases for 10-day intervals are plotted. Dots represent daily acrophases and arrows indicate the average acrophases represented as vector. The circle inside each panel represents critical values of Rayleigh test ($p < 0.05$). Z: wild boar sedated with zolazepam–tiletamine; Z + X: wild boar sedated with zolazepam–tiletamine–xylazine.

Table 2. Generalised additive mixed models predicting the activity (A) and movement (B) rates after capture in wild boar in the Oasi Alpe di Catenaia and Foreste Casentinesi National Park (Italy).

No. model	Model structure	AIC	Δ AIC	R^2
(A)				
3	AR~ Julian date + time of the day + temperature + precipitation + time since release × drug	–1426.2	0	0.423
2	AR~ Julian date + time of the day + temperature + precipitation + time since release	–1421.6	4.6	0.422
4	AR~ Julian date + time of the day + temperature + precipitation + study area	–1344.5	81.7	0.413
1	AR~ Julian date + time of the day + temperature + precipitation	–1344.5	81.7	0.413
(B)				
2	MR~ Julian date + time of the day + temperature + precipitation + time since release	18553.5	0	0.307
3	MR~ Julian date + time of the day + temperature + precipitation + time since release × drug	18559.0	5.5	0.307
4	MR~ Julian date + time of the day + temperature + precipitation + study area	18610.7	57.1	0.299
1	MR~ Julian date + time of the day + temperature + precipitation	18610.7	57.1	0.299

The best model was selected with the minimum AIC criterion [AIC = Akaike information criterion; ΔAIC = difference in AIC value between the AIC of a given model and the best model (with the lowest AIC); AR = activity rate; MR = mobility rate].

material Appendix 1 Fig. A3c). Wild boar movements increased together with total rain precipitation, showing a clear, although weak, positive pattern (Supplementary material Appendix 1 Fig. A3d). Finally, wild boar were found to cover short distances immediately after their capture and then they gradually increased their mobility until achieving a stable situation around 10 days after their capture, in accordance with the pattern found for AR (Fig. 4). The different mixture of drugs used for sedation was not included in the best model selected.

Discussion

Immediately after capture, wild boar showed low values of AR and MR. AR and MR highly increased during the first 10 post-capture days and then reached stable values. Capture was also found to produce a partial and variable effect on wild boar activity rhythms periodicity, affecting only some individuals of the study group.

More specifically, the analysis of daily activity rhythms showed a potential effect of capture and anaesthesia on wild boar periodicity: in six out of 16 wild boar, we observed locomotor arrhythmicity or inversion of activity pattern from nocturnal to diurnal, considering a unimodal and nocturnal activity pattern as the standard baseline condition in our study area (Brivio et al. 2017). Different investigations in invertebrates and vertebrates, including humans, clearly demonstrate that general anaesthesia disrupts or alters behavioural circadian rhythms (Dijk and Lockley 2002, Chassard et al. 2007, Poulsen et al. 2018). In this respect, marked differences related to the time of drugs administration were found. For instance, general anaesthesia during the active phase highly altered daily activity rhythms (Mihara et al. 2012, Anzai et al. 2013). Both honeybees and rats treated with isoflurane or ketamine, two general anaesthetics commonly used, showed a phase-shifts in the locomotor activity if the treatments were applied during the daytime (Cheeseman et al. 2012, Ludin et al. 2016). Conversely, administration of anaesthesia during the resting period appeared to have minor effects on activity rhythms (Prudian et al. 1997, Mihara et al. 2012). It is worth noting that different anaesthetic drugs and different durations of the anaesthetic treatment may induce diverse species-specific reactions. Although the drug mixture was administered when wild boar typically start their resting period (i.e., in the early morning), the changes in the circadian behaviour observed in this study provide a piece of evidence in the complex puzzle of how anaesthetics can affect the circadian timekeeping system in large wild mammals.

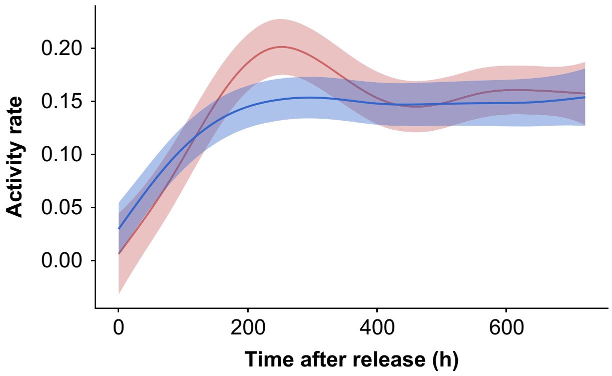

Figure 3. Effect of the interaction between time after release (hours) and the drug mixture on wild boar activity rate after capture. The values of activity rate reported were predicted by the best generalised additive mixed model (see the text for more details). Wild boar treated with zolazepam–tiletamine only and with the mixture of zolazepam–tiletamine and xylazine are represented by the blue and by the red line, respectively. Estimated standard errors are represented by the colour-shaded areas.

An alternative explanation for the behavioural pattern observed after capture and anaesthesia is a direct effect of stress on the regulation of circadian clocks. At the best of our knowledge, this has been observed in rodents, under controlled laboratory conditions only (Koch et al. 2017). Ours is one of the first findings on how stress can affect the circadian clocks in free-ranging large mammals. Since the affected wild boar were few (n = 7), we were not able to detect any clear effect of age, sex, study area, drug mixture used or season of capture. Nevertheless, our results remark the strong potential stress effect of a capture event on animal behaviour, as it may affect both pattern (arrhythmia) and phase (inversion) of the activity rhythms, therefore influencing both internal and environmental-related aspects of activity rhythms. Cortisol concentration significantly increases in wild boar after stressful situations (Morton et al. 1995, Gentsch et al. 2018) and this endogenous signal could alter the circadian timekeeping system (Kalsbeek et al. 2012, Dickmeis et al.

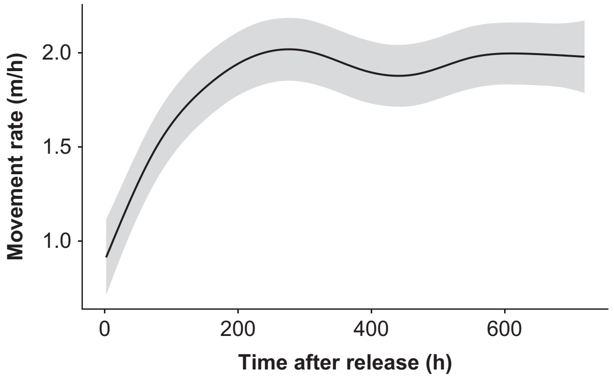

Figure 4. Effect of the time after release (hours) on wild boar movement rate. The values of movement rate reported were predicted by the best generalised additive mixed model (see the text for more details). Estimated standard errors are represented by the colour-shaded areas.

2013). Brivio and colleagues (2017) also observed an inversion of activity patterns in female wild boar in the same study area, occurring likely during the weaning period. Thus, we can conjecture that the wild boar may switch its activity periodicity when under stress.

Our results are in accordance with our first prediction: wild boar exhibited low activity and movement rates after the capture and the achievement of stable AR and MR values followed a gradually increasing pattern that lasted about 10 days, consistently with the range observed in other species (Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014, Brivio et al. 2015). In particular, the reduction of activity in wild boar lasted longer than in Alpine ibex (Brivio et al. 2015), but it was relatively short if compared with the 36 days of perturbation observed in black bears' movements (Cattet et al. 2008), hence confirming the high heterogeneity of results obtained by different studies on different species. So far, studies evaluating the capture effect on mammals are still few, making the comparison of results unfeasible. For the same reason, it is difficult to attribute the variability of results in the available studies to systematic, environmental or drug-related factors. Thus, our aim in this study was to understand wild boar reactions to capture in different study areas and to two different drug mixtures. Eventually, though, AR and MR best models did not include the study area, probably because of the fair similarity between OAC and FCNP. Anyhow, any slight difference in environmental factors characterising the two study areas would have had an attenuated effect, as we already took into account environmental factors variability by including some climatic variables (air temperature, humidity and precipitation) in our models.

As to the effect of sedation, we found a weakly significant different effect of the two drug mixtures only on wild boar activity, while the different drug mixtures used similarly affected MR. AR and MR reached the stable values after the same amount of post-capture hours with either one of the drug mixtures. The wild boar sedated with a mixture of zolazepam–tiletamine–xylazine showed a slightly more irregular AR pattern compared with individuals treated with zolazepam–tiletamine only. This weak effect can be due to the presence of xylazine in the mixture administered, and/or to the potential synergistic effect potentially triggered by its association with zolazepam–tiletamine. Nevertheless, the addiction of xylazine did not affect the total duration of the period required to restore stable activity levels. In the light of this, we can speculate that the length of the restoration period was likely driven by the overall stress caused by the capture and/or by the administration of zolazepam–tiletamine, as the latter was used for all individuals.

Nowadays, wildlife managers and researchers encounter a wide variety of circumstances in which the capture of animals is required. Whichever the purpose for the capture, the lightest and shortest capture effects would be desirable for ethical, conservationist and management reasons. Animals' welfare is a fundamental issue in wildlife research and management, but capture events can threaten it both directly and indirectly: capture can induce mortality (Kock et al. 1987, Beringer et al. 1996, Arnemo et al. 2006, Jacques et al. 2009) and cause a decrease in activity and mobility (Cattet et al. 2008, Morellet et al. 2009, Northrup et al. 2014, Brivio et al. 2015, this study), thus increasing the risk to be predated or involved in collisions with vehicles. Moreover, stronger capture effects result in significant distortions of the data acquired within a research project. The comparison of our results with those of other studies shows a remarkable heterogeneity in capture effect duration, which can arise from systematic, environmental and method-related factors. Further accurate investigations on the role of the method used for capturing, handling and releasing wild animals could permit to establish standard field protocols with minimum stress effects. Further studies should therefore focus on methodological aspects such as capture method, time spent in the trap, time of total handling, number of operators and kind and dosage of the drugs administered.

