



## Research paper

# Living in habitats affected by wind turbines may result in an increase in corticosterone levels in ground dwelling animals



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## ABSTRACT

Environmental changes and disturbance factors caused by wind turbines may act as potential stressors for natural populations of both flying and ground dwelling animal species. The physiological stress response results in release of glucocorticoid hormones. We studied two rodent species of the agricultural landscape (the common vole *Microtus arvalis* and the striped field mouse *Apodemus agrarius*) and tested the hypothesis that living in habitats affected by wind turbines results in an increase in corticosterone levels. Rodents were trapped at sites near wind turbines and in control areas. Faeces samples were collected from traps where the targeted animals were caught. For the analysis of corticosterone concentrations in the faeces, we used ELISA tests with antibodies for this hormone. The common vole showed a distinct physiological response – the individuals living near the wind turbines had a higher level of corticosterone. The striped field mouse did not show a similar response. We pointed out the main factors increasing corticosterone levels in voles and features of the studied species that may determine the differences in their reaction including: the width of the ecological niche, spatial mobility, and predation pressure. This is the first study suggesting impact of wind farms on physiological stress reactions in wild rodent populations. Such knowledge may be helpful in making environmental decisions when planning the development of wind energy and may contribute to optimization of conservation actions for wildlife.

## 1. Introduction

There are a variety of potentially negative effects of wind power on wildlife. The most thoroughly studied is the impact on flying animals – birds and bats. Their direct mortality via collisions with rotors, avoidance of foraging near wind turbines, and other detrimental effects have already been described (Kuvlesky et al., 2007; Smallwood et al., 2009; Garvin et al., 2011; Arnett and Baerwald, 2013). Much less is known about the wind energy effect on terrestrial, non-volant wildlife. Potentially, these animals can be affected by factors connected with the construction of wind turbines e.g. destruction of habitat, vibration and noise effects, visual impacts, higher direct mortality on wind farm roads, and an increase in human activity within the wind farm area (Helldin et al., 2012; Lovich and Ennen, 2013). As a result sites near the turbines or whole wind farm area may presumably become less suitable as potential habitats and be less frequently inhabited than more optimal (unaffected) habitat patches.

Recent studies showed that, in most cases, wind farms had no

significant effect on ground-dwelling animals (de Lucas et al., 2005; Walter et al., 2006; Helldin et al., 2012; Łopucki and Mróz, 2016). There are only few papers on the significant effect of wind power development on such animals e.g. increased mortality on wind farm roads in a desert tortoise population (Lovich et al., 2011), antipredator behaviour of ground squirrels – a higher level of overall alertness at the turbine site (Rabin et al., 2006), or avoidance of wind farms by large or medium-size mammals during the construction or operational phase (Helldin et al., 2012; Łopucki et al., 2017).

The most commonly analyzed parameters regarding the impact of wind farms on ground-dwelling animals include species composition and space use (de Lucas et al., 2005; Helldin et al., 2012; Lovich and Ennen, 2013; Łopucki and Mróz, 2016). Behaviour (Rabin et al., 2006), diet quality (Walter et al., 2006), survival (Winder et al., 2014; Agha et al., 2015) or growth and demography (Lovich et al., 2011) have been analysed less frequently. The effects of wind energy development on more subtle aspects of animal response such as physiological changes e.g. stress reactions are unknown. The home ranges of ground-dwelling

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animals, in many cases quite small, may entirely overlap with the wind farm area and therefore the animals may be permanently exposed to the potentially stressful impact of wind power. Especially, areas in the immediate vicinity of wind turbines are exposed to a relatively high level of noise, vibration, and electromagnetic disturbances (Rogers et al., 2006; McCallum et al., 2014; Xi, 2014; Arana et al., 2015). Although short-term stress exposition is not harmful to animals, chronic stress may cause inhibition of reproduction, growth, and survival as well as a decline in fitness (Harper and Austad, 2000; Sapolsky et al., 2000; Atanasov et al., 2015).

