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RESEARCH

A report of short-term aversive conditioning on a wolf documented through telemetry

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Abstract

Predation by large predators on livestock is one of the main concerns in species conservation as it elicits prompt and effective retaliations. Therefore, conflict mitigation is essential to ensure long-term coexistence of predators with humans. We performed aversive conditioning (AC) with rubber bullets on one collared wolf that had become particularly bold toward a transhumant shepherd and had preyed on livestock. By exploiting the unique fine-resolution location data available before and after the AC event, alongside careful retrospective field investigations, we were able to analyse the effects of AC on wolf behaviour. Our study revealed that after just a single AC event, the wolf modified its spatial and predatory behaviour: the wolf changed its use of space by increasing distance from humans and ceased to attack farms in the following 2 months; actually, the only livestock preyed after AC was represented by a sheep and two goats lost by shepherds that had left alpine pastures. This study represents a first step to increase knowledge on AC effect on the wolf. Additional researchers are encouraged to conduct and publish findings on this topic in order to provide a useful and widely tested array of tools to promote wolf conservation in human-dominated landscapes.

Keywords Aversive conditioning · *Canis lupus* · GPS collars · Livestock · Predation

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Introduction

An increasing number of large carnivores in some areas of the world has been reported over the past years (Chapron et al. 2014) as well as human-predator conflicts have been increasingly mentioned (Lute et al. 2018; Hoffmann et al. 2019; Pettersson et al. 2021; Bogezi et al. 2021). Effective strategies to mitigate these conflicts should involve decisions balancing the efficiency and costs of management actions, while taking also into account animal welfare, social and ethical acceptability (Breck et al. 2017; Moreira-Arce et al. 2018; Sampson and Van Patter 2020). Where possible, non-lethal interventions should be preferable to lethal control, as it is increasingly being advocated by the conservation community (Dubois et al. 2017). Moreover, in many European countries, large carnivores are protected by law, making unapplicable lethal management techniques for those problematic individuals, as it is instead commonly practiced in North America.

The debate on alternative non-lethal methods to manage and mitigate the conflict between large carnivores and humans is growing (Shivik 2006; McManus et al. 2015; Blackwell et al. 2016; Berzi et al. 2021), largely due to a public opinion increasingly opposed to lethal interventions. There are

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several non-lethal deterrent methods to avoid conflicts between predators and humans (Shivik 2006; Blackwell et al. 2016; Sampson and Van Patter 2020). These methods are based on two main approaches (Shivik and Martin 2000): disruptive-stimulus approaches and aversive-stimulus approaches. Former approaches act by disrupting predator access to resource and scaring the predator away (e.g. community-level hazing, Bonnell and Breck 2017). The second method aims to modify predator behaviour through conditioning: such methodology aims to make the predator associate a physical pain and/or discomfort feelings (e.g. illness, nausea, vomiting) with an environmental element such as scent, taste and human presence. As a result of this negative reinforcement, the animal should avoid the previously attractive stimulus (McCarthy and Seavoy 1994; Linnell et al. 1996; Tobajas et al. 2020) re-creating the landscape of fear (Gaynor et al. 2019). While there is an extensive knowledge on some non-lethal techniques (electronic guard: Linhart et al. 1992; guard dogs: Fritts et al. 2003; fladry: Musiani et al. 2003; hard plastic collars: King 2004; community-level hazing: Bonnell and Breck 2017), a lack of knowledge is evident for techniques that use aversive conditioning (e.g. electric shock collars: Shivik et al. 2002; rubber bullets: Beckmann et al. 2004; conditioned food aversion: Tobajas et al. 2020). For instance, the use of rubber bullets to scare the predator is poorly documented even though several European countries, such as France, Italy and Sweden, can use them for management purposes. On this topic, there is a wide anecdotal knowledge while scientific literature is very scarce (Rauer et al. 2003). One of the few published studies tested rubber bullets in different situations on brown bear, with some radiomarked individuals that were studied after the aversing conditioning event (Rauer et al. 2003).

On several other problematic species, such as the wolf, the scientific literature is very scarce. Through this study, we aim to fill these gaps by publishing the first results obtained from an aversive conditioning experiment performed on a problematic wolf accustomed to the presence of shepherds. We predicted that after the aversive conditioning intervention the wolf increased human avoidance and decreased its predatory activity upon livestock. Our goal is also to spur other authors who have comparable data to replicate the experience in other contexts and to publish and share their results.

