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A new large-scale index (*AcED*) for assessing traffic noise disturbance on wildlife: stress response in a roe deer (*Capreolus capreolus*) population

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Abstract Anthropogenic noise is a growing ubiquitous and pervasive pollutant as well as a recognised stressor that spreads throughout natural ecosystems. However, there is still an urgent need for the assessment of noise impact on natural ecosystems. This article presents a multidisciplinary study which made it possible to isolate noise due to road traffic to evaluate it as a major driver of detrimental effects on wildlife populations. A new

Highlights - Noise modelling tools help to assess noise pollution impacts on natural habitats
- Low-traffic roads may degrade large natural areas
- *AcED* index may assist in conservation and transport infrastructure planning
- *AcED* and FCM analysis are useful indices for ecological monitoring in large areas
- Traffic volume might be associated with FCM concentration level in ungulates

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indicator has been defined: *AcED* (the acoustic escape distance) and faecal cortisol metabolites (FCM) were extracted from roe deer faecal samples as a validated indicator of physiological stress in animals moving around in two low-traffic roads that cross a National Park in Spain. Two key findings turned out to be relevant in this study: (i) road identity (i.e. road type defined by traffic volume and average speed) and *AcED* were the variables that best explained the FCM values observed in roe deer, and (ii) FCM concentration was positively related to increasing traffic volume (road type) and *AcED* values. Our results suggest that FCM analysis and noise mapping have shown themselves to be useful tools in multidisciplinary approaches and environmental monitoring. Furthermore, our findings aroused the suspicion that low-traffic roads (< 1000 vehicles per day) could be capable of causing higher habitat degradation than has been deemed until now.

Keywords Cortisol metabolites · Noise modelling · Physiological stress · Low traffic road · National park · Road ecology

Introduction

Land protection has become an increasingly common strategy for conservation and the global network of protected areas cover more than 12% of the planet's land surface (Geldmann et al. 2013; McDonald and Boucher 2011). However, managing and conserving nature are not easy tasks and monitoring and research are considered to be among the main weaknesses in relation to natural areas management and governance (Françoso et al. 2015; Leverington et al. 2010). In parallel, ecotourism rates in national parks have increased and global development of road networks and the growth in motor vehicle use are damaging nature and threatening ecosystem functions at continental scales (Eagles 2002; Ibisch et al. 2016). Also, most visitors to national parks usually arrive by car and this also extends these negative impacts in supposedly low altered protected areas (Garriga et al. 2012; Mace et al. 2013; Pettebone et al. 2011). Roads affect biotic and abiotic components of ecosystems, nd traffic noise pollution is regarded as being one of the most widespread impacts due to road use (Coffin 2007). Indeed, transportation infrastructures have dramatically changed the acoustic environment to the extent that the ecological effects of anthropogenic noise have emerged as being a major conservation issue in from urban to aquatic and terrestrial natural ecosystems (Barber et al. 2010; Farina 2014).

A large body of research showing the effects of noise pollution on wildlife has been published during the last two decades (McClure et al. 2013; Ware et al. 2015). However, most terrestrial studies are focused on bird species that rely on vocal communication while other taxa are underrepresented in published literature (Shannon et al. 2016). In general, researchers usually refer to a road-effect zone due to emissions from traffic in which ecological impacts extend outwards from a road (Jaeger et al. 2005; Shanley and Pyare 2011). Road-effect zones are correlated with a decline in species richness and density in road surroundings (Forman and Alexander 1998; Forman et al. 2003; McClure et al. 2013; Parris 2015) and traffic noise is suggested as being the primary cause (Ware et al. 2015). Although contrasting examples can be found, in particular a relatively frequent intense use of road verges by some carnivore species (Mata et al. 2017). Anyway, this zone of disturbance is frequently alluded to as constant-width bands and, depending on traffic volume, a road-avoidance zone several hundred meters wide (of between 100 and 5000 m) has been suggested for ungulates (Forman et al. 2003; Leblond et al. 2013). However, traffic noise patterns fluctuate in time and space, and contour lines representing equal levels of noise exposure (isolines or isophone curves) on a map should not simply be plotted as parallel lines to roads, even less so in the case of non-flat terrains (Coffin 2007). In addition to traffic volume, noise emissions also depend on the type of vehicle, average speed, road slopes, type of pavement and the surrounding environment (orography obstacles, vegetation, buildings, background noise, etc.) (de Kluijver and Stoter 2003; Iglesias Merchan and Diaz-Balteiro 2013). Therefore, reporting on traffic disturbance on wildlife may be misleading in the absence of more descriptors than traffic volume. Thus, developing indicators that simultaneously address such a variety of parameters is a key challenge for the assessment of road traffic annoyance to wildlife.

Up until now, few studies have isolated the impacts of noise from confounding factors (Blickley et al. 2012; Shannon et al. 2014). High ambient sound levels may

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inhibit perception of sounds used for communication, orientation or detection of predators and this disturbance may cause an increase in energy expenditure by animals dealing with noise pollution (Brumm 2004; Farina 2014; Parris et al. 2009). On the other hand, the stressful non-auditory effects of chronic exposure to noise has been documented on the basis of laboratory and animal experiments (Babisch et al. 2005; Wright et al. 2007; Blickley et al. 2012), despite animals may show a rapid habituation to noises that do not pose a threat to them (Pater et al. 2009). This makes it particularly complex to find a link between relevant parameters for free-living animals and exposure to noise levels (Coffin 2007). The lack of any noise reference levels or dose-response data constitutes a weakness in the knowledge of behavioural and physiological stress reactions in wild mammal populations and the intensity of road disturbance (Leblond et al. 2013; Navarro-Castilla et al. 2014). Consequently, the long-term effects of noise pollution on the health and wellbeing of animals is one of the least understood and most common threats that remains unattended in protected natural areas (Brown et al. 2012; Lynch et al. 2011; Pater et al. 2009; Wright et al. 2007). However, insight into such potential effects over long time periods (e.g. seasonal, yearly) and/or at the landscape scale under natural conditions is crucial (Slabbekoorn et al. 2010; Shannon et al. 2014, 2016).