A useful indicator of physiological stress in animals are changes in the levels of glucocorticoids in blood plasma or their metabolites in faeces (Harper and Austad, 2000; Good et al., 2003). In field studies, faecal hormone glucocorticoid assays are currently considered the most reliable, most practical, and least invasive method for measuring chronic stress. A faeces sample contains an accumulative hormone level from the previous several hours and for this reason is more representative of an individual's general hormone exposure than point sampling with bleeding methods (Blondel et al., 2016). This non-invasive method was successfully used in many field studies on physiological responses of rodents or other mammals to various natural or anthropogenic stressors (Ylönen et al., 2006; Navarro-Castilla et al., 2014a,b; Blondel et al., 2016).

The aim of our study was to test the hypothesis that occurrence of small mammals in habitats affected by wind turbines is associated with an increase in their corticosterone levels. We studied two rodent species typical of the agricultural landscape: the common vole *Microtus arvalis* (Pallas, 1778) and the striped field mouse *Apodemus agrarius* (Pallas, 1771). These mammals have relatively small home ranges and if they live near wind turbines they may be permanently exposed to the potentially stressful impact of wind power. We expected higher corticosterone levels in individuals living near wind turbines than in individuals from unaffected control areas.

## 2. Methods

### 2.1. Study area

The study was conducted in south-eastern Poland (Europe) in the foothills of the Outer Western Carpathian Mountains, at a wind farm near Rymanów town (N49°36'; E21°50') and at control site about 8 km east. The studied wind farm has been in operation since 2013. It consists of 13 Repower MM92 wind turbines with tower heights of 100 m, rotor diameters of 92.5 m and single-turbine capacities of 2.05 MW. The total area covered by this wind farm reaches 270 ha (measured as a minimum convex polygon of external turbines), but the potential area of the physical (e.g. acoustic) impact is much larger. The wind farm and the control area were located at a similar altitude of approximately 300 m a.s.l. in arable fields and meadows with small groups of shrubs located along the access roads, bounds, and on non-managed patches of land. They were situated at least 500 m away from human settlements and 300 m from local paved roads.

### 2.2. Animal trapping

Small mammals were trapped simultaneously at sites in the vicinity (up to 100 m) of the wind turbines and in the control area. Patches with dense vegetation cover of wild (non-cultivated) plants (weeds, herbs, grasses, and shrubs) were selected as trapping sites. Mammals were captured in wooden box live traps (88 × 80 × 200 mm) provided with food bait (oats). We usually used 80–100 traps per turbine site and per trapping session. The traps were spaced about 3 m from each other. Such a large number and density of traps set at the same time in a relatively small area provides high probability of capture of a majority of rodents occurring there. At the control sites, we also set a similar number of traps (about 80–100) per one site, spaced about 3 m from

each other.

In each site, the traps were set in the evening, but we kept them closed until midnight. After midnight, we opened the traps only for 5–6 h. After this time, the traps were checked and all captured animals were described in terms of species, sex, reproductive activity, and body mass ( $\pm 1$  g) and subsequently released at the site of capture. Faeces samples were collected from traps where the targeted species were caught (see below). After the session, all traps were gathered, cleaned (if necessary), and transported to a new location (another turbine or another control site). We used such a short period of trap exposure because of the requirements of the sampling procedure for testing the corticosterone level (see below). Additionally, we wanted to collect samples from the population disturbed by the trapping procedure as little as possible; therefore, we did not mark the animals and did not repeat trapping at the same site. Trapping sessions were carried out after the breeding season of small mammals in October of 2016. The total capture effort at the wind turbines sites was 760 trap-days and at the control area 1000 trap-days (where “day” means 5–6 h).

### 2.3. Collection of the faecal samples in the field

Faeces samples were collected only from traps containing the targeted species, i.e. the striped field mouse or the common vole. From all such traps, we collected as many faecal pellets as possible if they were fresh (not dried) and not contaminated with urine, according to the procedure used by Navarro-Castilla et al. (2014a, 2014b). They were placed in an Eppendorf tube and immediately cooled in ice. For 1–2 h after checking the traps, the faecal samples were stored in a freezer at  $-20$  °C until analysis.