Study area

The study was conducted during summer 2021 in the North-Eastern Italian Pre-Alps (45°52′24 N, 11°47′57 E; Fig. 1) on the Grappa Massif. The study area (35,008 ha, altitudinal range: 110–1775 m a.s.l.) is characterized by an oceanic climate according to Köppen's classification (Pinna 1978), also referred to as Cool temperate (Cf) based on the Köppen–Geiger scheme (Fratianni and Acquaotta 2017). Winter is the coldest season, with the mean temperature of the coldest month ranging

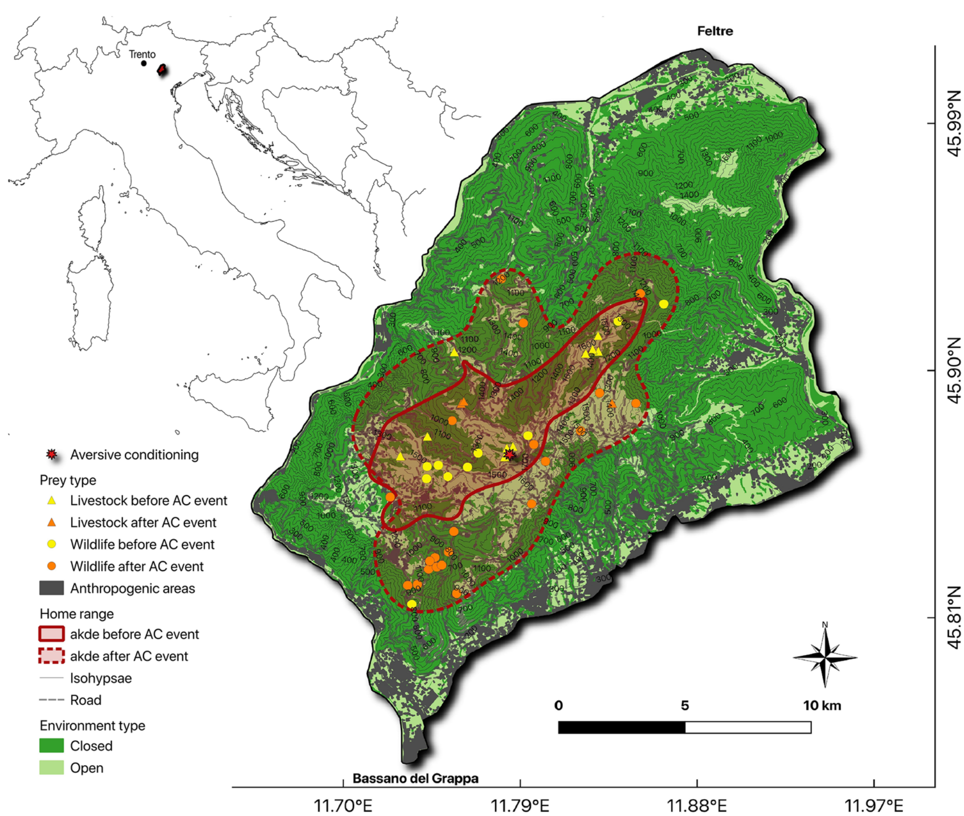

Fig. 1 Map of Italy (top-left) showing the localisation of the study area, located in the north-eastern Italian Pre-Alps, and an enlargement of the map displaying the localisation of the aversive condition event (AC), the home range of the wolf before (from 07/06/2021 to 19/08/2021) and after the AC event (from 19/08/2021 to 17/10/2021), and the positions of the wolf's preys observed in the period before and after AC. The map was generated in Qgis

from 0 to −3 °C. Summer is the warmest season, with mean temperature of the hottest month ranging from 15 to 19.9 °C (Fratianni and Acquaotta 2017). Annual precipitation amounts to 1200–1500 mm, with no significant differences among seasons. The study area is mainly covered with forest (deciduous forest 54%; mixed forest 14%). The open areas occupy about 30% of the study area and included meadows, pastures, arable land and anthropized areas. Livestock breeding is a relevant economic activity, with some thousands of sheep spending late spring and summer on the Grappa massif. The time spent in high-elevation pastures by sheep flocks starts in June and might last over a long time, with conclusion ranging from the end of August till mid-October, depending on environmental conditions in different feeding areas. The study area is characterized by a considerable human presence in the spring–summer period both due to tourism and to livestock grazing. The area has been recently occupied by wolves: the first reproduction was documented in 2017 with a pack consisting of two adults and six pups (Avanzinelli et al. 2018). Roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and chamois (*Rupicapra rupicapra*) are the most abundant ungulate species, but also Mediterranean mouflon (*Ovis aries*) and wild boar (*Sus scrofa*) can be found in low densities.

