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Roe deer stress response to a wind farms: Methodological and practical implications



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ABSTRACT

There is an insufficient number of field studies on the impact of wind farms on ungulates despite the rapid expansion of world wind energy. The results of previous studies suggests a possible impact of power plants, but they are divergent and do not take into account the size of wind farms. We examined the impact of wind farms on the stress levels in roe deer based on seven wind farms of various sizes (of 12–27 turbines) in eastern Poland. Fecal cortisol concentration was assessed with the ELISA method in droppings collected during the winter period. We found that the roe deer exhibited an elevated stress level in the area of larger farms, but such response was not found in the case of smaller wind farms. The roe deer were also characterized by a higher stress level in areas of wolf predation, but this factor was less important. Both the area of the farm and the number of turbines explained the phenomenon of the increased stress in the roe deer. We estimated 824 ha or 18 turbines as a threshold level of the impact of wind farms on the cortisol concentration in the roe deer. We conclude, that turbines should be concentrated in the smallest possible area. In such conditions, roe deer will probably be able to find appropriate refuges. We recommend further studies on wind farms older than 4 years and the distribution of turbines.

1. Introduction

Wind power is one of the fastest-growing renewable sources for electricity production in many countries (REN21 2019). It is commonly presented as a technology with a low environmental impact, especially when compared to the impacts of fossil fuels (Guezuraga et al., 2012). Paradoxically, despite the potentially positive impact of wind power on global warming, improperly located wind turbines may have a negative effect on the local biodiversity of wildlife (Allison et al., 2019).

There is a rich literature on the significant influence of turbines on birds and bats, which can be affected directly by collisions with the rotor or indirectly when they are forced to exclude wind farms from their foraging areas (Kuvlesky et al., 2007; Smallwood et al., 2009; Garvin et al., 2011; Arnett and Baerwald, 2013; Schuster et al., 2015; Dai et al., 2015). However, the adverse consequences of wind farms may also potentially affect terrestrial non-flying species (Lovich & Ennen, 2013), but the response of these animals is much less frequently studied than that of flying species (but see Agha et al. 2015; Lopucki & Mróz 2016; da Costa et al 2018; Lopucki & Perzanowski, 2018; Keehn et al. 2019). The fact that the wind energy effects on terrestrial animals are poorly known is constantly raised in many review papers (Helldin et al., 2012; Lovich & Ennen, 2013; Allison et al., 2019; Korfanta & Zero, 2019) nevertheless, this does not intensify field work to fill this gap in the knowledge, even in the case of economically important or protected species. Korfanta and Zero (2019) literally point out that, given the economic and cultural importance of ungulate species and their known sensitivity to other forms of anthropopressure, understanding wind energy impacts on these species should be a high priority. It should be remembered that wind farms are often built far away from human settlements, i.e. in wildlife refuges (Helldin et al., 2012). Therefore, the introduction of wind farm infrastructure into such a refuge can potentially disturb wildlife populations.

The impact of wind energy on ungulates was studied only for a narrow group of species. Relatively the most research was done for the reindeer *Rangifer tarandus*. It was found that semi-domestic reindeer in an enclosure located close to a wind turbine showed no negative behavioral response and little or no aversion towards a turbine rotor movement, probably due to rapid habituation in a small enclosure with

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continuous wind turbine exposure (Flydal et al., 2004). In turn, in the case of semi-domesticated free-ranging reindeer, the construction phase of even small wind farms was found to cause a reduction in the use of movement corridors and grazing habitats and increased the fragmentation of the reindeer calving ranges (Skarin et al., 2015). During the operation phase, both avoidance and lack of influence of wind farms on reindeer have been reported (Skarin & Alam 2017; Tsegaye et al., 2017). In a paper summarizing multiyear research on different phases of wind farm development (before construction, construction, and operation), Skarin et al. (2018) showed a shift in home range selection by reindeer as an effect of the presence of wind turbines. They also found that the operation phase of the studied wind farms had a stronger adverse impact on reindeer habitat selection than the construction phase.