In this context, non-invasive methodologies have been developed to index physiological stress levels for an array of different animals (Keay et al. 2006; Millspaugh and Washburn 2004). In particular, measuring glucocorticoid metabolites concentration in faecal sample collections is a good choice for assessing physiological status of wild animal populations from ethical standpoints (Barja et al. 2011, 2012; Zwijacz-Kozica et al. 2013), because it does not require capturing and handling animals, unlike plasma cortisol concentrations. Thus, the assay results are not altered because of stressful events such as venipuncture (Brearley et al. 2012; Keay et al. 2006; Touma and Palme 2005). Besides, it allows the sampling of wide-scale territories with an affordable time and resource investment (Escribano-Avila et al. 2013). Consequently, in recent years, a large variety of studies have been conducted in order to assess the physiological stress response of wild mammals that cope with their challenging environment through a less invasive way. As for instance ungulates in relation to their predation risk, habitat suitability, seasonality, reproductive condition, diet quality and human disturbance (Creel et al. 2009; Escribano-Avila et al. 2013; Taillon and Côté 2008). Ungulates are among the vertebrate species with strongest responses to road disturbance and they have been reported as avoiding proximity to even small roads (Fahrig and Rytwinski 2009; Gagnon et al. 2007; Rytwinski and Fahrig 2012). Among ungulates, the roe deer (*Capreolus capreolus*) is a species that typically inhabits forested habitats and their populations are highly affected by road presence, with fewer individuals in the proximity of roads and fragmented populations (Coulon et al. 2006; Fahrig and Rytwinski 2009; Hewison et al. 2001). Also, it has been shown that road proximity is an important factor increasing roe deer stress levels (Zbyryt et al. 2017).

The aim of the present study is to propose a new ecological indicator that contributes to measuring the impact of noise pollution on wildlife. We investigated the disturbance effects on wildlife due to traffic noise pollution on the basis of common methodologies for environmental noise assessment and non-invasive measurements of the physiological status of wild animal populations in large areas. The potential association between faecal cortisol metabolite (FCM) levels and traffic noise pollution from two low-traffic roads was analysed in samples from a wild roe deer population located within a protected natural area in Central Spain. We defined a new ecological indicator called acoustic escape distance (*AcED*), in order to assess the potential detrimental impact of noise pollution on wildlife, in terms of the effort to be done for avoiding disturbance through an acoustic tension zone due to road traffic. We hypothesised that the higher *AcED* levels, the more likely it will be for the FCM concentration to be increased.

Methods

Study area

The study area covers almost 15,000 ha of the Upper Lozoya valley (Spain). The latter is located between the two mountain chains making up the Sierra of Guadarrama, which forms part of the Spanish Central System. It is a predominantly siliceous valley and its altitude ranges approximately between 1100 and 2400 m. In the study area, there are four main vegetation formations. Lower areas are characterised by Pyrenean oak forests (*Quercus pyrenaica*), locally replaced by Scots pines (*Pynus sylvestris*) or shrub communities at altitudes of between approx.1700 and 2100 m. Finally, summits are dominated by open montane grasslands, although the altitudinal range vegetation limits result from a long-term human interference (Mugica et al. 1998).

Scots pine and Pyrenean oak woods cover the greatest extension of favourable habitats for roe deer within the study area (Fig. 1). The abundance of roe deer in the study area is well known (ranging between 4 and 7 roe deer per 100 ha) because the valley's population has been monitored by different sampling methods for almost a decade (Horcajada-Sánchez and Barja 2015). Its abundance is directly related to forest size, with similar roe deer densities being detected in oak and pine forests, and significantly fewer individuals being found in shrublands or valley bottoms (Horcajada-Sánchez and Barja 2015; Sáez-Royuela and Tellería 1991). Finally, high mountain shrubs and pastures on the summits are dominated by an over density population of the Iberian Ibex (*Capra pirenaica*). Regarding mortality risks, the only natural predator in the study area is the red fox (*Vulpes vulpes*) and several studies have reported a close correlation between young survival and fox abundance (Jarnemo and Liberg 2005). However, red fox predation incidence is considered to be occasional and the range of roe deer distribution in Sierra of Guadarrama is higher today than during previous decades (Escribano-Avila et al. 2013). Also, hunting is allowed in the Park, so that this species abundance has been quantified in detail for decades. The hunting season for roe deer males usually begins on 1st April and ends on 30th June in the Region of Madrid and both, males and females, can be hunted from 1st to 30th September. Lastly, despite roe deer being an ungulate frequently involved in vehicle collisions throughout European woodlands (Coulon et al. 2008; Kämmerle et al. 2017; Malo et al. 2004), a specific study on the incidence of roadkills of vertebrates in the park made during 2 years revealed, on average, one casualty of roe deer per road and year (Espinosa et al. 2012).

The study area is currently under Sierra of Guadarrama National Park authority management and there is an intensive recreational use due to its proximity to Madrid. The Upper Lozoya valley is crossed by two regional roads (M-604 and M-611), which are two-lane, narrow, paved mountain roads, with an annual average daily traffic (AADT) of approximately 850 vehicles (M-604) and 400 vehicles (M-611) respectively according to the Regional Transport and Infrastructure Department official data. Heavy vehicles represent about 6% of AADT on both roads, and traffic speed has been estimated at 60 km/h (50 km/h for heavy vehicles) on road M-604 and at 55 km/h (45 km/h for heavy vehicles) on road M-611. Finally, it is worth mentioning that the valley is recognised as being an excellent example of a multiple use forest (e.g. conservation, recreation, timber, grazing, hunting, mushrooms) (Caparrós et al. 2001).