Methods

Data collection

We captured five wolves between 2019 and 2021 by using foothold traps (FremontTM Humane Foot Snare Wolf/Cougar 1/8 7×7 and FremontTM Humane Foot Snare Fox/Coyote 3/32 7×7). Once captured, the wolves were immobilized with a mixture of anaesthetic drugs (medetomidine-ketamine) using a syringe blowpipe (Telinject). We collected biometric data and we took biological samples; we weighted animals and fitted them with GPS collars (VERTEX Plus Vectronic Aerospace GmbH). At the end, we injected atipamezole in order to reverse the effect of anaesthetic drugs and we monitored the wolves during recovery. We programmed the collars to record a localization every 2 h during the day, from 6:00 am to 6:00 pm UTC, and every 30 min during the night. We set the collars to transmit data twice a day (IRIDIUM transmission).

We tracked all collared wolves remotely and by using their location data we identified cluster sites (i.e. a minimum number of two locations no more than 200 m apart; Sand et al. 2005) to be checked by means of direct field surveys to identify wolf predation. We distinguished livestock and wildlife prey using prey phenotypic characteristics (hairs, antlers/horns). One of the aims of the study was to test aversive conditioning methods that can be helpful in reducing predation on livestock and therefore conflicts with humans. To this purpose, we used virtual fences traced around the sheep farms that are warned if the wolves entered the virtual perimeter around the farm; in addition, we implemented a dissuasion system consisting of a proximity sensor combined with a sound and light emitter (commonly known as the radio-activated guard "RAG"; Breck et al. 2002).

During summer 2021, one of the monitored wolves (an adult male) became particularly confident towards a transhumant shepherd in the alpine pastures (henceforth referred to as reference shepherd). The wolf, together with other members of its pack, visited the enclosure trying to get the sheep out of the fence, despite the presence of the shepherd with 4 dogs (breed Pastore Bergamasco), and an electrified fence. The formerly mentioned dissuasive and warning systems were effective to signal the wolves' presence and to deter the entry in the sheep corral but were ineffective in achieving the result of wolves leaving the area. From May 25 to July 4, we observed 18 cases of wolf approaches up to 10 m from the shepherd with reduced escape distances. Their presence induced panic within the sheep herd in the corral causing some sheep to die crushed by the others and some to jump out of the corral being promptly preyed upon by waiting wolves. To dissuade the wolf from approaching the area used by the shepherd, we planned to implement an aversive conditioning action (AC). This was anticipated by a legal procedure involving the preparation of a report on the case and on the aversive conditioning action planned for the regional Government that approved it and sent it to the national Ministry of Environment; the Ministry approved the dissuasive action after a positive evaluation from the Italian Agency for Nature Protection (ISPRA). Following the project approval, from August 19 to September 24, two rangers from the provincial police stayed from 7 p.m. to 12 p.m. near the electric fence that protected the sheep observing the surroundings by means of an infrared-camera (Flir Scout II-640 9 Hz) with the aim to shoot the approaching wolf with rubber bullets (Fiocchi 12 bore single bullet) by using a shotgun (Franchi, Alcione model). In a first period (19–29 August), the survey was conducted every day, while in the second period (30 August–24 September), the survey was conducted with a random frequency (N = 16). The survey protocol was to shot targeting the wolf's thigh, only when the wolf was less than 30 m away from the sheep fence displaying a predator-like behaviour. After the AC event, we monitored the wolf intensively by using telemetry and 13 camera traps (Browning spec ops advantage) opportunistically distributed inside the study area on the main transit points of wolves.

Data analysis

Using location data recorded by the collar, we compared a series of parameters useful to understand the effect of AC on the target animal. We assumed that the presence of the reference shepherd had an influence on the wolf from the first seasonal predation (07/06/2021) up to the last day that it spent in the alpine pastures before beginning transhumance outside the pack's territory (17/10/2021). A total of 132 days were included in the analysis, 73 before the AC and 59 after the AC. To avoid the influence of uncontrolled variables on this large temporal scale, we also performed the analyses on a short temporal scale and considered a period of 20 days before and 20 days after the AC event (from 30/07/2021 to 08/09/2021).