In conclusion, any capture event that includes chemical immobilization is likely followed by behavioural alterations of not negligible duration and the most evident effects are exhibited in the first hours after the release. Here, we showed that in wild boar this alteration consists, at the least, in a partial periodicity modification and in a depression of activity and mobility rates for a long period. Since captured individuals are not fully alert when handling is concluded, they should be released in places that are free from risks. This surely includes high traffic roads, but also lakes, streams and gorges as well. Moreover, the presence of predators is likely to affect released wild boar survival rate. While dangerous human or geographical elements may be avoided by simply displacing the releasing site, though, the stable presence of a predator would be more difficult to elude. Finally, since the addition of xylazine to a tiletamine/zolazepam protocol did not affect the long-term behavioural alteration time, its use needs to be considered a strictly veterinary issue, not providing any clear biological advantage or disadvantage. Hence, carefully evaluating drug combination and dosages for sedation appears to be a useful strategy to minimize capture effects. In this context, as the individual's state of stress at the moment of the drug injection presumably affects its response to anaesthesia, capture-handling protocols should be designed to reduce stress even before the starting of handling (i.e. when animals are still awake). Reduced initial state of stress could thus permit lighter dosages with still adequate anaesthetization and safe manipulation, which, in turn, will likely produce lighter long-term stress effects as well.

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Conflict of interest – The authors declare that they have no conflict of interest.

Ethical standards/permits – This study complies with all national and regional laws dealing with ethics and animal welfare. Capture and manipulation protocols were approved by Tuscany Regional Administration (no. 103/5936/152 – 13/03/2002). The research adhered to the ASAB/ABS Guidelines for the Use of Animals in Research.

Author contributions – SG and FB originally formulated the idea. RB, SL, NCa, EB, SG and MA implemented the animal captures. NCa and EB were the vets responsible in FCNP and OAC capture sections, respectively. MA and CP contributed materials/analysis tools. RB and SL conducted fieldwork. FB, SG and CB collaborated in imaging analysis. RB, FB, SG, CB and MB performed statistical analyses. RB, SG, CB and FB wrote the manuscript. MA and NCo provided editorial advices.

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Supplementary material (available online as Appendix wlb-00497 at <www.wildlifebiology.org/appendix/wlb-00497>). Appendix 1.

DISCUSSION AND CONCLUSIONS

My results shed light on several aspects of wild boar reproductive and behavioral ecology with a proper consideration of its temporal dimension, opening interesting perspectives for a more effective management of this species. Chapter 1 showed that adult males traded their food intake for mating opportunities, predominantly fueling their reproductive effort with resources previously acquired and stored in body reserves. Taking part in reproduction seemed a priority also for female wild boar, as highlighted by the results of Chapter 2: resources availability influenced their reproductive timing and synchrony, but all mature females reproduced every year regardless of the environmental harshness. Chapter 3 suggested that food resources, not hunting avoidance, drove temporal patterns of protected areas use, demonstrating that the common belief of reserve effect concerning all wild boar populations should be taken with caution. In Chapter 4 I showed that wild boar dealt with the risk avoidance - food intake trade-off by means of two main risk-taking strategies, but they were characterized by compensation, instead of consistency, among different traits. Finally, the research performed in Chapter 5 showed that, after being captured and released, wild boar gradually recovered their movement rates as soon as they did with activity, progressively exposing themselves to the risk to encounter predators.

Despite food resources were particularly abundant in autumn (see the data reported in Appendix for the magnitude of the seed biomass produced during the mast), adult males lost body weight during the reproductive season. Such phenomenon was likely driven by a feeding reduction aimed at allocating more time to their reproduction effort. The adoption of a capital breeding strategy likely made the reproductive outputs of adult males highly resilient against environmental perturbations (Stephens et al. 2009, Kerby & Post 2013). In this context, their effectiveness in fertilizing females should not be negatively affected by harshness conditions experienced during the rut, as adult males can exploit the whole pre-reproductive period to acquire resources to support their reproductive effort. Moreover, I showed that subadult males renounced to gain body weight during the most food-abundance period (coinciding with the rut), likely sustaining an income breeding strategy in an attempt to achieve an immediate reproductive success. This evidence is in accordance with the hypothesis of subadult male wild boar taking part in reproduction previously suggested by Šprem et al. (2011) and further strengthen the resilience of male reproductive effectiveness, as the lack of adult males would be compensated by more subadult males taking part in reproduction. By a management point of view, this means that an adult male-skewed harvest producing adult male scarcity (the typical outcome of wild boar drive hunting, Fernandez-Llario & Mateos-Quesada 2003, Toïgo et al. 2008) do not substantially undermine the reproductive potential of wild boar populations and it can neither limit their growth. Despite female breeding strategy could not be directly investigated in the research of Chapter 1, female body weight gain during the rut was a signal of an autumnal energy storing, likely foreshadowing a subsequent reproductive investment of it. Indeed, it concerned also fully developed adult females, highlighting that it could not represent a growth investment. Female wild boar were thus capital breeders just as adult males, although they differed in the timing of their reproductive investment (autumnal rut for males; pregnancy, birth, and weaning during winter and spring for females) and thus in the timing of their reproductive resource acquisition (pre-rut for males, i.e., spring and summer; the period between weaning and the pregnancy of subsequent year for females, i.e., autumn). Autumnal food resources thus potentially represent a fundamental driver of female wild boar reproductive patterns. Chapter 2 substantially contributed to disentangle the real effect of these and other environmental factors on female reproductive outputs. Indeed, in Chapter 2 I showed that environmental conditions (mainly spring temperature and autumnal mast seed) strongly influenced the reproductive timing and synchrony of wild boar populations, without really affecting the ratio of reproductive females. The potential to plasticly regulate their reproductive timing allowed to almost all subadult and adult females to reproduce even in bad years (cold spring, low mast seed production), by delaying ovulation and pregnancies until sufficient body conditions were achieved. This evidence can substantially modify the interpretation of autumnal harvest data for management planning: years with low ratios of reproductive females (those carrying signs of ongoing ovulation or pregnancy) are not necessarily years with low reproductive output (as they were previously interpreted, e.g., Fonseca et al. 2011, Bergqvist et al. 2018, Touzot et al. 2020), but simply years with a delayed breeding season. Prediction may eventually be made on offspring survival, which is known to be lower after late and asynchronous reproductive seasons in other species (Côté & Festa-Bianchet 2001), despite further researches are needed to confirm that such effect occurs in wild boar populations as well. In accordance to results of Chapter 1, the average number of available adult males per female did not affect the likelihood of females to ovulate or get pregnant, remarking the male reproductive resilience against environment and a male-biased harvest. The potential of females to plasticly displace the breeding season timing and to adjust its duration provided a maximized reproductive output under optimal conditions but also made it more resilient against ecological perturbations and environmental changes, if compared to species with a reproductive timing which is more rigidly constrained by photoperiodism. In the context of the global climate change, this skill is likely to prevent temporal mismatches between birth seasons and resources optimum for offspring, and thus represents a characteristic making wild boar "preadapted" to global change (Vetter et al. 2015, Touzot et al. 2020).

Further than playing a major role for wild boar reproductive ecology (Chapters 1 and 2), the autumnal mast seed strongly affected its spatial movements in autumn. Results included within Chapter 3 indeed showed that resources availability, not hunting avoidance, drove temporal patterns of the use of large size protected areas by wild boar. Thus, despite protected areas are often blamed for offering refuge to pest species, and to wild boar as well (Tolon et al. 2009), I demonstrated that the potential occurrence of reserve effect needs to be verified on a local scale. A first evaluation of the overall proportion of time spent inside and outside protected areas showed that only a given number of individuals actually used both protected and unprotected lands, while others remained on protected lands all-year round. This effect is likely to be important especially within protected areas with considerable size (i.e., offering a sufficient amount of resource inside their borders to fully sustain at least part of the population). In case of management plans aimed at reducing wild boar damages on agricultural lands which are close to large protected areas, removing individuals inside the latter seems to be unjustified a priori. A part of the effort spent for such operations would indeed be used for culling or capturing individuals which did not use the unprotected agricultural lands, and the harvesting operation may affect other no-target species (Grignolio et al. 2011). Removing individuals directly from the agricultural landscapes where damages occur would result much more efficient. The same may be said in cases of protected areas near to urban settlements, roads, and any other circumstances hosting human-wild boar conflicts. This management implication is in accordance with a previous study showing the importance to selectively remove only those individuals really responsible for the damage when managing wild ungulate populations (Honda & Iijima 2016). Moreover, I showed that even those wild boar moving across the large protected area borders did not use it as a refuge to escape hunting disturb. This result is in apparent contrast with those reported by Tolon et al. (2009), but it is worth noting that only a methodological approach based on high-resolution spatial data and on the knowledge of the local activity habits, like that used within Chapter 3, could have allowed to correctly interpret the autumnal increased use of the protected area by wild boar as driven by food resources distribution rather than by hunting avoidance.