The trapping procedure used ensured that the maximal time that an animal spent in a trap was 6 h (practically it was much lower), and all samples were collected at a similar period during the circadian rhythm of the studied rodents. This was important because it reduced the possible effect of capture upon the corticosterone level and daily differences in hormone excretion (Harper and Austad 2001; Touma et al., 2003, 2004; Navarro-Castilla et al., 2014a, 2014b).

In total, 693 small mammals were captured during the entire trapping period. About 69% of all captured animals were individuals belonging to the two targeted species. A large number of samples collected in the field allowed us to select only samples of the highest quality for analysis and obtain similar sample sizes based on sex and age. We also chose a similar number of samples from different trapping sites to avoid over-representation of the samples, e.g. from the vicinity of particular wind turbines. Among all 173 captured common voles, we assessed the faecal corticosterone concentrations for 154 individuals. Regarding the striped field mouse, we used samples from 146 individuals out of 303 that were captured.

### 2.4. Measurement of faecal corticosterone

Each faecal sample was weighed using an XA 100 3Y.A analytical balance (Radwag, Poland) with an accuracy of 0.001 g and only subsamples of 0.08 g for the striped field mouse and 0.12 g of wet mass for the common vole were taken to analysis. The remaining parts of the samples were weighed, dried for 8 h in 45 °C and weighed again to determine their dry mass contents.

For the analysis of corticosterone concentrations in the faeces, we used a commercial test kit with antibodies for this hormone (CORT ELISA Kit No. EU3108; Wuhan Fine Biological Technology Co.). The advertised sensitivity (minimal detectable dose) was lower than 46.87 pgCORT ml<sup>-1</sup>. Analysis was performed according to the manufacturer's protocol. The samples were mixed with 0.5 ml of physiological buffered saline (PBS) without calcium and magnesium ions (pH = 7.4  $\pm$  0.2) in an Eppendorf tube and shaken first by hand and then for 30 min in a multivortex. Then the mixture was centrifuged using a Heraeus Megafuge 11R centrifuge# (Thermo Fisher Scientific, Germany) at

room temperature, running at 9000 rpm for 10 min. The supernatants obtained were immediately used for analysis. After the initial washing of the plates with wash buffer using an ELx50 microplate strip washer# (BioTek Instruments, Inc. USA), 50  $\mu$ l of sample supernatant and 50  $\mu$ l of Biotin-detection antibody were added into appropriate wells and incubated for 45 min at 37 °C using the ELMI DTS-4 digital thermostatic microplate shaker# (USA). Next, the washing procedure was repeated, and then 100  $\mu$ l of a Horseradish Peroxidase (HRP)-Streptavidin Conjugate (SABC) working solution was added to each well and incubated for 30 min at 37 °C using the same thermostatic microplate shaker as mentioned above. After aspiration and the re-washing process, 90  $\mu$ l of Tetramethylbenzidine (TMB) Substrate were added to each well and incubated again for 10 min at 37 °C. Afterwards, 50  $\mu$ l of Stop Solution were added to each well and then the optical density (OD Value) was immediately read at a wavelength of 450 nm using the Synergy 2 multi-mode microplate reader# (BioTek Instruments, Inc. USA).

To validate the experiment operation and the appropriateness of the sample dilution proportion, a pilot experiment was conducted with 8 samples using standards. The pilot experiment showed that the values obtained for samples with undiluted supernatant were within the calibration (standard) curve and such undiluted supernatants were used in analysis. The range of the standard curve was from 78.12 to 5000  $\text{pg ml}^{-1}$ . We conducted separate pilot experiments for the samples obtained from both studied species.

To account for the differences in the mass of the analysed samples, faecal corticosterone concentrations were expressed as nanograms of CORT per one gram of dry mass of faeces ( $\text{ng g}^{-1}$ ).

### 2.5. Statistical analyses

All statistics were performed using SPSS software (23.0). The data was not normally distributed, and since they presented gamma distribution, we applied a multivariate generalized linear model using a gamma distribution with a log link function to analyze skewed data. For the faecal corticosterone concentrations in both species separately (as a response variable), location (wind farm and control) and sex were assessed as fixed factors, while density was estimated as a covariate. We compared various model types and a null model to achieve the best fit model using Akaike information criterion (AICc) in backward elimination. Akaike weights 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 (only models within  $\Sigma w_i = 0.955$ ).