Faecal sample collection

We established seven plots located up to a distance of 1000 m from the road margins. Plots ranged from 105 to 170 ha (mean area 140 ha/plot) and covered approximately 1000 ha of roe deer potential habitat surrounding the roads. Sample collection was performed monthly from October 2009 to March 2010. Within each plot, pathways typically used by roe deer were intensively surveyed for fresh scats at dawn and sunrise (only fresh faeces with a moist layer of green mucus and no signs of dehydration were collected). In order to control the effects of anonymous sampling, we established 15 linear transects to maximise the sampling area and the number of animals sampled in each plot. We followed the methodology recommended by Huber et al. (2003) who found no significant differences in FCM levels when comparing known and anonymous samples from a population, and they declared the technique as being reliable. Six pellets of each faeces were collected with gloved hands and placed in plastic tubes that were immediately stored in a portable freezer at −20 °C. We obtained between 9 and 15 samples in each plot area and the size of the plotted ellipses in Fig. 1 represents the real fieldwork effort made to collect them because of the variables influencing it, such as accessibility, animal density, forest cover, etc. In total, 81 faecal samples were collected and their location coordinates were annotated. In this way, 57 samples were assigned as being closer to road M-604 and 24 were assigned as originating from closer to road M-611 according to their Euclidean distance measurements. Finally, the samples were taken to the laboratory and maintained at −20 °C in a conventional freezer until assayed (Sheriff et al. 2011).

Faecal cortisol metabolite assay

Cortisol metabolites were extracted from faecal samples according to the procedure described by Escribano-Avila et al. (2013) and were assayed by a specific

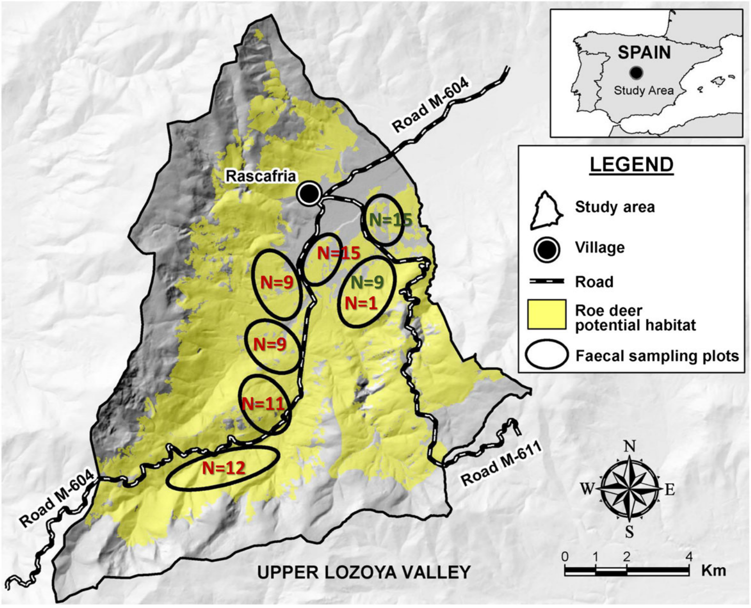

Fig. 1 Study area location and faecal sampling plots in potential roe deer habitat (8300 ha of pine and oak woods). Note: N = number of faecal samples per plot and assigned road (red colour = samples closer to road M-604; green colour = samples closer to M-611)

commercial enzyme immunoassay (EIA, DRG Instruments GMBH, Marbug, Germany), previously validated by ACTH-challenge as an indicator of physiological stress in roe deer (Escribano-Avila et al. 2013). A parallelism test of serial dilutions of extracts was performed with ratios of 1:32, 1:16, 1:8, 1:4, 1:2, 1:1 and curves parallel to those of the standard ($p > 0.05$) were obtained. Intra- and interassay coefficients of variation were 7.1 and 10.9%, respectively. The cross-reactivity of the antibodies with other substances according to the manufacturer was reported when the percentage was over 1%: Cortisol: cortisone 45%, progesterone 9%, deoxycortisol < 2%, dexamethazone < 2%; progesterone: 11-desoxycorticosterone 1.10%. The assay sensitivity was 2.5 ng/mL. FCMs are expressed as nanograms per gram of dry faeces (ng/g).

Noise modelling and sound pressure level increase

Strategic noise maps are elaborated at European Union level for the global assessment or prediction of people exposed to environmental noise pollution in a given area. As a result, a set of noise maps are calculated using the harmonised noise indicators L_{den} (day–evening–night equivalent sound pressure levels), to assess global annoyance, and L_{night} (night-time noise indicator), to assess sleep disturbance (EC 2002). L_{den} is considered to be the A-weighted long-term average sound level index (in decibels, dB) characterising a 24-h period (24 h) in a typical year. However, it is defined by a formula that includes human-perceived subjective penalties (Paunović et al. 2009). In this regard, the European Noise Directive 2002/49/EC allows Member States to use supplementary indicators in order to monitor or control special noise situations, and a weak treatment of noise pollution has been detected in relation to natural areas in Europe (EEA 2014). Alternatively, the equivalent continuous sound pressure level index (L_{eq}) is considered the most commonly used descriptive environmental noise index not including human-perceived subjective penalties (Cowan 1994; Pater et al. 2009; Paunović et al. 2009). Noise maps allow the study of large areas; they are mostly made by computation and, whenever possible, are validated by measurements performed at certain locations (Makarewicz and Galuszka 2011; Mioduszewski et al. 2011). Therefore, a L_{eq} 24 h noise map (that represents an average 24-h period in 2010 within the study area) was calculated from the set of strategic noise maps for roads in the park (Iglesias Merchan and Diaz-Balteiro 2012) and noise levels at faecal sample sites were extracted. The L_{eq} 24-h calculations were performed with Predictor™ Analyst 7810 software version 3.2 (Brüel and Kjær 2012). In this way, we obtained the sound-pressure level caused by traffic roads at each faecal sampling location on an average day (Supplementary data, Appendix A). We also took field measurements in three monitoring stations considering the European Good Practice Guide

(WG-AEN 2006) in order to validate the noise map (Supplementary data, Appendix B).