Besides providing valuable information on the use of protected areas in relation to the hunting disturb, high-quality spatial data on animal movements may also be used to investigate the behavioral strategies exhibited by wild boar to cope with the human-induced risk of mortality. Analyses reported within Chapter 4 demonstrated that tracking animal movements on a very fine temporal scale can provide robust measurements of the individual willingness to take risks across different contexts. The detected risk-induced resources selection revealed that wild boar were actually subdivided in groups sharing homogeneous risk-taking strategies, but these were constituted by compensating risk-taking traits. Individuals did not adopt extreme strategies in terms of the willingness to take risks, but rather balanced risk-prone behaviors in specific contexts (e.g., proximity with humans and low selection of familiar habitats) by exhibiting prudent behaviors in others (e.g., selecting covered habitats and moving short distances). These strategies likely allowed an optimal foraging, especially on anthropogenic resources, while ensuring a sufficient short-term survival. Moreover, results reported in this chapter provide a number of specific implications for the management of this species. First, wild boar which are in close contact with humans seems the most difficult to manage, on account on their low mobility and preference for covered habitats even during active periods. Since ordinary control plans are thus likely to prove inefficient in removing these individuals, specifically designed management practices are required to substantially reduce human-wild boar contacts. This may be the case of capture and culling plans preferably performed as close as possible to human infrastructures and covered habitats. Second, if a substantial reduction of wild boar numbers on a large spatial scale is required (but see below the feasibility limits of such an approach), managers should apply highly differentiated control methods. For instance, capturing or culling individuals only in open habitats would likely result ineffective for whole parts of wild boar populations. Conversely, by means of differentiated control methods, they would have the chance to remove all individuals, irrespectively of the risk-taking strategy they adopted.

An effective wild boar management may thus require integrating culling activities with the capture of alive individuals, and high-resolution spatial data can also profitably used to investigate the behavioral reaction to this management practice. Analyses reported within the Chapter 5 showed that wild boar (commonly believed as a robust animal, unresponsive to handling and manipulation) is sensitive to the capture-induced stress, which reduced both activity and mobility with almost identical temporal patterns. After the release, individuals initially allocated more time to rest but then progressively recovered their movement patterns as soon as they did with activity, implying that they did not trade the optimal foraging for predation avoidance. This behavior may expose released wild boar to eventual encounters with predators during a period in which they are still recovering the full ability to flee. Moreover, the evidence of reduced activity and mobility lasting up to ten days calls attention on the capture and release protocols, which should be designed to minimize capture-induced stress, alterations of behavior, and risks of injuries for released individuals. First, time spent into the traps by animals and that of their manipulation by humans should be reduced as much as possible. Second, the drug mixture should be chosen carefully (despite we did not detect substantial differences in the post-release behavior when zolazepam-tiletamine or zolazepam-tiletamine-xylazine were used) and administered with the minimum possible dosage. Third, release should be performed in safe places, preventing stressed, movement-inhibited wild boar to face dangerous environmental elements (e.g., roads, streams or lakes, gorges) and to meet people. An encounter with a wild boar enabled to flee would indeed represent a serious threat to human safety.

A comprehensive interpretation of the set of evidence included within the different chapters can provide a synthetic overview of the trade-offs among resources acquisition, survival, and reproduction that wild boar face during their life. Chapters 1 and 2 showed that the opportunity to take part in reproduction overruled both the current resources acquisition and the medium-term survival, remarking that wild boar tend to maximize the immediate fitness outcome rather than investing in the long-term reproductive success. Since the reproductive effort must be sustained with energy reserves, food resources acquisition seems in turn to prevail over the avoidance of risks for the individual survival, as suggested by the results of Chapters 3 and 5. Consistently, the compensation of risks taken over different contexts (Chapter 4) likely optimize the intake of resources while allowing a sufficient short-term survival. Despite apparently maladaptive, the relatively low importance given to the medium-term survival is in fact consistent with the high investment for a short-term reproductive outcome. This synthetic interpretation of wild boar ecological trade-offs provides an explanation for the overall deficiency of culling plans effectiveness. Counting on an early, immediate, and maximized reproductive success, wild boar populations are somehow adapted to undergo high mortality rates as is typical for r-strategist. While they may still be effective in particular contexts (e.g., for the removal of specific individuals, as explained above), culling plans aimed at reducing the overall density over a large spatial scale would likely result inefficient. Significant effects may eventually be achieved only by means of a huge removal effort, which is typically hard to implement in the context of a generalized reduction in the number of hunters (Massei et al. 2015).

In conclusion, the proper consideration of the temporal dimension when studying wild boar ecology substantially enlarged the available information on this species. This approach advanced the knowledge of several aspects of wild boar reproductive and behavioral ecology, but at the same time achieved an overview of its ecological trade-offs over a wide spectrum of situations. The results included in this thesis provided several implications to improve the reliability of wild boar management planning and the effectiveness of the existing practices, on both a large and a small scale.

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Non a tutti ma a ciascuno di voi dedico la mia più sincera gratitudine.

Appendix

Evaluating sampling schemes for quantifying seed production in beech (*Fagus sylvatica*) forests using ground quadrats

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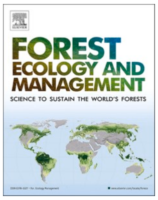

Evaluating sampling schemes for quantifying seed production in beech (*Fagus sylvatica*) forests using ground quadrats

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ABSTRACT

Accurate estimates of seed production are central for understanding mast seeding mechanisms at tree and forest scales, and for designing sustainable management strategies. As trees are long-lived organisms, a long-term perspective is required to understand how reproduction acts during the life cycle of a tree. However, long-term series of seed production are challenging to obtain, as the available seed count procedures strictly rely on field methods, which are cost- and time-consuming, inherently limiting their widespread use at extensive spatial and temporal scales.

In this study, we proposed a simple, rapid and flexible field method based on counting the seed in mobile ground quadrats (GQ), which was tested in beech forests. Quadrat measurements were first validated against reference measurements obtained from litter traps (LT) in three permanent plots. Results indicated that GQ provides robust and reliable estimates of seeds, which are not affected by seed predation occurring at the forest floor.

Additional quadrat measurements were performed to evaluate the influence of sampling schemes (random, regular, systematic) on the estimation of mean seed production at the plot scale. One hundred quadrats were collected in 0.25 ha beech plots and considered as a reference for evaluating the different sampling schemes and sampling sizes. Measurements were performed in October (three plots), which represented the peak of seed fall, and November (two plots). Results indicate that about 25 randomly located measurements allowed to characterize plot-level mean seed production with an acceptable error below 20%, regardless of the different mean seed production observed between the studied plots and the sampling periods. If the 25 sampling points are arranged in a grid, the obtained mean estimates are within the confidence interval of the reference plot-level values.

1. Introduction

Mast seeding, also known as masting, is the synchronous intermittent production of large seed crops in populations of perennial plants (Kelly, 1994). This reproductive strategy, which is typical of many anemophilous tree species or those dispersed by food-hoarding animals (Bogdziewicz et al., 2018a), has cascading effects on overall ecosystem functioning. Indeed, masting affects plant regeneration (Cutini et al., 2015), tree species composition (Lichti et al., 2014; Yang et al., 2019), and reverberates across trophic levels, driving population dynamics from primary resource consumers (Bisi et al., 2018; Cutini et al., 2013) to top predators (Masaki et al., 2020), also including those carrying human infectious diseases (Bogdziewicz and Szymkowiak, 2016; Rubel and Brugger, 2021). Therefore, the understanding of masting patterns has relevant management implications (Ascoli et al., 2020; Azad et al., 2017; Cutini et al., 2015; Wagner et al., 2010).

The scientific interest related to mast seeding has proliferated in recent years. A literature search was conducted in SCOPUS using the keyword combination KEY (“mast seeding” OR “masting” OR “tree mast”) for the period 1990–2020. Results indicate that over the last thirty years, a total of 651 documents have been published (Fig. 1). The number of items (*Nd*) significantly increased through the years N_y

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($N_d = 1.6 \times N_y - 4.5, R^2 = 0.86, n = 30$) and the number of citations N_c followed the same positive, significant trend ($N_c = 69.0 \times N_y - 451.1, R^2 = 0.90, n = 30$).

Notwithstanding the high scientific, environmental and societal relevance of masting, the knowledge about the mechanisms driving seed production in masting tree species is still incomplete (Pearse et al., 2016; Bogdziewicz et al., 2018b, 2020). As trees are long-lived organisms, a main challenge in studying masting is that a long-term perspective is required to understand how reproduction acts during the life cycle of a tree (Pesendorfer et al., 2020). Although long-term series have been recently become available (e.g. Ascoli et al., 2017; Chianucci et al., 2021; Clark et al., 2019), the spatial and temporal coverage of existing datasets is often not suited to address larger-scale scientific questions.

The availability of long series of annual seed records is hampered because quantifying seed production is a cost and time-consuming task. While direct measurements are hindered by the difficulty to access tree crowns, the available solutions to assess tree seed production strictly rely on field measurements. So far, the litter trap (LT) is considered the most accurate method (Perry and Thill, 1999) as it can provide quantitative measurements (number and biomass) of seed production. However, LT is limited by the cost and time needed for collecting and processing litter data, and by regulatory constraints (Tattoni et al., 2021), which limit its larger-scale deployment. As an alternative to LT, visual surveys have been often considered in many studies and are also employed in long-term research programs (e.g. Nussbaumer et al., 2018); however, visual methods are limited by the subjectivity of measurements, which are also not replicable, and the difficulty to apply them to tall trees, particularly those with small seed size or in denser stand and canopy conditions (Perry and Thill, 1999). Attempts to derive masting information from remotely-sensed data have been recently proposed by some authors (Bajocco et al., 2021; Camarero et al., 2010; Garcia et al., 2021), but the proposed solutions need further testing to make these methods operational. This calls for quick and low-cost alternatives for obtaining accurate field estimates of seed production at both trees and stand scales.