In addition to the above-described work on the reindeer response to wind energy, similar studies have also been carried out for other ungulates, such as: pronghorn Antilocapra americana, roe deer Capreolus capreolus, and rocky mountain elk Cervus elaphus. It was found that the winter survival of the pronghorn was not influenced by the exposure to the wind energy infrastructure; however, the authors suggest that the survival may be influenced by larger-scale wind energy developments than those examined in their study (Taylor et al., 2016). A tendency to avoid wind farm interiors and direct proximity to turbines was exhibited by roe deer (Lopucki et al., 2017). Rocky mountain elk were not adversely affected by a wind farm development, as determined by the home range and dietary quality (Walter et al., 2006). In the case of two other ungulate species (mule deer Odocoileus hemionus and Peninsular bighorn sheep Ovis canadensis nelsoni), only data on population ecology were collected in areas where wind farms are planned to be built and had no contribution to the knowledge presented above (Buchalski et al. 2015; Webb et al., 2013a; Webb et al., 2013b). It can be claimed that ungulate species indeed use wind farm areas, but there are data suggesting that wind turbines may affect their space use and habitat selection. However, no in-depth studies have been conducted and the behavioral and physiological mechanisms of this phenomenon remain unknown. Yet, only elucidation of the mechanisms of the effects of wind power on terrestrial wild animals will predict negative changes and contribute to better planning of new wind farms.

The aim of this study is to examine whether roe deer (a species in which a negative response to wind turbines has already been found in previous studies, i.e. tendency to avoid proximity to turbines) shows physiological changes (increased levels of stress hormones) associated with their occurrence in wind farm areas, compared to animals living away from the farms. We tested not only the stress response in the roe deer but we also wanted to determine the size of the wind farm that may evoke such reaction in order to be able to formulate guidelines for the sustainable development of wind energy, which would also take into account the impact on terrestrial animals.

2. Methods

2.1. Study sites

The field study was carried out between January 27 and March 9, 2018 in an area of seven wind farms and seven control areas in the eastern part of Poland (Fig. 1). All areas were under hunting pressure, however the hunting season ended on January 15. All selected power plants have 12 – 27 turbines. We assumed that an area of a wind park larger than 10 turbines is sufficiently large to have a permanent impact on roe deer due to the home range size of this species, i.e. from 57 to 88 ha (Cederlund 1983; Saïd et al., 2009; Morellet et al., 2013). The area of the studied power plants was calculated as a minimum convex polygon (MCP) of location of the turbines. Two separate groups were distinguished: smaller farms covering 513–624 ha and larger farms covering on average of 1500 ha (Table 1). The farms were generally new, as they had been in operation for maximum 4 years.

A factor that differentiated the farms was also the predator pressure.



Fig. 1. Location of study sites in the eastern part of Poland.

The two farms were located in areas of a strong wolf population: Orla in Podlaskie Voivodeship (northern part), and Łańcut in Podkarpackie Voivodeship (southern part). There are no current studies on the wolf population in eastern Poland; although the population of these animals in Poland exhibits stable growth (Reinhardt et al., 2015). Based on the information from the latest census (https://ibs.bialowieza.pl), Podlaskie and Podkarpacie Voivodeships are mainly inhabited by wolves and high predatory pressure of wolves can be expected in these areas. Therefore, we assumed a high probability of predation on roe deer by wolves in the areas of Orla and Łańcut wind parks, which is also confirmed by cases of frequent wolf attacks on domestic animals in those areas (Wierzbowska et al. 2016). The other five wind farm areas were assumed to be under minor or no wolf predation.

A control area was selected for each studied wind farm. The control areas were located 2–6.6 km from the closest turbines of each wind farm. They were selected in respect of the highest similarity to the power plant area (structure of land cover, altitude, distance from technical infrastructure, human settlements and others). The control areas were also under similar wolf pressure, and if the farm was regarded as under the pressure, it also concerns its' control area.

2.2. Collection of fecal samples in the field

The central part of each farm and each control area were scanned with binoculars to find herds of roe deer. Each encountered group was observed from a distance to estimate the number of individuals. After that, fecal samples (fresh droppings) from roe deer were collected in areas where the animals were observed. The central part of the site of sample collection was marked with a GPS receiver. The weather conditions, i.e. the snow cover and air temperature, were recorded. The fecal samples were cooled in a portable fridge, transported to the laboratory, and stored at -20 °C until analysis. In total, 234 samples were collected (130 in the wind farms and 104 in the control areas). The samples were collected at midday (11-14 a.m.) on the same day in the control areas and in the wind farm areas. The sampling period and exact hours of sample collection was precisely chosen. The collection of samples was carried out after the hunting season to exclude a higher stress level resulting from disturbances caused by hunting. The time of collection during winter and early spring ensured collection of high quality material. The roe deer were aggregated in groups that were sufficiently visible for distant identification. The droppings were well

Table 1

Main characteristics of wind farms covered by the study (1Area measured as minimum convex polygon (MCP) of turbine location).