As a first step, we analysed whether the wolf changed spatial behaviour in the period after AC with respect to the period before AC. By using the autocorrelated kernel density estimation method (AKDE; Fleming et al. 2015), we calculated the home range before and after the AC and evaluated their degree of overlap, using the *akde* function of the package "CTMM" in R (Calabrese et al. 2016). Then, we calculated the mean daily distances travelled in the two time periods (before and after AC) by using a continuous-time speed and distance (CTSD) estimation method implemented by means of the *speed* function of the "CTMM" package in R (Calabrese et al. 2016; Noonan et al. 2019). This allowed to overcome some shortcomings of the straight-line displacement estimation, as CTSD method is more accurate and unbiased because considers autocorrelation and tortuosity of location data (Noonan et al. 2019). Next, to investigate the environmental characteristics of wolf locations and whether the monitored wolf selected particular environmental features differently during the two monitored periods, we adopted the resource selection function (RSF) approach with the "use availability design" (Manly et al. 2007). Accordingly, we matched wolf locations (hereafter referred to as "used" locations) to randomly selected locations (hereafter referred to as "available" locations). We sampled available locations generating independent random points inside the 100% minimum convex polygon (MCP), estimated by using the relocations of the wolf collected throughout the monitoring period (07/06/2021 to 17/10/2021). We used a use-available ratio of 1:20 considering it large enough, due to the complexity of the environment in the study area. We then paired available locations to used ones and each pairing (ratio 1:20) was assigned a unique identification code (stratum-ID). Date and time of observation were assigned to its respective used location, as well as to its corresponding available locations. Subsequently, by using GIS software we assigned to all locations (both used and available) the following environmental covariates: altitude (Alt), the environment type (EType), classified in open environment (i.e. meadows, pastures and arable land) and closed environment (i.e. mixed and deciduous forest); the distance to (i) the reference shepherd (dSH), (ii) anthropogenic and residential areas (dAA) and (iii) the nearest road (dR) were calculated as well. Due to the Vaia storm that hit the study area during autumn 2018, 1% of the trees in the forest area had fallen creating optimal shelter sites for several animals, including wolves. Consequently, as a further environmental covariate to be considered in the analysis, we also calculated the distance of each wolf location to the nearest area with fallen trees (dFT). Finally, we assigned to each location (both used and available) a binary variable time related to the AC event date (tACevent): "before" and "after".

We built resource selection functions (RSFs) by using the wolf locations. RSF coefficients were estimated by fitting generalized linear mixed models (GLMMs) with a binary response variable (used = 1, available = 0). We fitted GLMMs by using the glmer function of the lme4 package. Predictor variables such as dSH, dAA, dR, dFT, Alt, EType and tACevent were used in the model. The stratum-ID was included as a random effect in the model. All numerical predictors were scaled [(x-mean)/SD] before running any model to improve glmer convergence (Bates et al. 2015). The variable screening revealed no collinearity (Pearson coefficient $|r_p| < 0.6$) and multicollinearity (Variance Inflation Factor, VIF < 3) among predictors.

We first created a GLMM with a full model structure, based on our expectation on the effect of the predictors in driving wolf resource selection. As we were interested in evaluating whether the selection by the wolf changed after the AC event, all environmental predictors (dSH, dAA, dR, dFT, Alt, EType) were included in the model in interaction with the binary variable tACevent (before/after). We chose the best model by applying a manual step AIC procedure, iteratively removing the worse predictor (that with the highest *P*-value) and re-running the model until achieving a model with the lowest AIC. In so doing, we removed all the predictors that contributed to increase the model AIC from the best model. Finally, the beta coefficients estimated by the most parsimonious model were entered into the resource selection function to obtain RSF scores, which are proportional to the probability of selection. The RSF scores were used to represent the scenarios predicted by the model. We implemented the RSF separately at both temporal scale (large: using data from 07/06/2021 to 17/10/2021, short: from 30/07/2021 to 08/09/2021).