Recently, two studies have developed and tested the use of “ground quadrats” (GQ; (Touzot et al., 2018; Tattoni et al., 2021). The GQ method is based on counting the number of seeds after their falling on the ground, using quadrats of a given area. The method can be considered a floor-level variant of litter trap, but it has several advantages in terms of reducing time and costs of field procedures compared to LT, as demonstrated by previous studies (Touzot et al., 2018; Tattoni et al., 2021), which, in turns, potentially allows for a larger-scale field deployment of (mobile) ground quadrats compared to (fixed) litter traps.

The above-cited studies have successfully validated the GQ method against benchmark LT measurements in deciduous oaks (*Quercus cerris* L. and *Q. petraea* L.) and chestnut (*Castanea sativa* Mill.) forests, i.e., tree species having large (and thus easily detectable) seed size. A still open question is therefore whether GQ could be considered a reliable method for quantifying seed production in small-sized masting tree species like beech (*Fagus sylvatica* L.), since the small size of beechnuts may complicate the retrieval of seeds on the ground. In addition, as the previous studies focused on pure methodological differences, more investigations are needed to assess the sampling efforts (scheme and number of samples) required to obtain reliable estimates of seed production at the plot level.

Beech is a monoecious, wind-pollinated tree species. It has a

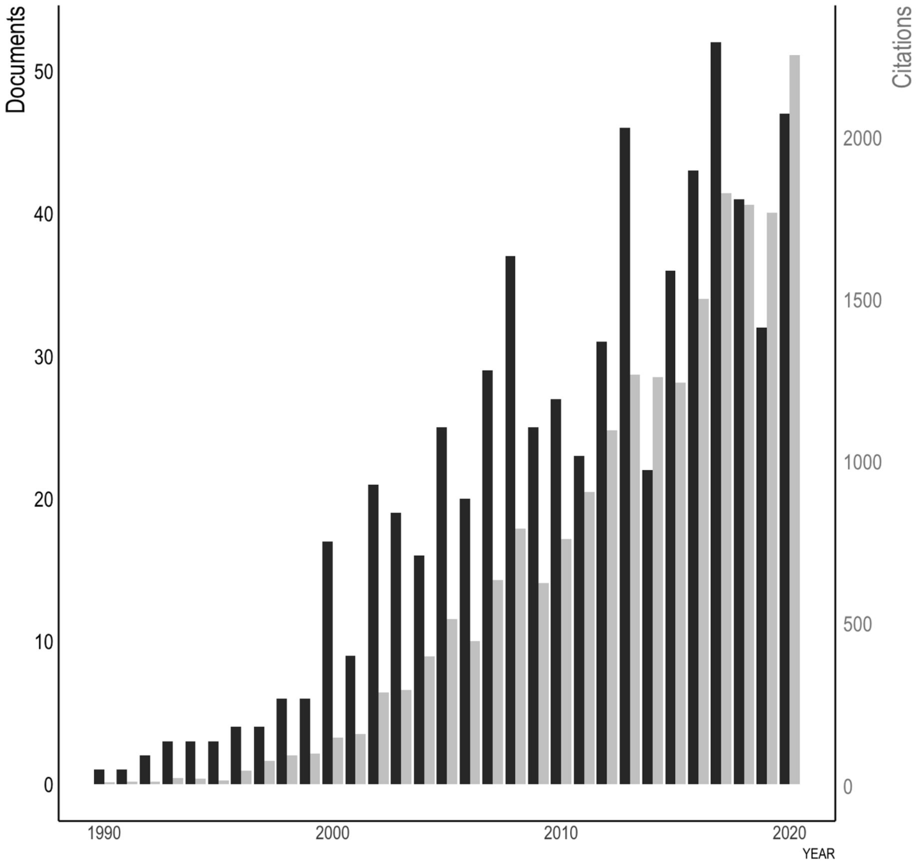

Fig. 1. Yearly published items and citations related to mast seeding over 1990–2020. (The search was conducted on January 1, 2021, using SCOPUS with the keyword combination KEY (“mast seeding” OR “masting” OR “tree mast”).

flowering masting strategy, in that the seed crop is determined by the flowering effort (Pearse et al. 2016). The time of flowering is April-May, depending on the climatic conditions. Female flowers usually developed earlier than male flowers, to reduce self-pollination (Nielsen and Schaffalitzky De Muckadell, 1954). Once pollinated, female flowers turn into a protective cupula, to protect the two beechnuts, and develop during the summer, although abortion can occur in adverse summer conditions. In autumn, often after the first frost, the cupulas open and the seeds start to fall (Simak, 1993). Mast years in this species occurred irregularly at interval of 3–10 years (Övergaard et al., 2007).

The aim of this study was to evaluate the reliability and effectiveness of the GQ method in beech forests, which were sampled in a mast year (which is an essential requirement of beech reproductive strategy) and evaluating optimal sampling procedures using this method for estimating plot-level beechnut production. Our specific questions were:

1) validating GQ measurements against benchmark values obtained with the LT method;
2) assessing how many random measurements are needed to obtain an estimate of seed production at the plot scale with a predefined precision (random sampling);
3) assessing how the precision varies with the different spatial arrangement of measurement points (regular and systematic sampling).

2. Materials and methods

2.1. Study sites

Field data was collected in three study sites located in the Alps and Northern Apennines, Italy (Fig. 2), to sample a comprehensive range of stand conditions in beech forests.

Two independent field campaigns were conducted in the period October-November 2020 to either i) validate the GQ method against LT measurements either ii) test different sampling strategies for quadrat sampling. The first campaign was performed in three permanent beech plots (Chianucci et al. 2019) sampled in Northern Apennines ("Alpe di Catenaia" site, Fig. 2). The second campaign was performed in an Alpine ("Val di Sella") and two Apennines ("Alpe di Catenaia", "Foreste Casentinesi") sites (Fig. 2).

2.2. Ground quadrat (GQ) measurements vs litter trap (LT) method

Paired ground quadrats-litter trap measurements were carried out in three permanent 0.5 ha monitoring beech plots in the Alpe di Catenaia study site (see Chianucci et al., 2019). In the sampled plots, three different silvicultural treatments were applied, including natural evolution pattern (unthinned control), two periodic thinnings, and four periodic thinnings. As a result, the plots varied in stand structure (stand density ranged between 412 and 2046 trees ha^{-1}, while basal area ranged between 29.7 to 48.2 m^2 ha^{-1}), crown and canopy conditions, which yielded different seed production (for details, see Cutini et al., 2015; Chianucci et al., 2016; data available from Chianucci et al., 2021).

In the original design, nine litter traps, each 0.25 m^2 in size, were systematically distributed inside each plot. However, at the time of sampling, five traps were temporally moved in a plot to ease trees removal after the last recent thinning. Sampling was performed on October 17 (day of year (doy) 291) and November 16 (doy 321) 2020, for a total of 44 paired measurements. Litter was collected in each trap, and then carried to the laboratory for separation and seed counting.

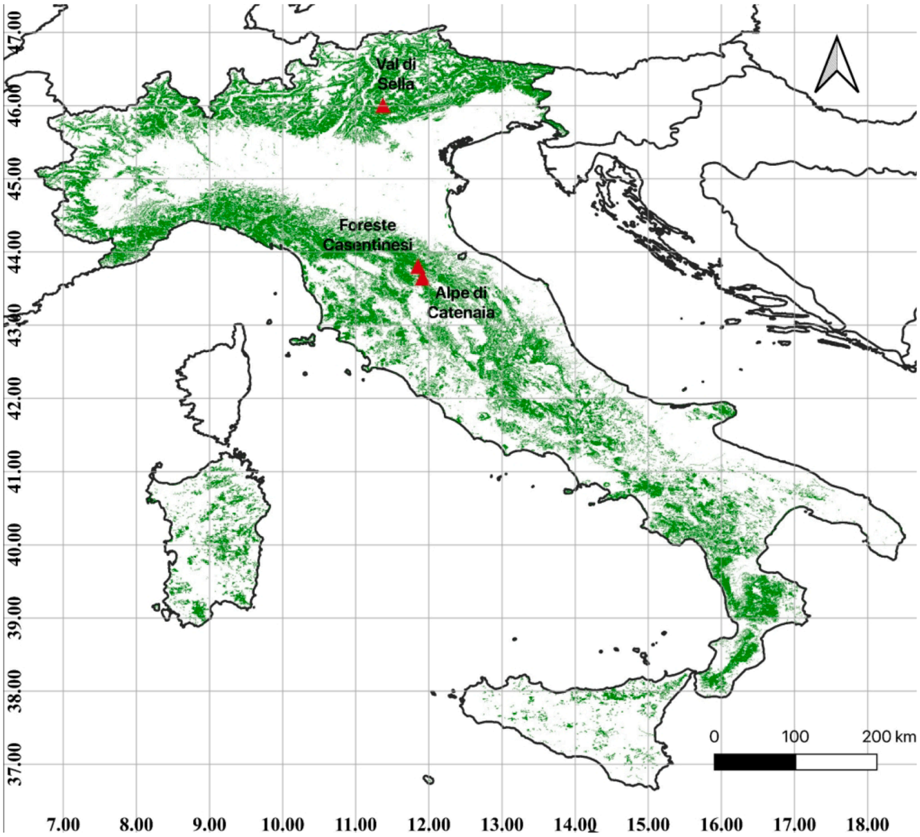

Fig. 2. Study sites. Green represents the forest coverage according to the Corine Land Cover (CLC) Level IV data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Immediately after each trap collection, a 0.25 m^2 was placed in the ground, at a distance within 1 m north from the trap, and quadrat seeds were counted. These paired GQ-LT 0.25 m^2 data were then used for comparing the two methods and validating the ground quadrat counting.