Name of wind farm	Location(GPS)	Turbines(N)	Category of wind farm size	Area(MCP, ha) ¹	Time since launch (years)	Wolf occurence
Iłża	51°10′50″N 21°11′2″E	27	Larger	1357	4	No
Jarczów	50°27′43″N 23°36′55″E	15	Smaller	605	3	No
Lubartów	51°30′47″N 22°17′24″E	16	Larger	1553	3	No
Łańcut	50°3′3″N 22°17′34″E	19	Smaller	624	3	Yes
Orla	52°42′20″N 23°18′10″E	15	Larger	1664	4	Yes
Tomaszów	50°26′7″N 23°30′39″E	12	Smaller	511	3	No
Tyszowce	50°36′5″N 23°44′13″E	15	Smaller	583	2	No

visible on the snow cover and maintained their quality due to low temperatures (slower bacterial degradation of cortisol metabolites). The similar hours of collection of the material in each study site ensured temporal standardization.

2.3. Laboratory analysis

The concentration of cortisol in the feces samples was assessed with the ELISA method (competitive ELISA variant) using a non-specific commercial kit with antibodies for this hormone (COR ELISA Kit No. EU0391, Wuhan Fine Biological Technology Co.). The analysis was performed according to the manufacturer's protocol. Before the measurements, mixtures of feces were prepared. Briefly, 100 mg of feces weighed using an XA 100 3Y.A analytical balance* (Radwag, Poland) were mixed with 0.4 ml of Dulbecco's phosphate-buffered saline (DPBS, Thermo Fisher Scientific) without calcium and magnesium (pH = 7.0-7.3) in an Eppendorf tube and shaken for 10 min in a multivortex. Next, the resulting suspension was centrifuged at room temperature (at 10000 rpm for 20 min) using a Heraeus Megafuge 11R centrifuge* (Thermo Fisher Scientific, Germany). Afterwards, the obtained supernatants were immediately used for analysis using a Synergy 2 multi-mode microplate reader* (BioTek Instruments, Inc. USA) equipped with an automated microplate strip washer* (ELx50, BioTek Instruments, Inc. USA) and an ELMI DTS-4 digital thermostatic microplate shaker* (USA). The concentration of cortisol in the samples was determined by comparing the optical density (OD) of the samples to the standard curve, the range of which was from 0.391 to 25 ng/ml. The OD values of the samples were measured at 450 nm at room temperature. The fecal cortisol concentrations were normalized per weight of feces and finally expressed as nanograms of cortisol per one gram of dry mass of feces (ng/g). All samples with results within the range of the standard curve (222 samples after exclusion of 12 samples below the range of the curve) underwent statistical analysis.

2.4. Statistical analysis

To analyze the hormonal response of roe deer to wind farms, we used a generalized linear model, because the data did not meet the assumptions of the general linear model. We compared various distributions and link functions comparing the values of Akaike information criterion (AIC). Gamma distribution with log link function had the lowest AIC values and was used in the final model. We used the AIC value threshold greater than two. The level of cortisol in each sample was set as a dependent variable in the model. The covariates included the time of sample collection, snow cover, air temperature, and size of the roe deer group. The wind farm size (smaller farms, larger farms, and control), predation (two groups of wind farms: under predation or no predation), and interaction between them were the factors in the model. The time of sample collection was used in the model as the number of the day in the year. The snow cover was assessed only as a percentage of the area covered by snow, regardless of the snow depth. We compared various model types and a null model to achieve the bestfit model using AIC in a backward elimination procedure. The model with the lowest AIC value was selected. Akaike weights $(\Sigma \omega_i)$ of each variable were calculated to assess their relative contribution. The assessment was based on 95% confidence set of models by starting with the highest Akaike weight and adding a next model with lower Akaike weights in a sequence to exceed a sum of 0.95. The Akaike weights of each variable were a sum of model weights containing that variable (models within $\Sigma wi = 0.99$, because the last model added to exceed a sum of 0.95 resulted in $\Sigma \omega_i = 0.99$). Higher Akaike weights indicated a higher contribution of the variable. We also used a pairwise comparison with Bonferroni adjustment of groups in factors that were statistically significant in the model.