Noise disturbance assessment

In spite of the common worldwide use of L_{eq} index for assessing environmental noise pollution, it cannot be considered to be a direct measurement of annoyance, although annoyance is dependent on the noise level (Brüel and Kjær 2001; Ouis 2001). In this framework, considering physical models based on the spreading losses in outdoor sound propagation could help to assess the potential impact of noise pollution (Barber et al. 2010; Reed et al. 2012). Thus, we proposed to calculate the sound pressure level increase (N, in dB) caused by traffic noise over the characteristic sound level of the natural environment (L_{nat}) within the study area, which represents the quiet environment in the absence of road traffic noise. A general expression for spreading loss (N) between any two positions for a receiver at distances d_1, d_2 (in metres) from an acoustic linear source can be given in the form (Crocker 1998):

$$N = 10\, log_{10}\left(\frac{d_2}{d_1}\right) \tag{1}$$

where d_1, d_2 are the distances between the noise source (i.e. the road) and the closest (i.e. receiver at point location 1) and farthest (i.e. receiver at point location 2) positions respectively (Fig. 2). In our case, d_2 is the unknown variable that means the theoretical maximum distance needed to walk away from road margins to completely avoid traffic noise interference. Solving Eq. (1) for the distance d_2, we obtain Eq. (2):

$$d_2 = 10^{\left(\left(\frac{N}{10}\right)+log_{10}(d_1)\right)} \tag{2}$$

Finally, analogously to terms like 'flight distance', 'escape distance', 'distance to refuge', etc. frequently used to measure flight distances in wild animals (Stankowich 2008), we have called 'acoustic escape distance' (*AcED*) the difference of value between distances d_2 and d_1 as illustrated in Eq. (3), representing the distance to which animals should go to keep themselves in a comfortable area of natural sounds audibility:

$$AcED = (d_2 - d_1) \tag{3}$$

Thus, the variable *AcED* gives the distance from faecal locations (point location 1) to which animals should move in order to totally reduce the increased N decibels due to traffic noise over the natural acoustic ambient (Fig. 3). *AcED* values were calculated considering each sample location in ArcGIS 10.5.1 (ESRI 2017).

Determination of the natural ambient sound level (L_{nat})

The calculation of sound pressure level increase (N) caused by traffic noise over the characteristic sound level of the natural environment requires establishing a reference value for the latter, which we called L_{nat} and which was measured in decibels. However, natural ambient sounds may vary per vegetation cover (that attracts different animals), running water, terrain features, weather conditions, time or season, among other factors (Pijanowski et al. 2011) and a clear, consensual method for assessing natural quietness or natural ambient sound levels is still lacking (de Coensel and Botteldooren 2006). Besides, natural areas are supposed to be under human activities causing little disturbance and, therefore, the natural ambient sound concept frequently becomes equivalent to the background noise level (Gjestland 2008), which in practice has to do with managing quietness in the countryside in accordance with the criteria for quiet areas in Europe (EEA 2016). In this sense, EEA (2016) suggests a reference value L_{90} of 30 dB. The annotation L_{90} refers to the sound level (L), in decibels, exceeding 90% of a measurement time. Nevertheless, a continental scale map of the natural sound levels recently published by the National Park Service of the USA was calculated on the basis of L_{50} (50th percentile). Therefore, considering field work constraints such as absence of anthropogenic noise and of accessibility for instrument placement, as well as the need to balance the efforts of the working scale and details required for this study, we decided to search for a single site location which fulfilled two key criteria for estimating natural quietness within

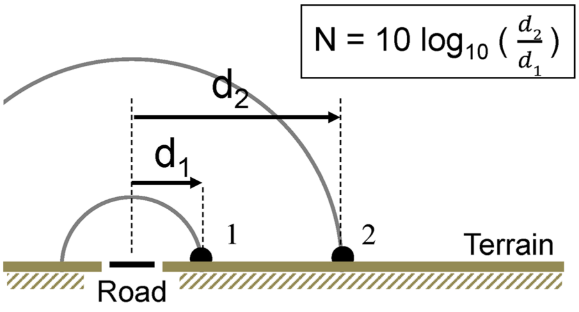

Fig. 2 Hemispherical sound propagation from a linear source

the study area: first, it should be located relatively close to a road and secondly, it should offer time interval opportunities for perceiving enough quietness at the same time. We also took into account the recommendations on principles and methods stated in ISO 9613-2 in field measurements.

Field measurements for adopting a global reference value of the natural ambient sound level consisted of three measuring and recording intervals 5 min long (sound pressure level logged in slow response mode, every 1 s) alternated with 5-min interval breaks between records. Finally, we estimated a global reference value for L_{nat} and we adopted the decibel value at L_{75} based on the method described by Falzarano (2005) for characterising the natural sound level. The measurement station was located in a potentially quiet area in the Scots pine wood (Supplementary data. Appendix A) and L_{nat} resulted in being approximately 30 dB (Supplementary data. Appendix B). That value is usually considered to be a valid reference level for natural

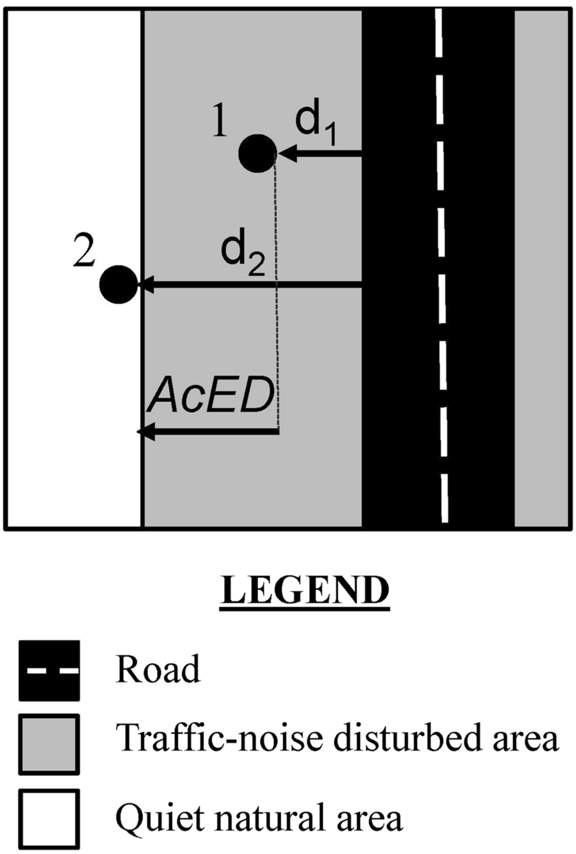

Fig. 3 Graphical representation of the acoustic escape distance (*AcED*) based on the sound propagation loss