2.3. Sampling strategies for GQ measurements

2.3.1. Data collection

Additional quadrats measurements were performed in three square 0.25 ha beech plots, which were located in three sites in the Alps and in Northern Apennines (Fig. 2 and Table 1). Given that no previous pilot studies have ever evaluated the number of ground quadrats needed for plot-level seed sampling, we first collected a very large set of measurements, by aligning a 10x10 grid inside each plot, with sampling points spaced 5 m apart (Fig. 3), to ensure sample independence (Cottrell, 2004).

Each measurement point was identified by a unique numeric ID, and the number and position of each sampling point were labelled and marked in the ground prior to sampling (Fig. 3). We assumed that 100 measurements points in a 0.25 ha plot were representative of the target population (plot-level mean seed production), considering that comparable studies using litter traps usually recommended 10 to 30 litter traps for sample areas of variable size (McShane et al., 1983; Morrison, 2011; Finotti et al., 2003), which is also in line with the 10–30 range of measurements recommended by ICP for litter trap measurements in Level II plots (minimum sample size 0.25 ha; Ukonmaanaho et al., 2016).

In the two Apennine sites, quadrat measurements were performed twice between October and November, with an interval of about one month between repetitions. Measurements in the Alpine site (“Val di Sella”) was performed only in October.

2.3.2. Sampling size and schemes

For each plot and sampling period, we tested different sampling schemes using a procedure inferred from comparable field sampling procedures (Cottrell, 2004; Koenig et al., 2011; Majasalmi et al., 2012; McShane et al., 1983; Metcalfe et al., 2008; Zou et al., 2020). We firstly evaluated the number of measurements needed in random sampling for comparison with regular and systematic sampling. Determination of sample size needed to obtain estimates with predefined precision requires information about the variability among sampling units. In this case, the sampling units were the 100 quadrat estimates, and variability among these sampling units was characterized by the coefficient of variation (CV). Plot-specific CV, expresses as a percentage, was calculated as:

$$CV = \left(\frac{\sigma}{\mu}\right) \times 100 \tag{1}$$

where μ is the mean seed number obtained from 100 quadrat measurements, and σ is the standard deviation. As mean seed varied between the sample plots, we determined the minimum sample size (n) for random sampling based on the maximum allowable error (AE), using a common formula for a finite population of plots (Shiver and Borders, 1996):

$$n = \frac{4N(CV)^2}{(AE)^2 N + 4(CV)^2} \tag{2}$$

where 4 is the squared t-value per $\alpha = 0.05$ and N equals the 100 measurements. Using this formula, we evaluated how n varied with a predefined acceptable level of errors ranging between 5% and 30% (with a step of 5%). We further explored how the number of random samples influence the standard error of measurements by applying a bootstrapping procedure. For each plot and sampling period, we created 1000 subsets of varying sample sizes (n = 10–95 with a step-size of 5) by randomly selecting values from the original population (N = 100 measurements). Standard error was then calculated as (Eq. (3)):

$$SE = \frac{SD_i}{\sqrt{n_i}} \sqrt{\frac{(N - n_i)}{N - 1}} \tag{3}$$

where SD is the standard deviation and n is the sample size for each considered i scheme. The second square root term in Eq. (3) is the finite population ($N = 100$) correction factor.

Table 1
Main stand characteristics of the sampled plots. D: quadratic mean diameter at breast height; N: tree density; G: basal area.

Site	D (cm)	N (N ha^{-1})	G (M^{2} HA^{-1})
Foreste Casentinesi	43.1	240	35.0
Alpe di Catenaia	31.3	274	21.1
Val di Sella	23.8	648	28.9

As random sampling in forest is often impractical, regular sampling was then evaluated by considering collecting quadrat counts progressively, moving from the centre to the edge of the plots. Sample size was increased by adding individual measurement points to the sample by moving circularly clockwise until the whole grid was covered.

Finally, systematic sampling was considered using nine different types of predefined sampling schemes: grid, square transect and cross (Fig. 4). The number of sample points for these sampling schemes ranged from 9 (i.e. SS9) to 25 (i.e. SS1). The square schemes (SS4 and SS5) were considered as the sampled plots were concentrated respectively close to the centre and the edges of the plot. As the original grid have even number of columns and rows, the selection of quadrats started from the top-left side of the plot when an odd number of columns and rows was selected in the systematic sampling schemes.

2.4. Statistical analyses

We first used paired GQ-LT seed data to validate the quadrat method using simple linear regression. The influence of the month on the GQ-LT relationship was also assessed using an ANCOVA.

With reference to sampling schemes, we first explored how random sampling size influenced the acceptable error (AE) rate in the sampled plots (Equation (2)). We then assessed how the standard error (SE) varied according to the different sampling schemes and sample sizes in the same plots (Eq. (3)). Results from the schemes were also evaluated in terms of mean plot-level estimates and associated confidence intervals.

3. Results

3.1. Ground quadrat (GQ) measurements vs litter trap (LT) method

Quantitative analyses from litter traps confirmed that the year of sampling (2020) was a mast year (Fig. 5). In the three permanent plots, seed production ranged between 64 and 1196 n m^{-2} using LT (average ± standard error 390.5 ± 43.7 n m^{-2}), while it ranged between 64 and 884 n m^{-2} using GQ (253.6 ± 23.3 n m^{-2}), considering the whole sampling period.

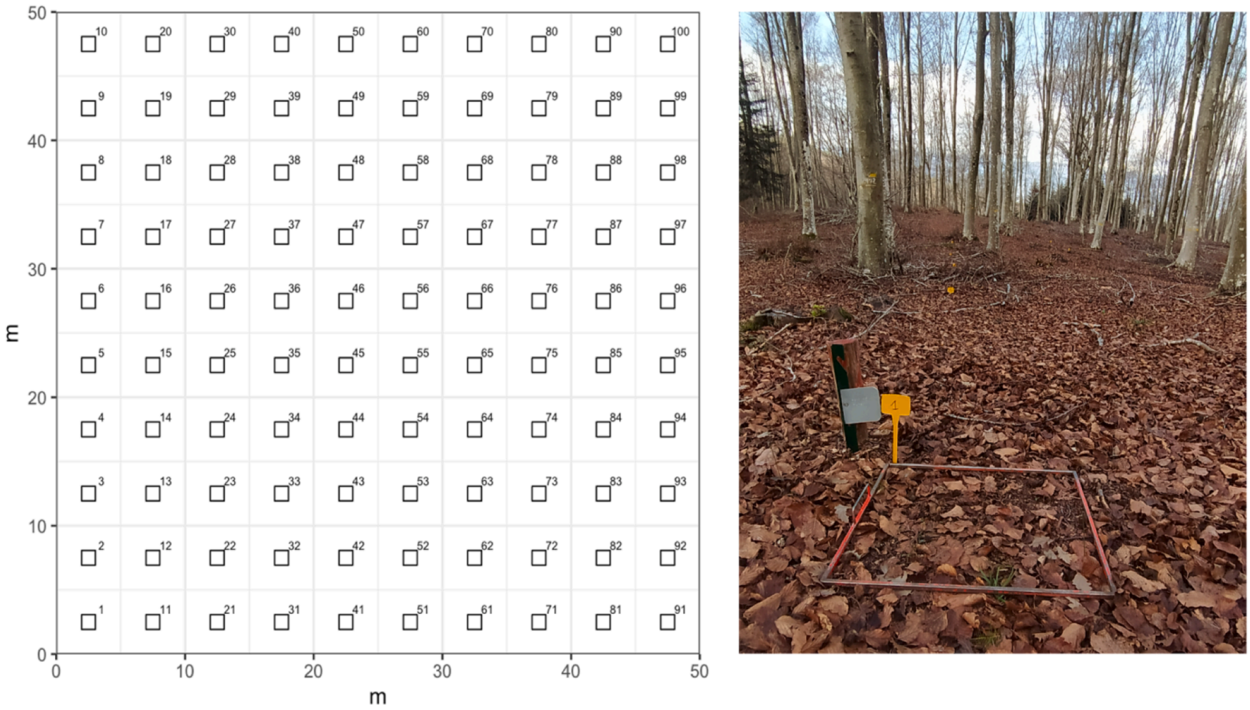

Fig. 3. Left: illustrative example of the 10x10 grid of points used for GQ sampling in 0.25 ha beech plots. Right: each sampling point number was labelled and its position marked in the ground using yellow posts. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Regression analysis showed that the methods were significantly correlated with each other ($p < 0.001$). The analysis also indicated that GQ on average underestimated seed compared with LT data (Fig. 6). Closer inspection in the plots revealed that the underestimation was observed in the first sampling in October, when on average quadrats have −39–51% lower number of seeds compared to traps. Conversely, the two methods yielded similar average values in the second sampling (November). This is confirmed by the analysis of covariance (ANCOVA), which revealed that the sampling period has an influence on the LT ~ GQ relationships ($p < 0.001$).