To determine the threshold of a significant impact of the wind farm depending on its size, we used a logistic regression model. The threshold of the cortisol values of each sample (collected in the wind farm area) compared to the median value of the cortisol level in the control areas was a binary dependent variable. A value that was higher than the mean in the control areas was marked with 1, whereas a value that was lower than the mean in the control areas was marked with 0. The analysis only included farms that were not under predatory pressure (compare Table 1) to assess the impact of only the farms, without consideration of the other known factors that could have a statistically significant effect on the stress level. For this analysis, we have developed three separated models: one with the farm area (expressed with MCP in ha), the second with the number of turbines, and the third with the impact area (expressed with the total area covered by a 500-m buffer of all turbines of the wind farm). The minimum convex polygon (MCP) and impact area were calculated in Quantum GIS (3.4.5 Madeira). All statistical analyses were performed using SPSS software (version 24.0, IBM Corporation, Armonk, NY).

3. Results

The response of roe deer to wind farms in the best-fit model included the statistically significant size of the wind farm and predation (p < 0.001 in both cases) (Table 2). The model included also the size of the roe deer group, but this covariate was not statistically significant (p = 0.089). The other covariates: time of sample collection, air temperature, snow cover, and interaction of the wind farm size and predation were excluded by the AIC procedure. The feces samples from the smaller wind farms exhibited a similar level of cortisol to the sampled from the control areas (FCC = 1.26 ng/g and 1.30 ng/g respectively). However, the cortisol concentration in both areas was statistically

Table 2

Effects of the wind farm size, predation, and size of the roe deer group on the cortisol concentration in fecal samples ($\chi 2 = 36.59$; df = 4; p < 0.001). Non-significant variables: time of sample collection, air temperature, snow cover and interaction of predation and wind farm size were excluded by the AIC procedure.

Source	Intercept	Wald χ ² 12.25	df 1	p < 0.001	$\Sigma\omega_i$
Covariates Factors	Group size Wind farm size Predation	2.89 23.75 8.28	1 2 1	0.089 < 0.001 0.004	0.59 0.99 0.93



Fig. 2. Fecal cortisol concentrations in 1 g of sample (mean \pm SE) with regard to the wind farm size ("control" – control areas, "smaller wind farms" – wind farms covering an area of 513–624 ha of MCP, "larger wind farms" – wind farms covering an area of 1357–1664 ha of MCP) and pairwise comparison with Bonferroni adjustment ($\chi 2 = 21.54$; df = 2; P < 0.001; n = 94 for the control, n = 39 for the smaller wind farms, and n = 88 for the larger wind farms).



Fig. 3. Fecal cortisol concentrations in 1 g of sample (mean \pm SE) with regard to the predatory pressure ("no predation" – areas with no predation from wolves, "predation" – areas with high predation from wolves) and pairwise comparison with Bonferroni adjustment ($\chi 2 = 6.32$; df = 1; P = 0.012; n = 191 for "no predation" and n = 30 for "predation").

lower than in the case of roe deer occupying the larger wind farms (FCC = 1.83 ng/g, p < 0.001 in both cases in pairwise comparison) (Fig. 2). The effect of predation had lower relative contribution to the model than the wind farm size ($\Sigma\omega_i = 0.93$ and 0.99 respectively). Nevertheless, roe deer occupying areas with predatory pressure exhibited a significantly higher fecal cortisol concentration, compared to areas without predation (FCC = 1.69 and 1.23 ng/g respectively, p = 0.012) (Fig. 3). The elevated stress level in areas with predatory pressure was shown regardless the presence of roe deer on the wind farm or control area.

The probability of a higher fecal cortisol concentration than the median value in an control areas increased with the wind farm size. This was confirmed by both logistic regression models. The model including the area of the wind farm expressed by MCP as an explanatory variable was statistically significant ($\chi 2 = 20.81$; df = 1; p = < 0.001); however the Cox and Snell R² was quite low (0.171) and the proportion of correctly classified cases equaled 72.1. The model with the number of turbines as a predictor of the stress level was similarly fitted, but Cox and Snell R^2 was lower (0.153) and correctly classified cases equaled 72.1% ($\chi 2 = 18.43$; df = 1; p < 0.001). The threshold of a significant impact of the wind farm on the cortisol concentration may be assessed based on the value of probability in both models. The probability of a higher of cortisol concentration in roe deer on the farm compared to the control areas is higher than 0.5 on farms exceeding 824 ha or 18 turbines (Fig. 4). A worse fitness to those from the model with the number of turbines and MCP was shown by the model with the impact area (expressed with 500 m buffer from each turbine). Cox and Snell R² equaled 0.123 and correctly classified cases equaled 72.1% ($\chi 2 = 14.54$; df = 1; p < 0.001).