### 3. Results

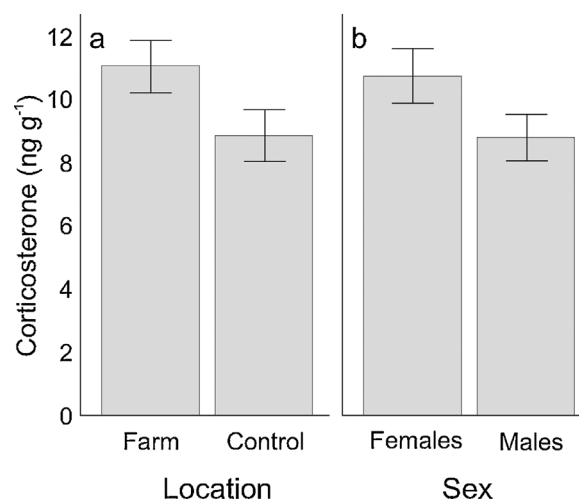
The best model for the common vole included the location, sex, and interaction between these two variables. The density (i.e. number of captured individuals per 100 traps) was not significant in the model and was excluded during the AICc procedure (Table 1). The location was a relatively most important variable, explaining most of the variability in the faecal corticosterone concentrations ( $\Sigma \omega_i = 0.955$ ). All factors were statistically significant ( $P \ll 0.05$ ).

The mean faecal corticosterone concentration in the common voles from the wind farm area reached  $11.01 \text{ ng g}^{-1}$  (SE  $\pm 0.70$ ) and was significantly higher than in the control area ( $\chi^2 = 4.99$ ;  $P = 0.025$ ), where it was  $8.82 \text{ ng g}^{-1}$  (SE  $\pm 0.69$ ) (Fig. 1A). The corticosterone level in the common vole differed also between the sexes, where females showed higher mean values ( $11.16 \text{ ng g}^{-1}$ ; SE  $\pm 0.78$ ) than males ( $8.70 \text{ ng g}^{-1}$ ; SE  $\pm 0.61$ ) (Fig. 1B). This difference was statistically significant ( $\chi^2 = 6.01$ ;  $df = 1$ ;  $P = 0.014$ ). The interaction between the location and sex was significantly different only between males in the control area and other groups, i.e. males in the farm area

**Table 1**

Effects of location, sex, and density on the faecal corticosterone concentrations in the common vole ( $\chi^2 = 14.53$ ;  $df = 3$ ;  $P = 0.002$ ) and the striped field mouse ( $\chi^2 = 7.82$ ;  $df = 1$ ;  $P = 0.005$ ) in a multivariate generalized linear model. In the common vole, non-significant density was excluded by AICc, in the striped field mouse non-significant location and sex were excluded by AICc; all factors in the table are statistically significant. Contribution of each variable in the models ( $\Sigma \omega_i$ ) within 95% confidence set ( $\Sigma \omega_m = 95\%$ ) was added for the common vole.

Species	Factor	Wald $\chi^2$	df	P	$\Sigma \omega_i$
The common vole	intercept	2079.73	1	0.000	
	location	4.87	1	0.027	0.955
	sex	6.19	1	0.013	0.918
	location*sex	6.23	1	0.013	0.918
The striped field mouse	intercept	13.02	1	0.000	
	density	8.34	1	0.004	



**Fig. 1.** Faecal corticosterone concentrations in 1 g of sample (mean  $\pm$  SE) in the common vole with regard to a) location: within the wind farm (Farm) and the control area (Control) and b) sex (Females and Males) and pairwise comparison in GLM with Bonferroni adjustment (for location:  $\chi^2 = 4.99$ ;  $df = 1$ ;  $P = 0.025$ ;  $n = 93$  for Farm, and  $n = 61$  for Control, for sex:  $\chi^2 = 6.01$ ;  $df = 1$ ;  $P = 0.014$ ;  $n = 80$  for Females, and  $n = 74$  for Males).

( $P = 0.007$ ), females in the control area ( $P = 0.017$ ), and females in the farm area ( $P = 0.002$ ). Other groups were not significantly different from each other ( $P \gg 0.05$ ).