3.2. Sampling strategies for GQ measurements

The three plots considered for quadrat sampling showed differences in the average seed production (Table 2 and Fig. 7), despite their similar coefficients of variation. ANOVA and Tukey's post-hoc test showed that seed production in October Val di Sella significantly differed ($p < 0.001$) from those of other plots, while the seed production is not significantly different between the other two plots (either considering the cumulated production or the ones separated for sampling period). In all plots, most of the seed were collected in the first sampling carried out in October (Fig. 7). The range of production found in these plots agreed with the values found in masting years from other studies in European beech forests (Table 3).

With reference to sampling design, statistical analysis (Eq. (2)) showed that the number of random samples required for achieving a predefined allowable error is similar across plots and sampling period (Fig. 8). Results indicated that about 50 samples are required for achieving an AE = 10%, about 30 samples are required for AE = 15%, and about 15–20 samples are required for AE = 20%.

Analysis of standard error from the bootstrapping subsets further allowed to quantify the number of samples needed to achieve the desired precisions. About half the number of original samples are required to resemble the original dataset (N = 100) variability (Fig. 9). Depending on the plot, about 40–45 samples are required for achieving an acceptable error of 10%, 25–30 samples are required for achieving an AE = 15%, 15–20 samples are required for an AE = 10% (Equation (2)).

While random sampling showed comparable results between the sampled plots, different pattern resulted from regular sampling (Fig. 10). In the plot sampled in Foreste Casentinesi, which was characterized by the lowest mean seed production, a lower number of seeds was found in the middle of the grid, and cumulative mean seed monotonically increased as sampling increased, particularly in the field sampling in October (Fig. 10). In the plot sampled in Alpe di Catenaia, a larger number of seeds were initially found in the middle of the grid, but the values then became stables (Fig. 10). In the plot sampled in Val di Sella, which was characterized by the highest mean seed production, there was no clear trend observed in cumulative mean seed counts (Fig. 10). Comparable results were also obtained for the sampling performed in November.

With reference to systematic sampling, we found that the different sampling schemes yielded different performance in estimating average seed production, depending on the site (Fig. 11). When considering sampling in October and average seed estimates, the SS1 (grid sampling, $n = 25$) yielded mean seed values which are within the confidence intervals of the original population ($N = 100$), irrespective of the sampled

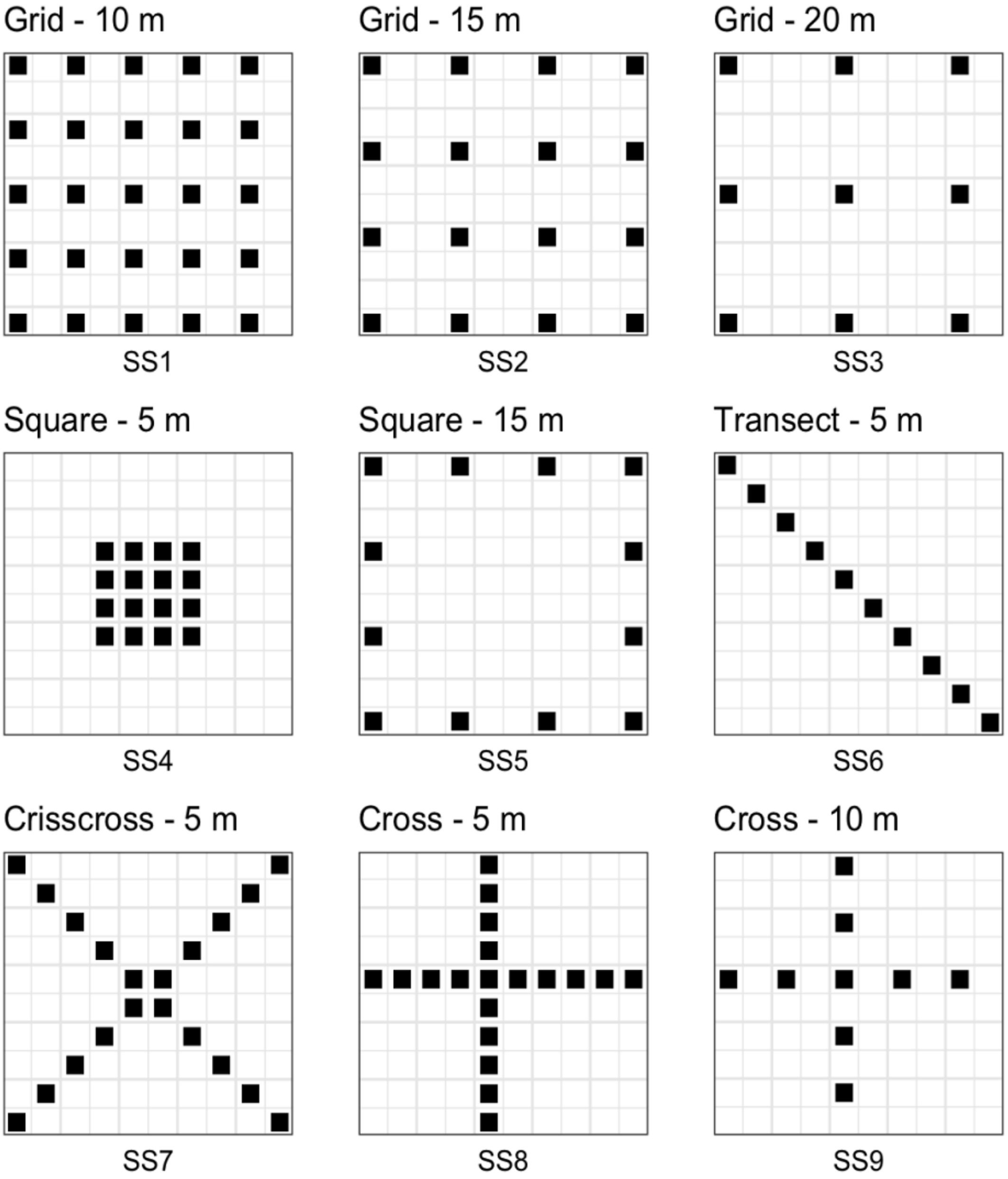

Fig. 4. Illustrative example of the systematic schemes tested for ground quadrat (GQ) sampling.

plot (Fig. 11). Similar results were found for the SS2 (grid sampling, $n =$ 16), although the scheme yielded larger error values than the previous one, and underestimated mean seed production in one plot (Alpe di Catenaia). In the plot sampled in Val di Sella, which was characterized by the highest seed production, grid sampling (SS1, SS2, SS3) yielded estimates closest to the mean population production, square sampling (SS4, SS5) yielded similar accuracy than grid sampling, transect sampling (SS6, SS7) underestimated mean seed production, cross sampling (SS8, SS9) overestimated mean seed production, as compared with population values. In the other two plots, square sampling (SS4, SS5) was poorly suitable for sampling seed, more likely because the different seed availability observed in these plots between centre and edge of the plot area (Fig. 10), while the other transect (SS6, SS7) and cross sampling schemes (SS8, SS9) yielded similar accuracy than grid sampling. Comparable results were also obtained for the sampling performed in November.

Finally, when comparing results in terms of standard error (Eq. (3); Table 4) SS1 was still the best scheme for Alpe di Catenaia and Val di Sella, with a CV% comparable with the original population values, while SS4 yielded lower absolute standard error in Foreste di Casentinesi, although the differences in standard error between SS4 and SS1 are small in this site. The lower standard error in SS4 was obtained in this site because it has a lower seed number at the centre of the plot, for which also the absolute error was smaller (Fig. 10). In all sites, also the crisscross (SS7) scheme yielded low S.E. and CV, although this scheme underestimated the mean seed production in Val di Sella (Fig. 11 and Table 4). Comparable results were also observed in the sites when considering sampling in November.

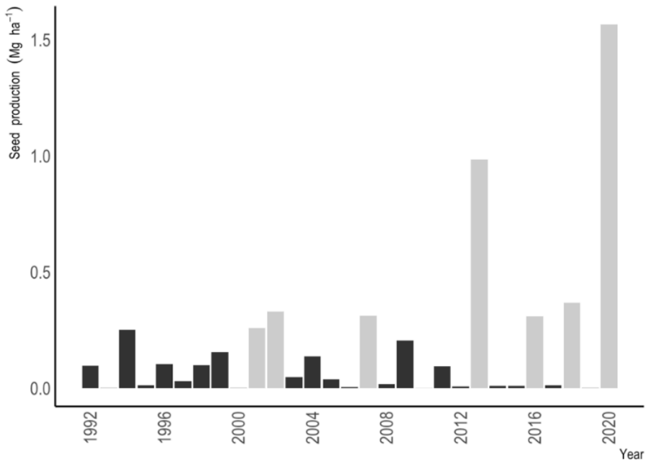

Fig. 5. Long-term (1992–2020) seed biomass production obtained from litter trap (LT) measurements in a permanent beech plot (unthinned control) sampled in the current study and a previous one (Tattoni et al., 2021). Grey columns indicate classified mast years (MY), while black columns indicate non-mast years; MYs are those whose annual production is larger than the 75° percentile calculated over the time-series (Bajocco et al., 2021). Data available in Chianucci et al. (2021).