4. Discussion

We expected that wind turbines can have a physiological impact on roe deer, and individuals living within the area of wind farms have higher cortisol levels than individuals inhabiting unaffected areas. This phenomenon has already been described in the literature on the endocrine ecology of small mammals living in wind farms (Lopucki et al., 2018). We also assumed a possibility of the absence of differences in hormone levels as an effect of physiological adjustment of roe deer stress response to disturbances caused by wind turbines during the several years of operation (similar to work by Flydal et al., (2004) on reindeer behavior habituation). We found that, from the group of factors we analyze, the size of a wind farm is a key factor affecting the probability of occurrence of increased levels of stress hormones in roe deer and that other ecological factors such as predation (possibly also the size of the roe deer group) may be of great importance in the studied phenomenon. Of course, we do not exclude the potential impact of other factors (e.g. competition with other ungulates, food base,



Fig. 4. Logistic response of the fecal cortisol concentration in the roe deer to the wind farm size based on: A) area (MCP) and B) number of turbines ($\chi 2 = 20.81$; df = 1; p = < 0.001 for A and $\chi^2 = 18.43$; df = 1; p < 0.001 for B, n = 111 in both cases).

tourism) but the impact of these factors has not been studied in our paper. Our result sheds new light on the issue of the reaction of ungulates to wind energy development and can be the basis for addressing three basic issues raised below.

4.1. Methodological issues - comparative studies on wind farms of various sizes are needed.

The small number of studies on the reaction of ungulates to wind energy carries the risk of incorrect extrapolation and interpretation of existing results. This is related to the fact that insufficient attention is paid to the influence of such factors as the size of the wind farm. Typical work on the response of terrestrial animal was done on one or at most several (usually similar) wind farms (Walter et al., 2006; Skarin et al., 2015; Taylor et al., 2016; Łopucki et al., 2017; Skarin & Alam, 2017; Tsegaye et al., 2017; Skarin et al., 2018) and the farm size factor was not analyzed. Our research shows that if only smaller wind farms had been selected in our study design, the result would have been "no effect on deer stress response". Obviously, reverse "incomplete" knowledge would have been provided if we had chosen only larger farms for the investigations. It is highly probable that such an "incomplete" result could later be extrapolated to other species and influence decisions taken during the design of wind farms and assessment of their environmental impact (Łopucki and Perzanowski (2018) and Korfanta and Zero (2019) pay attention to such risk). Since studies on the response of terrestrial animals to wind energy are only beginning, we strongly recommend conducting comparative studies for the same species on farms of different sizes and with more replications to avoid incorrect extrapolation and interpretation of existing results. It is also possible that stress response of roe deer indicated in our result was a local effect. For this reason similar research should be carried out on other ungulate species and in other regions.

4.2. Ecological issues – Why living on wind farms can increase stress levels in roe deer.

The hormonal response of the roe deer to wind farms presented in this study confirms the results reported by Lopucki et al. (2017) where avoidance of wind farm areas or turbine proximity by this species was indicated. Nevertheless, in our study, the smaller farms did not exert a significant impact on the cortisol level in the roe deer. It seems important to ask a question why smaller farms have no effect on the cortisol level and larger ones cause a significant increase in stress. This issue was not discussed by Łopucki et al. (2017), however, the main reason for the avoidance of wind farms by roe deer was indicated, namely the noise from the turbines, which probably disturbs perception of sounds in the environment. Roe deer rely mainly on hearing rather than other senses, particularly to avoid danger (Molinari-Jobin et al., 2004) and thus they are susceptible to the noise effect. However, according to the risk allocation hypothesis, animals can benefit from reducing high vigilance in areas of chronically high risk (Lima & Bednekoff, 1999), but this response shoud be reflected in behavioral adjustment (Courbin et al., 2013; Basille et al., 2015; Martin et al., 2015). In our opinion, such response may be expected in the presence of turbines, where habitat shifts have been observed, probably to lower vigilance (Lopucki et al., 2017). The effect of noise from wind turbines has also been demonstrated in other species (Rabin et al. 2006, Agnew et al. 2016).