The best model for the striped field mouse included only density as an explanatory variable. Location ( $5.60 \text{ ng g}^{-1}$ ; SE  $\pm 0.56$  for the wind farm,  $5.29 \text{ ng g}^{-1}$ ; SE  $\pm 0.51$  for the control area), sex ( $5.91 \text{ ng g}^{-1}$ ; SE  $\pm 0.57$  for females,  $5.01 \text{ ng g}^{-1}$ ; SE  $\pm 0.48$  for males), and interaction between these two variables were not significant in the model and were excluded from the AICc procedure (Table 1). The faecal corticosterone concentration increased with the estimated density of mammals, and this relation was highly significant ( $\chi^2 = 7.82$ ;  $P = 0.005$ ). The expected corticosterone level in the striped field mouse increased from  $4.00 \text{ ng g}^{-1}$  at sites where 22.5 ind. per 100 traps were captured to  $6.74 \text{ ng g}^{-1}$  at sites with 50 ind. per 100 traps (Fig. 2).

### 4. Discussion

We hypothesized that wind turbines have a stressful impact on small mammals and expected higher corticosterone levels in individuals living near the wind turbines than in individuals inhabiting the unaffected control areas. We supported this hypothesis only partially. One of the two studied species, the common vole, showed a distinct physiological response on the wind farm area, whereas the other species, the striped field mouse, did not. Therefore, two questions arise: (1)

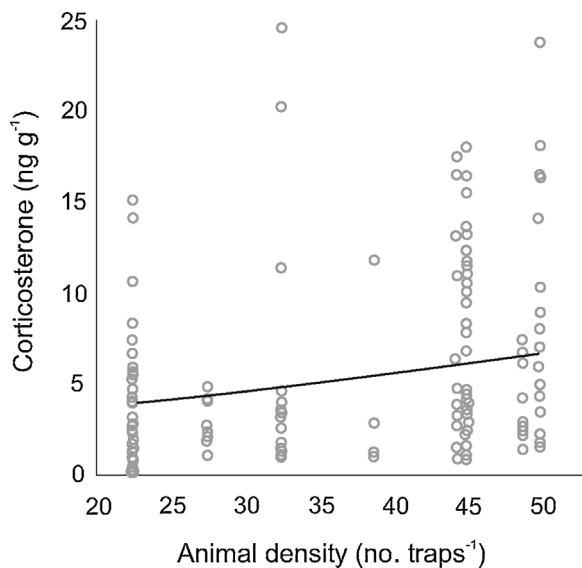


Fig. 2. Effect of animal density (number of individuals per 100 traps) on faecal corticosterone concentrations in the striped field mouse; the curve shows generalized linear model fit (likelihood ratio  $\chi^2 = 7.82$ ;  $P = 0.005$ ;  $n = 146$ ).

what is a key factor increasing the level of corticosterone in small mammals living near wind turbines and (2) why were such differences between the species observed? We try to answer these questions below.

#### 4.1. Potential factors which generate higher levels of corticosterone near turbines

##### 4.1.1. Predation risk

It is possible that small mammals living under the turbines are exposed to lower avian predation pressure. According to Garvin et al. (2011), Kuvlesky et al. (2007), and Smallwood et al. (2009), wind turbines reduce the presence of birds of prey both through direct mortality via collisions and because they avoid foraging near wind turbines. However, lower predation pressure should result in a lower level of corticosterone, while we observed an opposite response in the common vole. Possibly, the positive effect of lower avian predation pressure can be neutralized by terrestrial predators. However, the main terrestrial predator for rodents in our study area is the red fox *Vulpes vulpes*, which has a negative response to wind farms, as its occurrence is lower than in control areas (Łopucki et al., 2017). The differences in the corticosterone level observed in our study do not appear to be explained by the predation pressure.