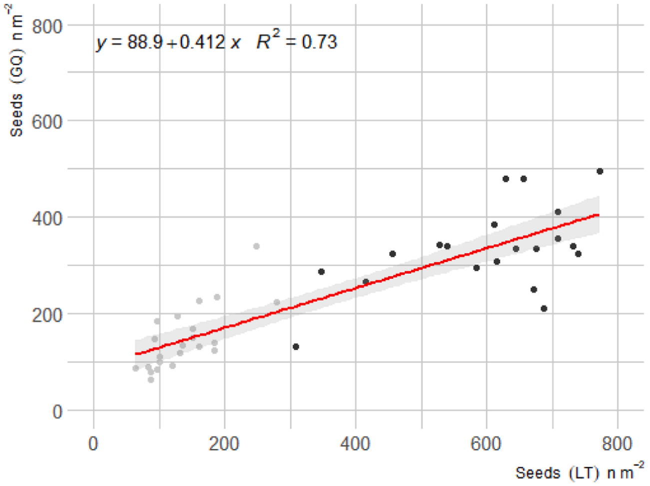

Fig. 6. Scatterplot of seed nuts counted in ground quadrats (GQ; y-axis) vs seed nuts counted in reference litter traps (LT; x-axis). Seed data are expressed per unit surface (n m^{-2}). The red line indicates the fitted regression, along with its 95% coefficient interval (shaded grey regions). Summary of regression fitting is also displayed at the top of the graph. Black circles indicate seeds sampled in October, while grey circles indicate seeds sampled in November. To improve readability, the axes have been cut at 800n m^{-2} (1 record not shown). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Summary statistics on seed production obtained in the sampled plots. To ease the interpretation of results, seed data are expressed per unit surface (n m^{-2}).

SITE	MONTH	MIN	MAX	MEAN	SE	CV (%)
ALPE DI CATENAIA	October	96	900	400.0	17.8	44.5
	November	12	364	143.2	6.9	48.0
FORESTE CASENTINESI	October	116	740	340.0	14.3	42.0
	November	20	344	140.8	7.1	50.0
VAL DI SELLA	October	100	1252	577.6	28.3	49.1

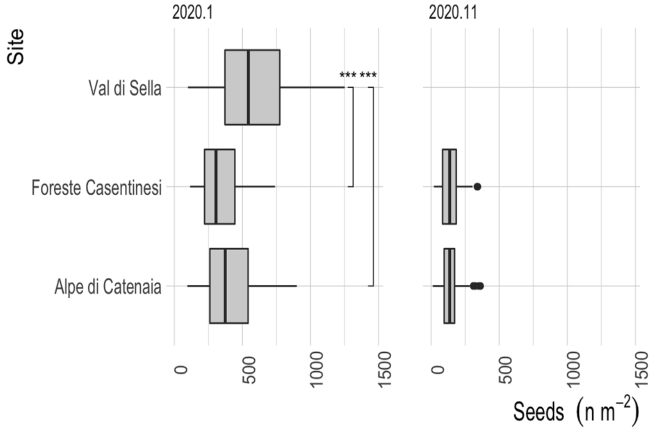

Fig. 7. Variability in seed estimates obtained from ground quadrats in the sampled plots in the different sampling periods. To ease the interpretation of results, seed data are expressed per unit surface (n m^{-2}). Asterisks indicate the sites with different seed production ($*** = p < 0.001$).

Table 3
Statistics on seed production, expressed per unit surface, (n m^{-2}), in beech forests sampled in Europe in previous studies. The year of the production were classified as masting year (MY = Yes) or non-masting year (MY = No), with the exception (*) of France, where the year was classified as intermediate masting year.

Site	Min	Max	Mean	MY	Source
Croatia	240	590	–	Yes	(Gavranović et al., 2018)
France	38	867	306	Yes*	(E Silva et al., 2012)
Germany	17	43	–	No	(Szwagrzyk et al., 2015)
Germany	277	437	–	Yes	(Szwagrzyk et al., 2015)
Germany	269	324	–	Yes	(Burschel et al., 1964)
Italy	0	64	6	No	(Tattoni et al., 2021)
Sweden	110	1010	530	Yes	(Övergaard et al., 2007)
Sweden	0	49	–	No	(Övergaard et al., 2007)
Sweden	–	–	370	Yes	(Simak, 1993)

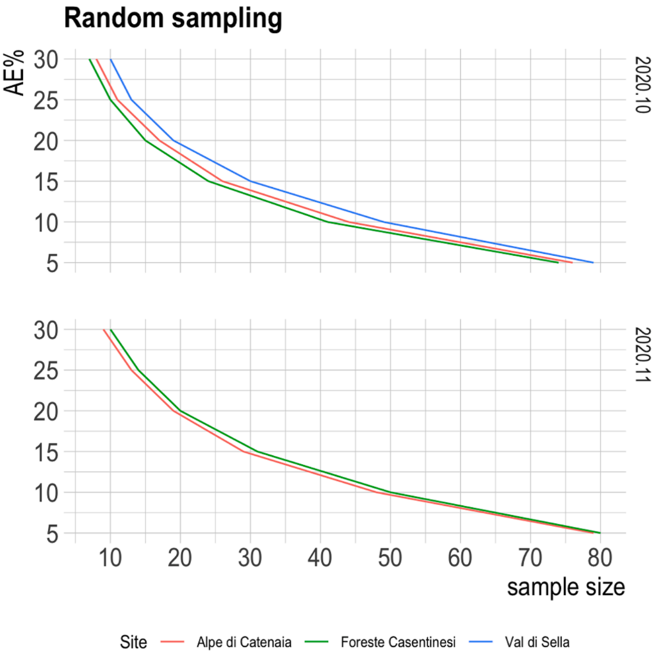

Fig. 8. Relationship between allowable error (AE) and random sampling size in the studied plots.

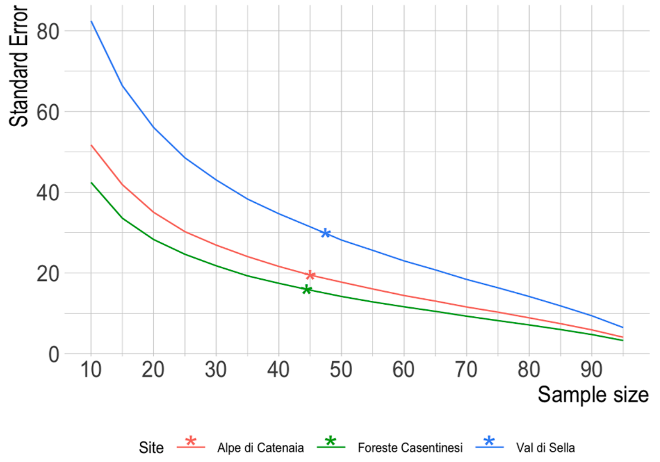

Fig. 9. Standard Error (Eq. (3)) as a function of sample size derived from bootstrap. The standard error is expressed per unit surface (n m^{-2}). Asterisks indicate the standard error of the original dataset (N = 100) in each plot. Only the results from October are shown here as sampling in November showed similar behaviour.

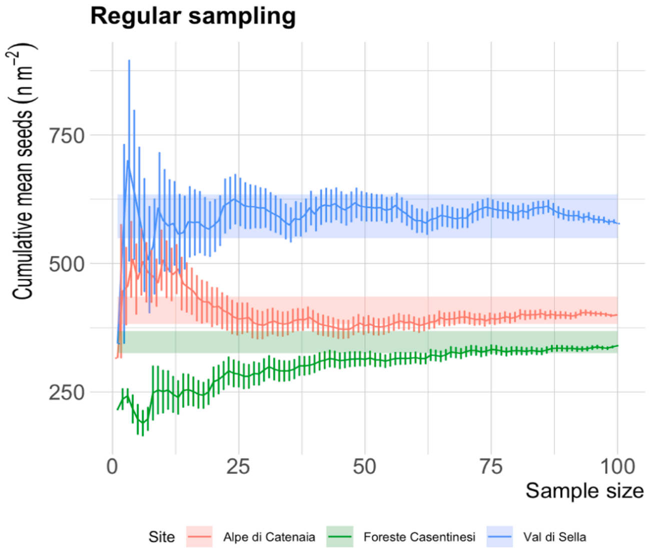

Fig. 10. Cumulative mean seeds (±standard error (SE); Eq. (3)) obtained from quadrats collected using a regular sampling (from the centre to the edge of the plots) scheme, considering sampling in October. Seed data are expressed per unit surface (n m^{-2}). To ease interpretation of results, shaded horizontal regions were depicted, representing the mean value ± 2 × *SE* calculated from the original population (100 quadrats for each plot).

4. Discussion

We demonstrated that ground quadrat (GQ) is a reliable method to assess seed production in beech forests. While broadly consistent with the previous findings of Touzot et al., (2018) and Tattoni et al., (2021), the outcome has relevant practical implications, in that it extended the applicability of GQ for sampling small-sized seeds, which are widespread in many masting tree species such as ashes, maples and hornbeams (Czeszczewik et al., 2020; Frey et al., 2007; Hoch et al., 2013). In such species, visual counting methods are hindered by the difficulty to detect small seeds while still on the trees, making counting after seedfall a more suitable option. In this line, the study provides evidence that small seeds can be efficiently and reliably counted at the floor level using quadrats.