As a final step, we investigated whether the wolf changed its predatory behaviour after the AC event. We analysed the number of wildlife prey and livestock prey found in the cluster sites identified by using the wolf location data before and after the AC event. We used a chi-square contingency test (with Yates correction) to test for a significant association between the two categorical variables prey type (livestock/wildlife) and period (before/after AC).

Results

The AC action using rubber bullets was successfully achieved on August 19, 2021, during the first day that operators attempted the AC approach. The operators fired two rubber bullets hitting the wolf on the thigh of the left hind leg both times, following the protocol. In the following days, the operators did not see the wolf during the survey. During AC, the wolf did not respond aggressively. It quickly fled over 800 m, where it lied down for about 30 min. It subsequently resumed running away at a slower speed until it arrived at the rendezvous site where it remained until the following night. Thanks to videos recorded in the days after the AC event by the camera traps, a vet was able to check the wolf's health condition: no evidence of injury was detected; this was also confirmed by proper movement parameters: it was possible for the wolf to double the distance travelled during the night after AC.

The spatial behaviour of the wolf changed significantly after the AC event with respect to the period before it, as evidenced by an absence of overlapping confidence intervals estimated for the home range size and the mean daily distance. At the large temporal scale, the home range size increased by 170%, from 38.36 km^2 (CI 95% = 32.71–44.95) to 103.58 km^2 (CI 95% = 125.06–84.09; Fig. 1). The overlap between the two home ranges was 98.47%, i.e. the former home range was included in the one after AC. After the AC event, the mean daily distance travelled by the wolf varied significantly, from 31.33 km/day (CI 95% = 30.35–32.31) to 42.88 km/day (CI 95% = 41.64–44.24) and from 26.82 km/day (CI 95% = 25.24–28.42) to 46.56 km/day (CI 95% = 44.27–48.88) when considering the large and the short temporal scale, respectively.

During the monitoring period, the localisations of the wolf were on a mean distance to the reference shepherd of 2464 ± 8 m before the AC and 3715 ± 11 m after it. The mean distance to roads was 109 ± 0.5 m before the AC event and 151 ± 1 m after it. Finally, the wolf before the AC event was at 435 ± 1 m away to anthropogenic structures, while after it the mean distance was 406 ± 1 m.

At the short temporal scale, the most parsimonious RSF model included dSH, dAA, dR, dFT, Alt, EType, tACevent and the interactions tACevent*dSH, tACevent*dR, tACevent*dFT, tACevent*Alt and tACevent*EType (Table 1). Predictions of this model showed that after the AC event the wolf increased the selection for higher altitudes and for areas close to fallen trees (Fig. 2). More in general, the wolf increased selection for open habitats (Table 1). While during the 20 days before the AC event, the wolf strongly selected areas close to reference shepherd, during the following 20 days this pattern of selection disappeared (Fig. 2). On the contrary, before the AC event the distance to roads seemed not to influence spatial selection by wolf, but during the 20 days after the AC event it clearly selected areas farther from roads (Fig. 2). The wolf showed a selection for areas distant from anthropogenic structures, regardless of the AC event (Table 1).

Analysis at the large temporal scale confirmed the results on the short scale, except that the most parsimonious RSF model also included the interactions tACevent*dAA

Table 1 Results of the most parsimonious resource selection functions model at the short temporal scale by the monitored wolf before and after the aversive condition event (AC), in the study area located in the north-eastern Italian Pre-Alps (from 30/07/2021 to 08/09/2021)

Coefficients	Estimate	Std. error	z-value	*p*-value
(Intercept)	−5.37	0.17	−30.66	< 0.001
Alt	−0.22	0.11	−2.04	0.042
dR	−0.08	0.06	−1.36	0.173
dAA	0.12	0.04	2.92	0.004
dSH	−2.43	0.14	−17.55	< 0.001
dFT	−1.36	0.11	−12.91	< 0.001
tACevent (After)	1.90	0.19	10.02	< 0.001
EType (Open)	−0.68	0.14	−4.75	< 0.001
tACevent (After) * Alt	0.91	0.13	6.93	< 0.001
tACevent (After) * dR	0.17	0.08	2.16	0.031
tACevent (After) * dSH	2.21	0.15	14.87	< 0.001
tACevent (After) * dFT	0.84	0.12	6.79	< 0.001
tACevent (After) * EType	1.07	0.18	5.85	< 0.001