##### 4.1.2. Aerodynamic noise from the blades

Small mammals living in close proximity to a turbine are permanently exposed to high levels of aerodynamic broadband noise (Katinas et al., 2016; Rabin et al., 2006; Jakobsen, 2012). For modern wind turbines (about a 100 m high tower and a similar diameter of blades), the noise level at the rotor axis height is 100–110 dB (Rogers et al., 2006), which usually gives (after applying proper parameters of the noise propagation model) a sound pressure level of about 60 dB(A) at the base of the tower. The power spectrum of aerodynamic noise generated by the wind turbine has usually the highest strength of sound in the range of low-frequencies (Katinas et al., 2016; Arana et al., 2015; MDEP, 2012).

The ears of mammals are designed to process a broad frequency range. The literature data on the hearing range in rodents show that these animals can be divided into two groups: those with extended low-frequency hearing (below 100 Hz) and those with restricted low-frequency hearing (not hearing appreciably below 1000 Hz) (Heffner et al., 2001; Engle and Barnes, 2012). Our surveyed species belong

rather to the second group and a large part of the noise spectrum produced by wind turbines is inaudible for these animals.

Low-frequency sounds or infrasound, although they are inaudible for some rodents, may be biologically significant. Du et al. (2010) showed a possible mechanism for infrasound-induced "stress" in laboratory rats – a high level of infrasound (16 Hz, 130 dB) activated microglial cells and up-regulation of CRH-R1 expression (corticotrophin-releasing hormone and its receptor). Branco et al. (2004) studied long-term exposures (2160 h) of rats to moderately intense (85 dB) low frequency noise ( $\ll 500$  Hz) and found changes in their lung parenchyma. However, long-term exposure to infrasound with frequencies and a sound pressure level typical for wind turbines and its effects on rodents have not been studied so far.

Audible sound may be significant for animals to even greater extent than infrasound (Kight and Swaddle, 2011). It is known from numerous studies that noise pollution causes changes in foraging, anti-predator behaviour, reproductive success, density and community structure of animals (Barber et al., 2010; Chan et al., 2010), and habituation or adaptation patterns may occur in response to noise stressors (Kight and Swaddle, 2011). Due to their continuous character, wind turbine sounds of higher frequencies can be potentially an important factor influencing the level of corticosterone in the surveyed small mammals. The most probable effect of continuous wind turbine sounds of higher frequencies is associated with increasing individual vigilance of mammals (Kern and Radford, 2016; Dukas, 2004; Barber et al., 2010), because aerodynamic noise masks other environmental sounds like that of an approaching predator. According to Rabin et al. (2006), ground squirrels at a turbine site exhibited a higher level of overall alertness, compared to animals from a control site, which indicates that they perceived themselves to be under higher risk. This phenomenon may occur also in the case of the voles from our study, since these social animals also exchange warning signals when a predator appears (Gerkema and Verhulst, 1990).

##### 4.1.3. Mechanical noise from wind turbine machinery

The aerodynamic noise from the blades is generally considered to be the dominant noise source, but for animals living near wind turbines the mechanical noise may be equally significant. The mechanical noise is emitted by the yaw motors, cooling fans, power converter, hydraulic pumps, and bearings. The yaw mechanism is responsible for turning the nacelle to a position perpendicular to the wind according to its direction. The pitch mechanism is used in power control – it turns the blades to limit the generated power or adjusts it to the nominal level. Cooling fans are responsible for extracting the heat generated during the work of the turbine and are activated or deactivated automatically (Arana et al., 2015). Such noises are much less predictable than the continuous typical "swishing" sound of the blades because they are generated suddenly during activations or deactivations of various parts of machinery. Such mechanical noises are ignored in the noise propagation model because their spatial extent is limited, but they are clearly audible next to the turbine (Arana et al., 2015). The mechanical noise consists of both high-pitched (high frequency) and buzzing sounds (low frequency), and can be easily heard by rodents. As a consequence, animals living near turbines are exposed to episodes of sudden noise repeated many times throughout the day. Such episodes may be a significant factor increasing the level of corticosterone in the surveyed rodents because the reaction to a sudden sound is an essential element of anti-predatory behaviour of small mammals. The higher vigilance of mammals exposed to anthropogenic noise described above (Kern and Radford, 2016; Barber et al., 2010), including the noise of wind turbines (Rabin et al., 2006), may additionally sensitize small mammals to sudden, unexpected sounds from the environment.