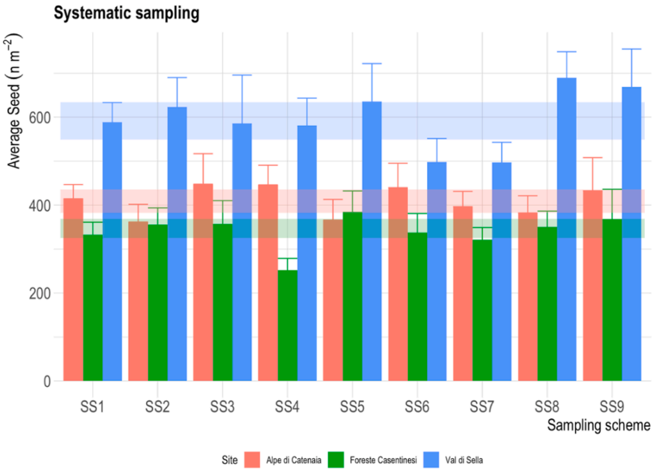

Fig. 11. Average (±standard error (S.E.); Eq. (3)) seed estimates obtained from quadrats collected using different systematic sampling schemes, considering sampling in October. Seed data are expressed per unit surface (n m^{-2}). To ease interpretation of results, shaded horizontal regions were depicted, representing the mean value ± 2 × *SE* calculated from the original population (100 quadrats for each plot).

Table 4
Standard error (SE; Eq. (3)) and coefficient of variation (CV%; Eq. (1)) of the mean seed production obtained from the different systematic sampling schemes in October. Seed data are expressed per unit surface (n m^{-2}).

		Alpe di Catenaia		Foreste Casentinesi		Val di Sella	
Method	n	SE	CV%	SE	CV%	SE	CV%
SS1	25	32.3	44.7	29.4	51.0	45.9	44.8
SS2	16	40.4	48.5	39.0	47.7	67.9	47.4
SS3	9	69.4	48.5	54.2	47.6	111.3	59.6
SS4	16	44.8	43.7	27.8	48.1	63.4	47.5
SS5	12	46.9	47.1	48.5	46.4	87.5	50.7
SS6	10	55.5	41.9	44.5	43.9	54.6	36.4
SS7	20	34.7	43.6	28.7^2	44.6	46.9	47.0
SS8	19	39.1	49.3	37.1	51.2	60.6	42.4
SS9	9	75.5	54.6	68.7	58.5	87.6	41.0

Long-term analysis revealed that the year of sampling was the heaviest mast year in the permanent plots over the 30 years of observations (Fig. 5). Quadrat count estimates also supported the uniqueness of this mast year, as the mean production ranges observed in October (340–578 seeds per square meter) were noticeably higher than the range of 150–300n m^{-2} found in previous studies on beech masting (Burschel et al., 1964; Schmidt, 2006, but see also Table 3). In addition, the maximum values are in line with heavy masts found by Övergaard et al. (2007) in Sweden, with the latter using traps which are likely less affected by seed predation than ground quadrats. Despite the large number and variability of seeds found in this study, we further demonstrated that GQ are robust in estimating seed production, as compared with LT, and the correlation between the methods was not affected by the intensity of the seed predation occurring at the forest floor, in accordance with the findings of Touzot et al., (2018). On the other hand, our results showed that the number of samples required for achieving predefined acceptable error rates (Fig. 8) is not affected by the different seed production observed between the plots and the sampling periods (October and November). This further confirmed that the GQ is a robust method, which is suitable for assessing seed production in different conditions, including long-term monitoring (masting and non-masting years) and situations with different mean production due to different stand age, productivity, fertility.

Besides the uniqueness of the 2020 seed production, the long-term series also showed an apparent increasing seed production in beech over the recent years (Fig. 5). The result agreed with a recent study

(Bogdziewicz et al., 2021), which interpreted such trend as increasing summer temperature, which is a weather cue for masting in beech, influences the length of the growing season and increase photosynthesis. We agree with such hypothesis as we observed in our experimental plots a larger duration of the growing season, particularly a later onset of leaf senescence in recent years after warmer summers. Conversely, the same authors observed a lower frequency of null masting events over time, which they still attributed to warmer summer temperature. In our sites, there is no strong evidence of decreasing null-masting frequency; conversely, the recent heavy masting years are usually followed by null or very scarce mast years; in our view, the outcome can be interpreted as the huge seed production consumed most of the available resources in the heavy mast year, which limited the resource budget for reproduction in the subsequent year (Allen et al., 2018).

With reference to seed predation, the observed systematic underestimation of seeds in GQ was in line with the rate of seed predation previously observed in the same plots (Cutini et al., 2007). In the study area, up to half of the number of beechnuts is predated during a mast year, of which about eighty per cent are consumed by wild boar (*Sus scrofa* L.) (Cutini et al., 2007). Interestingly, seed predation appeared to be concentrated in October, while its impact was negligible in November, i.e., when the availability of seeds was lower. This suggests that wild boar consumed most beechnuts when this food item was more abundant and temperature was still milder, while settling for lower-altitude resources like chestnut and oak seeds (Bisi et al., 2018; Canu et al., 2015; Cutini et al., 2013) once late winter low temperatures made beech habitats too harsh. These considerations highlight the importance of setting a proper timing for quadrat sampling, in order to account for the degree of predation in the estimation of seed production. For this reason, we recommend concentrating the sampling immediately after the main seedfall period, which occurs between late October – early November in temperate regions (Touzot et al., 2018).

While broadly consistent with a previous study, which found that ground quadrats allowed discriminating mast and non-mast year in beech forests, irrespective on seed predation occurring at the forest floor (Zwolak et al., 2016), our results further demonstrated that a combination of LT and GQ can potentially allow determining the intensity of seed predation. From an operational point of view, when the focus of the research is assessing seed predation, we suggest to cross-calibrate quadrat seed counts with measurements obtained from LT in reference plots (when available); GQ could then be used more intensively to assess seed predation at larger temporal and spatial scales. The proposed solution also holds potential to relate seed counts, as easily determined by GQ, with seed biomass estimates which can be retrieved from litter traps.

Many studies on masting or tree seed production have made use of data derived from litter traps. However – perhaps surprisingly – very few studies have evaluated the accuracy of sampling schemes on LT when the target estimate variable is seed production (see for example Cottrell, 2004; Stevenson and Vargas, 2008). Indeed, previous studies have assessed the ideal sampling sizes required to estimate total litterfall (Dellenbaugh et al., 2007; Finotti et al., 2003; Metcalfe et al., 2008), specific leaf area (Liu et al., 2021), soil nutrient (Yang et al., 2017), leaf litter (Yang et al., 2017) and leaf area index (Chianucci and Cutini, 2013; Metcalfe et al., 2008) from LT. On the other hand, some studies have been more focused on defining the proper timing and repetition for LT sampling to account for the contribution of reproductive (flowers and seeds) parts to total litterfall (Ovington, 1963; Kollmann and Goetze, 1998). Our results indicate that sample size and schemes have an effective impact on estimating seed production using GQ. Given that quadrats are comparable with (fixed) traps in terms of collector size and accuracy, we speculate that our findings apply to sampling seed from LT as well.

Regarding the sampling size, we found that about 20 randomly placed quadrats allowed to provide an estimate of seed production at the plot (0.25 ha) level, with an acceptable error within 20%, which represents an intermediate value between the error range 10–30% commonly expected in comparable studies and field protocols (Lucas-Borja and Vacchiano, 2018; McShane et al., 1983; Metcalfe et al., 2008; Ukonmaanaho et al., 2016). Random seed sampling has been considered in previous studies (e.g. Zwolak et al., 2016), but it is often impractical in the field; thus, alternative schemes may be preferable to optimize field measurements.

Considering regular sampling, our results indicate that this sampling scheme is not suitable in natural stand conditions, in situations where seed production differed between centre to the edge of the plots. This has been verified in the plot located in Foreste Casentinesi site (see Fig. 10), which was characterized by fewer, but larger trees (see Table 1), with presumably higher tree crown. Thus, the combination of lower tree density and higher individual crowns have likely created spatially variable canopy conditions (e.g., larger between-crowns gaps and higher canopy clumping; Chianucci, 2020) which explained the heterogeneous spatial distribution of seeds in this plot.

Compared with both random and regular sampling, systematic schemes are generally simpler to be applied in field conditions, which may partly explain why such schemes are often considered in many ground seed sampling studies (Cutini et al., 2013; Masaki et al., 2019; Swamy et al., 2011; Tiebel et al., 2019). Our comparison indicates that collecting 25 measurements arranged in a grid (SS1) provides reliable estimates of seed production at the plot scale (0.25) ha, which are within the confidence interval of the population means and appeared relatively robust to varying stand conditions. Crisscross sampling (SS7) can be considered an alternative reliable option in many situations, although the method showed a tendency to underestimate seed in Val di Sella, i.e. the highest seed production conditions.

5. Conclusion

We demonstrated that ground quadrats (GQ) are reliable tools to estimate seed production in beech forests. Compared to LT, the method is quicker, cheaper, and more flexible, as it does not require authorization for installing fixed traps, and counting seed was performed in the field, avoiding further laboratory steps (Tattoni et al., 2021). The simplicity of the method makes GQ highly suitable for long-term monitoring of seed production. From a practical viewpoint, we recommend arranging quadrats in systematic grids, with 25 measurements being suitable for sampling a 0.25 ha plot, concentrating the measurements soon after the seedfall peak (i.e. late October – mid November in temperate forests).

CRediT authorship contribution statement

Francesco Chianucci: Conceptualization, Data curation, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Clara Tattoni:** Data curation, Formal analysis, Writing - original draft. **Carlotta Ferrara:** Formal analysis, Writing - original draft, Writing - review & editing. **Marco Ciolli:** Data curation, Formal analysis, Writing - original draft. **Rudy Brogi:** Data curation, Formal analysis, Writing - original draft. **Michele Zanni:** Formal analysis, Writing - original draft, Writing - review & editing. **Marco Apollonio:** Writing - review & editing. **Andrea Cutini:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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