Alt altitude, *dR* distance from the nearest road, *dAA* distance from anthropogenic and residential areas, *dSH* distance from the reference shepherd, *dFT* distance from the nearest area with fallen trees, *tACevent* binary variable time of the aversive conditioning event (AC), by considering the recording date and time, and the time of the AC event: to all locations recorded before the AC event, we assigned the category "before", while to all locations recorded after the AC we assigned the category "after"; EType Environment type (open = meadows, pastures and arable, land or closed = mixed and deciduous forest)

(Online Resource, Table S1), suggesting a modification of the pattern of selection by the wolf also on account of the distance to anthropogenic structures. In general, the predictions of the best model were in accordance with predictions at the short temporal scale: the wolf increased selection for higher altitudes, for open habitats and for areas close to fallen trees (Online Resource, Figure S1). When considering 59 days after the AC event, a stronger selection for areas farther from roads and a weaker selection for areas farther from anthropogenic areas was detected (Online Resource, Figure S1). Moreover, predictions of RSF model at large temporal scale showed a weak selection for areas close to the reference shepherd (Online Resource, Figure S1).

We identified 49 predations by the monitored wolf: 25 during the period before the AC and 24 after the AC event. The relative number of livestock species (before: $N = 15$, after: $N = 3$) preyed by the wolf varied significantly after the AC event ($\chi^2 = 9.93$; $P = 0.002$), with an increase of predation on wild ungulates with respect to livestock (Online Resource, Figure S2, Table S2). However, it must be noted that the three livestock preyed (two goat and one sheep) were not included in any flock: they were alone in the woods as they had been lost by sheepherders that had moved to the plains. If we consider livestock of the reference shepherd only, the effect of the AC reset out the predations: five