Human-related noise may restrict habitat use and movement of species and represent a life-threatening hazard in predator-prey interactions, particularly in the case of herbivorous mammals (Bowles, 1995; Clevenger & Waltho, 2005). Therefore, refuge areas play an important role of compensation mechanisms, and human-related environmental conditions can be essential in fitness capabilities. In the aspect of wind energy, it can be claimed that smaller wind farms ensure larger available areas that can be used by roe deer as refuges. Consequently, in larger wind farms, such possibilities are inherently smaller; thus, the lack of refuges should increase the stress level in the animal and lead to further physiological consequences. This point of view is confirmed by the higher explanatory power of the area of the wind farm than the number of turbines. The turbines are located depending on the optimal use of energy (including cost effectiveness), legal standards, and local conditions (e.g. Wan et al., 2009; Herbert-Acero et al., 2014). For this reason, their spatial arrangement is the result of some consensus so that turbines can be spatially distributed in various ways. The way the wind farms are spatially designed is probably a matter of their size. In general, a smaller wind farm will occupy smaller areas with no essential impact on roe deer. However, in the case of larger ones, the importance of their spatial design regarding the influence on terrestrial mammals increases.

Our results indicate a significant impact of predatory pressure on the cortisol level in roe deer, but this factor was less important than the presence of the wind farms. The results are in agreement with studies reported by Zbyryt et al. (2017) which showed that human-related factors have a greater impact on the stress level in roe deer. Furthermore, game animals show higher vigilance during hunting season (e.g. Sönnichsen et al., 2013), and higher vigilance to the presence of humans than natural predators (e.g. Ciuti et al., 2012). This phenomenon is explained by the evolutionarily induced pray fittness to the predatory pressure (Kuijper et al., 2014; Périquet et al., 2017), which is not comparable to human-related factors (Zbyryt et al., 2017).

4.3. Practical application of the results - how to build wind farms more friendly for ungulates

An important aspect of our work was the formulation of guidelines for the sustainable development of wind energy, which would also take into account the impact on terrestrial animals. The roe deer is an important game species in Poland and Europe (Łabudzki et al., 2009; Burbaitė & Csányi, 2010) and a number of factors that should be considered in managing this species have been identified (Putmam, 1997; Cederlund et al., 1998; Hemami et al., 2004; Vospernik & Reimoser, 2008). The impact of wind farms, however, has not been considered despite the fact that they are usually located in areas of the occurrence range of roe deer.

The results of our work indicate that larger farms (over 824 ha or 18 turbines) can affect the stress level in this species; hence, smaller power plants would be preferable. Moreover, taking into account literature data (e.g. Skarin et al., 2018), it can also be pointed out that when designing the spatial arrangement of turbines, attention should be paid to leaving convenient habitats in the vicinity of the farm to enable the habitat shift of ungulates. The wind farm size indicated by us (824 ha or 18 turbines) cannot be treated literally, but as an approximate value. This is due to a limited sample and given parameters of logistic regression models. Our results, however, indicate that a better solution for ungulates is to concentrate wind turbines within the farm in the smallest possible area (taking into account technological considerations) than disperse them in the landscape. This conclusion is supported in our results by the best fitted model including the area of the wind farm expressed by MCP. Since we have studied relatively new farms, further research in an area of longer operating installations (older than 4 years) is needed. If such research shows that the stress response in deer is much lower on long-term wind farms, introduction of changes in deer management in areas where new wind farms have been built may be considered. These changes could include, for example, suspension of hunting for the first few years of the farm's operation. This would reduce the number of stress factors for roe deer and would probably limit the potential negative effects of stress (Sapolsky et al. 2000; Korte et al. 2005) on the condition of animals. Furthermore, new studies should be performed in a different wind farm areas and with focus on other ungulate species to verify our results, especially the farm size threshold.

5. Authors' contributions

Daniel Klich and Rafał Łopucki conceived the ideas, designed methodology, collected material and led the writing of the manuscript. Daniel Klich analysed the data. All authors preformed laboratory analyses and contributed critically to the drafts and gave final approval for publication.

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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