background noise in forests and rural lands in Europe (Gjestland 2008; Hernández et al. 2013). It is also the same value found by Iglesias et al. (2014) when characterising the acoustic environment beside the Lagoon of Peñalara, in spite of being a place very much frequented by tourists located in the study area, where people quietly rest for a while after a hiking route.

As the resultant sound pressure level of multiple sources is determined by logarithmic addition, it is noted that adding two sound pressure levels of an equal value (doubling the acoustical energy) will give a 3 dB increase at the measurement point. On the other hand, when adding two sound pressure levels that differ by 10 dB and over, the higher level is the resultant total level (and the sound pressure level remains unchanged at the measurement point). In other words, a change of 3 dB is considered as being just noticeable by a person with normal hearing, and a change of 10 dB is perceived as doubling or halving the sound level (Cowan 1994). Therefore, we also calculated the distance from faecal sample sites to the 20 and 30 dB contour lines (isophone curves from the road traffic noise map) as other potentially explanatory variables to be considered in our study. These variables were called, respectively, *Iso20* and *Iso30*.

Data analysis

Data were analysed by general linear models (GLM) with Gaussian error structure. Assumptions of normality and homogeneity of variances were checked in the residuals. A preliminary exploration of the data revealed that the variables *N* and L_{eq} and *Iso30* were highly correlated with distance to roads (*Distance*) from the sample sites ($r = -0.83$, -0.86 and -0.80, respectively), so we decided to include only *Distance* in the model.

In order to identify the variables most relevant to FCM levels, we developed a full model, including all the uncorrelated explanatory variables, and performed a model selection procedure with the Akaike Information Criterion for small sample size (AICc, Burnham and Anderson 2002). The full model for the stress levels (FCM) included the following explanatory variables: *AcED*, *Road* identity (subsamples M-604 and M-611), *Distance*, *Month* (subsamples October, November, December, January and February) and *Iso20* (for a full explanation about the variables, see above).

All the subsets of the full model were evaluated by AICc, and those within 7 points of AICc from the best model were retained for interpretation, as all of them receive some support from the data and should be considered competitive models for interpretation (Burnham and Anderson 2002; Richards 2005). The relative importance of explanatory variables was assessed through Akaike weights (*wi*), computed as the sum of the Akaike weights of the models containing the variable of interest. A higher value of the *wi* represents a higher relative importance of the variable in the dataset. All statistics were done in R (R Core Team 2017), using the library MuMIn for model selection and averaging (Barton 2017).

Results

Faecal sample sites showed a homogeneous distribution from both road (M-604 and M-611) margins (Fig. 4a) and the Mann–Whitney *U* test confirmed that faecal locations were not distributed statistically significantly closer to one road or another ($U = 669$, $p = 0.877$). Mean L_{eq} levels in sample sites were 32.8 dB in samples closer to road M-604 and 30.8 dB in samples closer to road M-611, ranging from 24.6 to 45.4 dB and from 22.4 to 43.9 dB, respectively. That means a mean sound pressure level increase (*N*) due to traffic noise over the sound level of the natural environment of about 5.1 and 4.3 dB in sample sites closer to roads M-604 and M-611, respectively.

In relation to cortisol metabolite data, the FCM mean concentration equalled 1213.7 (SD = 581.3) ng/g in the total population's faecal samples and it ranged from 287.2 to 3314.5 ng/g. When dividing the sample data into subsamples according to the categorical variables (Table 1), the mean FCM concentrations from samples closer to roads M-604 and M-611 were 1299 (SD = 78.2) and 1011.0 (SD = 105.4) ng/g, respectively, and their amplitude of values was clearly higher in samples closer to road M-604 (Fig. 4b). In relation to the sampling date, the mean FCM concentrations oscillated alternately in the different months (Fig. 4c). FCM maximum levels were reached in December (828.9 ng/g) and minimum levels in November (1470.3 ng/g) (Table 1).

The model selection identified 31 competitive models within 7 points of AICc (Supplementary data. Appendix C). Akaike weights of retained models were low, ranging from 0.140 in the best model to 0.005 in the last model selected. The null model, containing only the intercept was also included in the model selection table, as a competing candidate. All the explanatory variables included in the full model were represented in the selected models, although with varying degrees of relative importance, showing that although all the variables adjust up to a point to the data, some of them present higher relevance (Table 2). *Road* identity was the variable most relevant to the FCM values, followed by *AcED*. The variable related to the distance from sample sites to the 20 dB isophone curve (*Iso20*) was the least important variable.

Similarly, the model containing only *AcED* as an explanatory variable had an Akaike weight of 0.046, whilst models containing only *Iso20* presented weights of 0.010 (Supplementary data. Appendix C).

Although the averaged model only showed significant effects for *Month*, with higher values in November (Table 3), the coefficients of the variables pointed to higher stress levels for individuals next to road M604, a weak decrease in FCM levels with an increasing distance to roads (*Distance*) (Fig. 4d) and a stronger positive relation between FCM levels and increasing *AcED* values (Fig. 4e). Additionally, the coefficient for *Iso20* was almost zero, pointing to a lack of relationship between this variable and the FCM values (Fig. 4f).