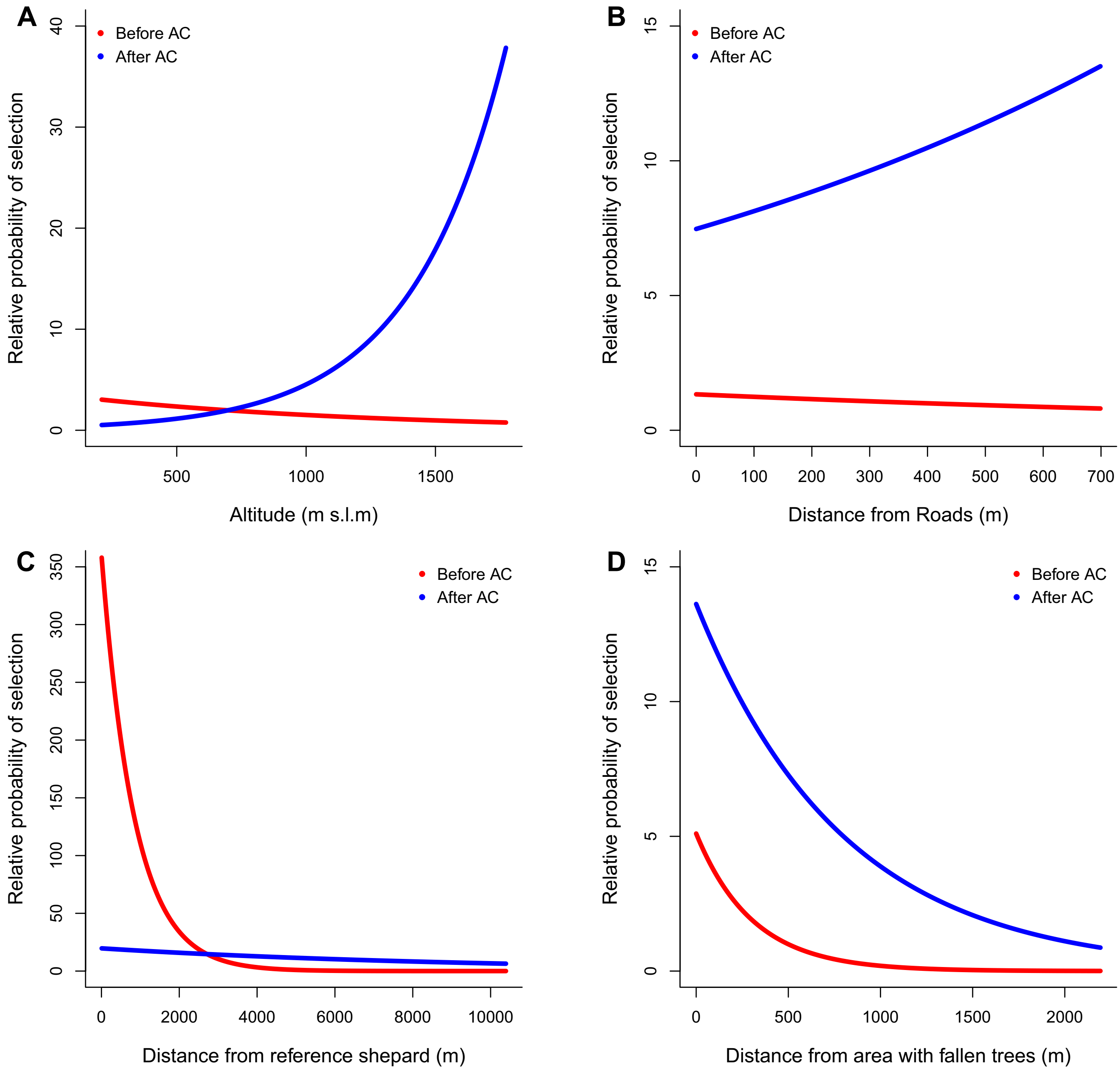

Fig. 2 Relative probability of selection as predicted by the resource selection function, which was built using the wolf observations collected from 30/07/2021 to 08/09/2021 (short temporal scale) in the north-eastern Italian Pre-Alps (Italy). Plots depict the effect of the interaction between time of the aversive conditioning event and A altitude, B distance from the nearest road, C distance from the reference shepherd, and D distance from area with fallen trees. The figure was generated in R

predations were recorded before the AC event and zero predations after it. The experimental wolf stayed with the pack till December 2021 and kept on preying on wild ungulates, then on the 22nd of January 2022, it left the pack and dispersed northward moving 80 km away, reaching an area where it stopped and where on March 14 was filmed with an adult female that became its mate giving birth to 5 pups in May 2022. The monitoring of the wolf ceased on 12 August 2022, when the battery of the collar run out, till then he had preyed upon wild ungulates only.

Discussion

Our study revealed that after just a single AC event, the wolf clearly changed its behaviour during the following 2 months. By taking advantage of unique fine resolution and detailed location data available before and after the AC event, together with a posteriori careful field survey, we showed that the wolf increased its movements, modified its resource selection increasing distances from the shepherd and from roads, and decreased its predation on livestock relying only on stray sheep and goats that were abandoned by shepherds, so ceasing any attack to livestock in farms.

After the AC event, the wolf increased significantly both mean daily distances travelled and the home range size, at both temporal scales (large and short). Daily distance travelled may represent a short-term measure of space requirements that at least in part reflects food resource distribution and foraging strategy (Garland 1983; Carbone et al. 2005). We conjectured that this significant increase in average daily distances travelled may be related to the type of food resource used by the predator. Results on kill rate support this hypothesis: as a matter of fact, the ratio of wildlife and livestock species preyed by the wolf varied substantially after the AC event. Before the AC event, the wolf mainly predated on livestock being typically concentrated in few spots known to the predator, which implied less time and shorter distances travelled to find them. Conversely, wild animals may require more efforts and more time for research since they are generally distributed more uniformly. Moreover, wildlife preys are accustomed to the presence of predators and are able to adopt effective anti-predator behaviours to avoid being predated (Laundré et al. 2001; Caro 2005; Bongi et al. 2008). It has been shown that the domestication process causes inhibition of some behaviours and changes in the quantitative and qualitative nature of responses, such as reactions to predators (Price 1999; Mignon-Grasteau et al. 2005; Brokordt et al. 2006; Ciucci et al. 2020). Thus, when livestock is not adequately protected and managed by farmers, it represents a more advantageous resource than wild prey in terms of cost and benefits trade-off. We hypothesized that the reduction of attacks on livestock by the wolf after the AC event was mainly driven by an increased risk perception around the anthropic areas and activities. However, we cannot exclude that the kill rate modification by the experimental wolf was also influenced by a modification of wild prey availability and behaviour. We cannot also exclude that other members of the pack, which were not collared, may have preyed upon livestock when the experimental wolf was not present, though it is worth noting that the experimental wolf never attacked livestock in farms after AC (but only a stray sheep and two goats) not only during the time he was in the pack area but also when he moved away to create a new pack 80 km north.