Discussion

Our results showed that *Road* identity was the variable most relevant to the stress values and *AcED* was more explanatory than other measurements of noise pollution (i.e. *Iso20*). In relation to *Road* identity, FCM concentration mean values were 1299.1 ng/g in subsample closer to road M-604 and 1011.0 ng/g in subsample closer to road M-611, which can be considered as being a just noticeable difference in GMN values compared with the very explicit work of Dehnhard et al. (2001). They assessed the physiological response of four roe deer exposed to controlled stressful situations (loading and transport) with and without the administration of a long-acting tranquilliser, and it resulted in a difference of about 550 ng/g in FCM concentration mean levels between both groups. This is a finding to be noted, because traffic volume is the main distinguishing characteristic between the roads M-604 and M-611 and traffic volume has been designated as being a key factor when assessing the ecological effects of roads and, particularly, in relation to road avoidance behaviour

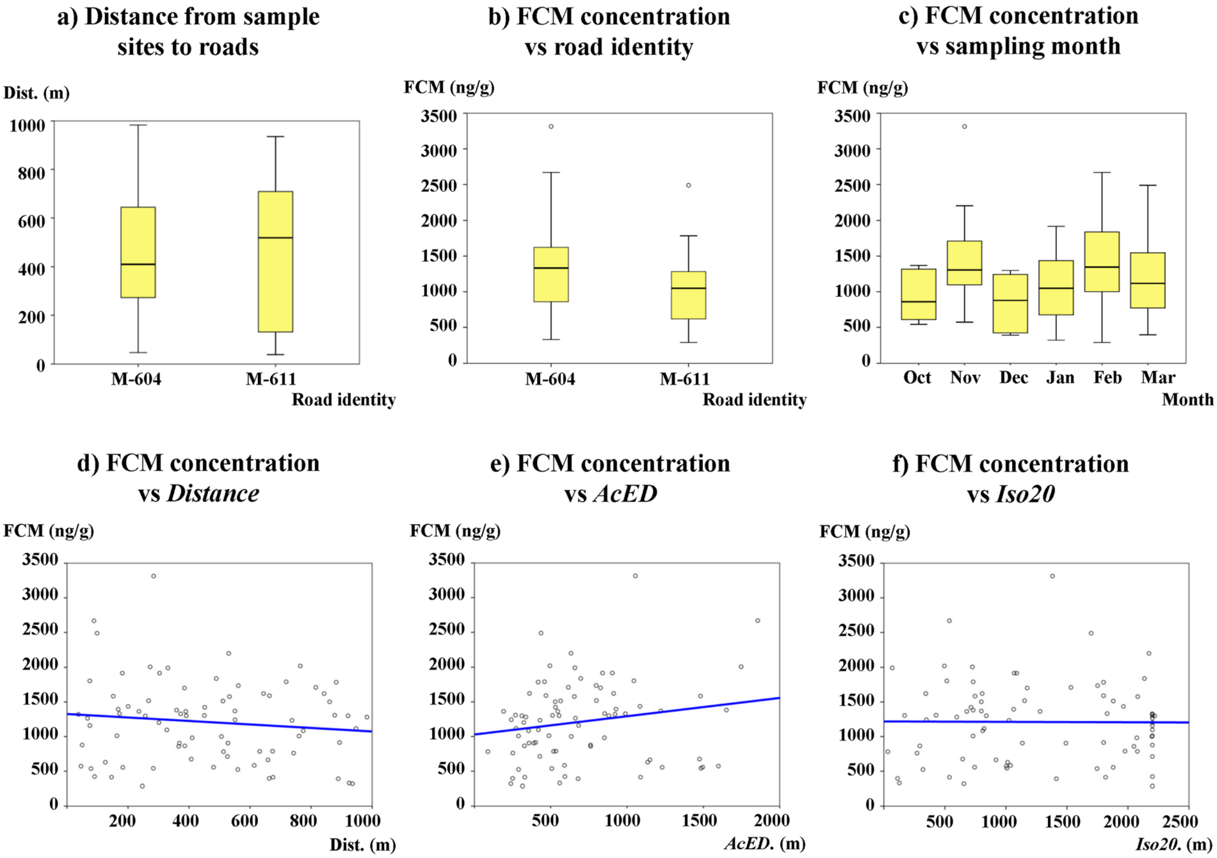

Fig. 4 Box-plot and scatter-plot diagrams. **a** Distance to roads from faecal subsamples grouped according to their closest road (*Road* identity). **b** Faecal cortisol metabolites (*FCM*) concentration in roe deer faecal subsamples grouped according to their closest road (*Road* identity). **c** FCM concentration in faecal subsamples grouped according to the sampling month (*Month*: October, November, December, January, February and March). **d** FCM concentration in faecal samples in relation to their distance to roads (*Distance*). **e** FCM concentration in faecal samples in relation to their acoustic escape distance (*AcED*). **f** FCM concentration in faecal samples in relation to their distance to the 20 dB isophone curve (*Iso20*)

Table 1 Levels of measured FCM (ng/g) in roe deer faecal samples

Variable	Sample/subsamples	n	Mean (range)
FCM	Total population	81	1213.7 (287.2–3314.5)
	(Road identity)		
	M-604	57	1299.1 (329.81–3314.5)
	M-611	24	1011.0 (287.2–2488.7)
	(Month)		
	October	7	946.1 (542.3–1369.3)
	November	17	1470.3 (572.4–3314.5.)
	December	9	828.9 (390.7–1299.1)
	January	18	1082.0 (320.0–1917.0)
	February	18	1381.9 (287.2–2668.6)
	March	11	1190.9 (395.7–2488.7)

FCM faecal cortisol metabolites

(Charry and Jones 2009; Grilo et al. 2015; Jaeger et al. 2005). Currently, a traffic volume of 1000 vehicles per day appeared to be a preliminary accepted threshold value to classify roads with regard to their potential for causing ecological effects (Selva et al. 2011). Nevertheless, AADT is approximately 850 vehicles in road M-604 and 400 vehicles in road M-611 within the study area.

Apart from that, the response to roads with a scant traffic flow is considered to be different from the effects of chronic disturbance along busier roads (Forman and Alexander 1998; Jaeger et al. 2005). On the other hand, 'busier roads' is a definition frequently used when referring to roads whose AADT is higher than 5000–10,000 vehicles in rural areas and higher than 20,000–50,000 vehicles in nearly urban areas (Forman 2000; Grilo et al. 2012; cita). Therefore, the resulting FCM concentration difference between both roads is a finding that strengthens the substantial evidence which already exists regarding the detrimental impacts of traffic disturbance on wildlife (Ware et al. 2015). Even more so when noting that the concentration of cortisol metabolites in a faecal sample is not an immediate response to stressors, because FCM production requires a species-specific time period (Creel et al. 2002; Möstl and Palme 2002). In this context, we proposed the acoustic escape distance (*AcED*) as a measurement of disturbance perception in relation to the energy cost needed to move away from disturbing noise sources. In short, *AcED* gives the maximum theoretical distance to which animals should move from a given location subject to a given sound pressure level to keep themselves in a comfortable acoustic environment of natural sounds stimuli without traffic noise disruption. Unlike *Iso20*, that is the shortest distance calculated from a given location to the actual 20 dB isophone curve independently of the sound pressure level to which the animal is exposed at the point of departure. The *Iso20 values* can be directly measured on a map, but it only considers a single possible direction of movement (the minimum distance) between all the theoretical possible routes to the aforementioned isoline. Nevertheless, *AcED* is not an actual distance to be mapped despite being measured in meters.

The fact is that *AcED* was the explanatory variable most relevant to the stress values after *Road* identity, showing a positive relationship between stress levels and increasing *AcED* values. Note that *AcED* means an estimation measurement of the potentially maximum effort to be made through a terrain of acoustic transition within the so-called road effect zone. The latter is where the literature refers to an increasing avoidance and vigilant behaviour in animals that may affect their nutritional and energy intake (Brumm 2004; Ciuti et al. 2012; Forman 2000; Jaeger et al. 2005; Parris et al. 2009; Shannon et al. 2014). Thus, noise pollution represents an invisible source of habitat degradation (Ware et al. 2015) and we can all imagine a road effect zone where sound fluctuations make it difficult to listen and correctly identify sound sources, which brings to mind a concept defined by Farina (2014): the *sonotone*. In this case, *AcED* has to do with the potentially maximum distance to be covered to avoid disturbance through an acoustic tension zone between two co-occurring *sonotopes*, the overlapping zone between the road traffic noise dominance and the more remote *sonotopes* of natural sounds. Therefore, *AcED* can also be considered as the perception of a potential distance, at which the sounds from the natural environment prevail over the road traffic noise in terms of energy. In this way, we tried to approach subjective notions such as noise disturbance or sound perception and to relate them to their potential non-auditory health costs, once that animals move through their home range and FCM levels could be interpreted as a response to repeated or chronic exposure in part of their territory (Blickley et al. 2012).

Despite our sampling protocol not being designed to assess the spatial distribution of animals around the roads, the distance of the faecal samples from sampling plots to the roads is a field datum that was homogeneously distributed from both roads. This is not at all

Table 2 Relative importance of each variable (wi: Akaike weight of the variable: #Models: number of models in the retained set where the variable was present)

Variable	#Models	wi
Road	15	0.66
AcED	15	0.38
Month	15	0.36
Distance	15	0.36
Iso20	15	0.24

Road road identity, *AcED* acoustic escape distance, *Month* faecal sampling month, *Distance* distance (d_1) from sample sites to roads, *Iso20* distance from faecal sample sites to the 20 dB isophone curve

Table 3 Averaged model coefficients

	Estimate	Std. Error	z value	p
(Intercept)	1148.22	309.21	3.690	< 0.001
Road [M-611]	− 293.81	154.56	1.873	0.061
AcED	0.20	0.20	0.985	0.325
Month [January]	171.03	235.85	0.714	0.476
Month [February]	450.05	242.04	1.830	0.067
Month [March]	340.55	250.63	1.336	0.181
Month [November]	543.22	243.50	2.196	0.028
Month [October]	− 34.02	306.41	0.109	0.913
Distance	− 0.27	0.27	0.953	0.340
Iso20	− 0.02	0.10	0.159	0.874

Road road identity, *AcED* acoustic escape distance, *Month* faecal sampling month, *Distance* distance (d_1) from sample sites to roads, *Iso20* distance from faecal sample sites to the 20 dB isophone curve significant and the importance of that particular faecal samples location should not be overestimated. However, it is worth noting as an unexpected finding that differs from the general statement that refers to a trend in ungulates to avoid road surroundings (Coulon et al. 2008; Shannon et al. 2014). On the other hand, we know that roe deer show high levels of site fidelity, dung pellets usually being located in their grazing places (de la Torre 2003). It has also been contrasted that roe deer may exhibit a certain degree of habituation to traffic disturbance (Kämmerle et al. 2017). As a result, individuals living closer to roads are theoretically subjected to greater traffic disturbance (Navarro-Castilla et al. 2014). In this sense, our results revealed an expected decrease in stress levels with increasing *Distance* to roads. However, *Distance* resulted in being the second least important variable to the stress value matched with *Month*.