Results of the RSF showed that also the spatial selection of the wolf changed significantly during the 2 months after the AC event with respect to the period before it. Most likely, through the AC event the wolf landscape of fear was reshaped (Gaynor et al. 2019). The presence of humans near the sheep's flock, armed with rifles firing rubber bullets, would be perceived by the wolf as a danger, and consequently, also the surroundings would be perceived as a high-risk area (Johnson et al. 2015). Our results support this hypothesis: while before the AC event the wolf strongly selected areas close to the reference shepherd (i.e. the shepherd who suffered more attacks by the wolf and owned the alpine pasture on which we carried out the AC event), during the following 20 days, this pattern of selection disappeared, suggesting that the wolf reduced or zeroed out its visits to the shepherd. The analysis on the large temporal scale showed a weak selection by wolf for areas close to the reference shepherd, suggesting a possible diluted effect of the AC over time. RSF analysis showed that the wolf selected areas distant from anthropogenic structures regardless of the AC event, while selection for areas further away from roads increased after AC, probably because they were perceived by the wolf as higher-risk areas associated to human activity (Eggermann et al. 2011; Muhly et al. 2019).

Our study revealed that a single AC event using rubber bullets succeeded on keeping the wolf away from the shepherd and more in general from preying upon livestock. Our results are consistent with Smith et al. (2020), who showed preliminary results in which in 81% of the cases aversive conditioning by using rubber bullets on wolves was successful in moving predators away from urban areas. This is in contrast with other studies on bears, which showed that the use of rubber bullets did not prevent the predator from returning to urban patches (Rauer et al. 2003; Beckmann et al. 2004; Mazur 2010). Furthermore, in our study case, the effect appears to last beyond a month, specifically for 59 days at least. This is longer than what reported by Beckmann et al. (2004) on black bears, where the AC impact was often not effective beyond a month. Unfortunately, we could not analyse the efficacy of AC in keeping the wolf away from the shepherd for a longer period, since the shepherd left the high-altitude pasture to descend to the valleys 59 days after the AC event. However, the monitoring of the hunting behaviour of the wolf after his dispersal and till the subsequent August allows us to conjecture a long-lasting effect of AC.

Management implications

With the return of large carnivores, there is an increasing need for prevention systems to solve conflicts with humans while preserving these species (Woodroffe 2000; McManus et al. 2015). The use of rubber bullets may be an effective aversive conditioning method to limit the damages caused by large carnivores, to prevent them from approaching urban areas and to avoid illegal solutions to manage the problems. It is, however, imperative that aversive conditioning is implemented as part of a comprehensive wildlife coexistence program (Sampson and Van Patter 2020). In this sense, the use of alternative less invasive preventive techniques (e.g. guard dogs, electrified fencing: Shivik et al. 2003; Shivik 2006; conditioned food aversion: Tobajas et al. 2020), together with the education and engagement of the community and stakeholders should also be promoted to obtain a more compassionate and ethically correct coexistence with wildlife (Bonnell and Breck 2017; Breck et al. 2017; Sampson and Van Patter 2020). A limitation of the present case study is that we were not able to verify the effectiveness of AC on a wider sample size; however, this study represents a first step in gaining knowledge that can be useful for comparison with other studies in the future. Further research evaluating other less risky and harmful aversive conditioning methods (e.g. conditioned food aversion: Tobajas et al. 2020, community-level hazing; Bonnell and Breck 2017) should be conducted to improve conservation of wild carnivores. We encourage other researcher to implement and publish similar studies in order to provide a useful and widely tested tool for administrations and operators involved in wildlife conservation.

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Author contribution Conceived and designed the experiment: M. A.; performed the experiment: M. Z., D. B., S. L., L. C., D. F.; analysed the data: M. Z., F. B.; wrote the paper: M. Z., F. B. All authors read and approved the manuscript.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics statement This research complies with all national and regional laws dealing with ethics and animal welfare. Capture and manipulation protocols were approved by The Italian Ministry of Environment on 5.7.2018 n 0014897 for wolves captures, on 16.8.2022 n 0089653 for the dissuasive action. The research adhered to the ASAB/ABS Guidelines for the Use of Animals in Research.

Conflicts of interest The authors declare no competing interests.

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