In relation to traffic disturbance, vehicle speed and traffic volume are considering key factors determining road influence on animals in roadside environments (Coulon et al. 2008). In this regard, it has been observed that the vehicle speed was very similar in both roads, M-604 and M-611 (detailed in 'Methods' section), but that traffic volume resulted double in road M-604 than road M-611. Noise levels are highly dependent on traffic volume and average speed and, consequently, traffic noise emissions notably varied between the neighbourhoods of both roads (Supplementary data, Appendix A). In addition, variations in topography along the roads and trace changes in curves and slopes make it possible for noise doses to be different for individual receivers located at the same distance from the same road and vice versa (Supplementary data, Appendix A. Inset map 'a'). Those scenarios generate a different exposure to noise levels along roads and aversion to disturbed road edges may appear either because of exposure to high noise levels (> 65 dB) or because noise levels heavily mask communication (Proppe et al. 2017). However, avoidance will not occur in the case of unavailable alternative habitats (Frid and Dill 2002) and roe deer may not change their behaviour in response to disturbances (Ward et al. 2004). It is for these reasons that faecal samples may have been found at a homogeneous distance from road margins. On the other hand, glucocorticoid responses may be adaptive in the short term (Creel et al. 2002), with the road traffic noise usually falling below this level before the 1-km road-effect zone (Proppe et al. 2017). Also, roads within the study area have been designated as having a low-traffic volume, because AADT is lower than 1000 vehicles (Eigenbrod et al. 2009; Sharma et al. 2000). Nevertheless, a mean sound pressure level increase (*N*) of about 5.1 and 4.3 dB resulted in sample sites closer to roads M-604 and M-611, respectively. Thus, it is worth mentioning two important facts in this regard. First, a change in sound pressure level of 3 dB is considered to be just noticeable and a change of 5 dB clearly noticeable (Cowan 1994). Second, but not least, it should not be forgotten that non-auditory adverse effects of noise on health are not subject to habituation (Stansfeld and Matheson 2003), so that, therefore, there are many lessons to be gleaned about how animals are affected by noise pollution (Kight and Swaddle 2011).

In this context, increasing exposure to sound pressure levels due to road traffic may become chronic for animals and the potential adverse effects of road disturbance may not be correctly measured whether it is only based in behavioural changes (Gill et al. 2001). Alternatively, it is well-documented that chronic exposure of animals to noise alters the reactivity of their hypothalamic-pituitary adrenal system and it increases the production of glucocorticoids which are broadly interpreted as being a stress response (Kight and Swaddle 2011). In spite of the fact that some authors have doubts about the overwhelming use of glucocorticoids to predict individual and population fitness (Bonier et al. 2009), studies have shown that faecal and plasma measurement of glucocorticoids in animals accurately reflect their physiological state. Furthermore, FCM concentration reliably tracks changes in free glucocorticoid concentrations (Sheriff et al. 2010), and hormonal responses are a reliable fitness indicator in predicting how animals cope with their changing environment at broad spatial and temporal scales (Escribano-Avila et al. 2013; Navarro-Castilla et al. 2014). Consequently, we expected to find a significant relationship between FCM concentration and traffic noise level in sampling sites (L_{eq}) or environmental noise level increase (*N*) due to road traffic. Nevertheless, *N* and L_{eq} were highly correlated with *Distance* (detailed in 'Methods' section), which was found to be the second least important explanatory variable in our models. On the other hand, according to Akaike weights of retained models, *Iso20* (the calculated distance from sample sites to the 20 dB isophone curve according to the road traffic noise map) was unexpectedly the least important among all the explanatory variables analysed. Even though *Iso20* was proposed in the spirit of giving information about the distance to the higher habitat quality in terms of lack of disturbance due to road traffic noise, in other words, the distance from road margins to areas where animals were able to reduce their vigilant behaviour mode and their subsequent potential health costs (Ciuti et al. 2012; Clair and Forrest 2009; Shannon et al. 2014; Stansfeld and Matheson 2003).

In general, our findings support the suggestion of expanding common methods and noise modelling tools, together with non-invasive glucocorticoid metabolite measurements, for detecting and monitoring effects of anthropogenic disturbances on animal populations (Creel et al. 2002; Iglesias Merchan et al. 2016; Blickley and Patricelli 2010). With regard to our particular case study, despite the fact that FCM concentration levels were higher in response to *Road* identity and *AcED* values, there was no evidence that current traffic volumes are affecting the population dynamics within the study area. This is in concordance with the findings by Creel et al. (2002) in relation to the use of snowmobiles and an elk (*Cervus elaphus*) population in Yellowstone National Park in the USA. However, we should not lose sight of the potentially chronic stressor role of transport infrastructures that might be crucial in terms of conservation and viability of roe deer populations in fragmented landscapes (Barber et al. 2010; Kuehn et al. 2007). Obviously, other unidentified factors (e.g. light and chemical emissions) may have contributed to the patterns observed (Jaeger et al. 2005) as well as habitat quality. Thus, we also underline, in accordance with Millspaugh et al. (2001), the need for caution when interpreting FCM measurements to assess wildlife adaptation to anthropogenic disturbances at present. Finally, also of interest is that our findings lead us to think that low-traffic roads (< 1000 vehicles per day) may noticeably degrade habitat quality and may potentially affect the physiological status of wildlife. This is in opposition to the potential role attributed to low-traffic areas of contributing to biodiversity conservation as relatively undisturbed natural habitats and functioning ecosystems (Selva et al. 2011). Therefore, our results reinforce the assumption that noise impact assessment and management on natural ecosystems is still an urgent conservation priority to be taken into account by practitioners and policy makers and that requires further research (Francis and Barber 2013; Parris 2015).

Conclusions

This study has made it possible to isolate noise due to traffic in order to assess it as a major driver of effects on wildlife populations at both large spatial and long-time (seasonal) scales, in response to the concern and demand from researchers in the literature. We have expanded the applicability of common noise modelling tools from transportation or industrial sectors to the field of ecology and nature conservation together with non-invasive methodologies to index physiological stress in animals. Its adaptation combined with GIS tools allows the calculation of a new original indicator, acoustic escape distance (*AcED*), focused on the potential detrimental impact of noise pollution on wildlife and natural habitat quality, due to noise disruption above the natural acoustic stimuli.

Finally, two key findings are especially relevant in this study: (i) *Road* identity and *AcED* were the variables that best explained the FCM values observed in roe deer, and (ii) FCM concentration turned out to be positively related to increasing traffic volume (*Road*) and *AcED* values.

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