##### 4.1.4. Vibrations of the ground

Vibrations may be perceived by animals as a mixture of auditory and tactile sensations. There are numerous papers that prove the effect

of vibration on mammals, e.g. those cited by Norton et al. (2011), Rabey et al. (2015) or by Atanasov et al. (2015). Wind turbines are large vibrating cylindrical towers, strongly coupled to the ground with massive concrete foundation, through which low frequency vibrations are transmitted to the surroundings even at a distance of several kilometres (Saccorotti and Piccinini, 2011; Xi, 2014). The vibration of the ground near a wind turbine may be not only mechanically but also acoustically induced. The turbine-dependent seismic amplitude increases with wind speed and vibrations at the turbine base may be about 10 000 times higher than a few hundred meters away (Xi, 2014). The question is whether vibrations at the levels generated by wind turbines may be biologically significant. Ground vibrations induced by wind turbines are rather small and only very sensitive seismographs can detect such seismic activity (Saccorotti and Piccinini, 2011; Xi, 2014). Vibration levels near turbines do not exceed 120 nm (the maximum motion that is induced at about 1 Hz), or  $10^{-12}$  m<sup>2</sup>/Hz if the units of power spectral density were used (MDEP, 2012; Xi, 2014). Botha (2013) found that ground vibrations induced by wind turbines (located 90 m away from the measurement point) are small and walking or running 50 m from the measurement point elicited larger vibrations than those produced by a wind turbine (Schmidt and Klokke, 2014). Such small vibrations of the ground probably do not exceed the threshold for tactile perception of vibration. Moreover, rodent's body is able to attenuate vibrations of high magnitudes 0.3–1 m/s<sup>2</sup> at many frequencies (Rabey et al., 2015). Hence, we can conclude that vibrations generated by wind turbines seem not to be a main factor affecting the level of corticosterone in the surveyed small mammals.

#### 4.1.5. Electromagnetic field

The electromagnetic field (EMF) around wind turbines was studied by McCallum et al. (2014). If transformers were located in the hub of the turbines, the mean EMF level (characterized by magnetic flux density) was 0.9 milliGauss (mG) at the base of the wind turbines, decreased to background levels (0.2–0.3 mG) within a 2 m radius. Wind turbines with pad-mounted transformers located at the ground level could potentially generate higher levels of EMF. For such transformers, the EMF level was at 67 mG, but its value decreased to the background level (0.2–0.3 mG) within 8–10 m. This indicates that, despite the type of the wind turbine (i.e., hub vs. pad-mounted transformer), the EMF levels in the vicinity of wind turbines are relatively low and are increased only within a small radius. Therefore, EMF produced by wind turbines is not expected to be a key factor increasing the level of corticosterone in small mammals.

### 4.2. Differences in the CORT level between species

The above considerations suggest that the main factors potentially able to increase corticosterone levels in the studied animals may be the permanent exposure to the aerodynamic noise of wind turbines and episodes of mechanical noise repeated many times throughout the day. Both species, however, are exposed to these noises in the same way. What makes them different in their reaction? There are a few possible explanations.

#### 4.2.1. Width of the ecological niche

Both study species are the most abundant and stable components of the small mammal fauna of agricultural landscapes in Poland. However, they differ ecologically and belong to different taxonomic groups. The striped field mouse belongs to the family Muridae and occupies a broad range of habitats and food niches. It inhabits farmlands, various types of ecotones, woodlands, dry and wet grasslands, marshes, and pastures and does not avoid habitats in the vicinity and within human settlements – gardens, storehouses, and dwellings (Gliwicz and Kryštufek, 1999; Horváth and Trócsányi, 1998). The striped field mouse is also known as the most synurbic species in central Europe (within its geographical range) and it can occur in urban green areas even in the

centre of the city, often as the only rodent species there (Łopucki et al., 2013; Łopucki and Kiersztyn, 2015). Such a wide range of potentially suitable habitats reflects high ecological plasticity of this species and its high ability for adjustment or habituation to various anthropogenic disturbances. In turn, the common vole belongs to the family Cricetidae; it prefers mainly open habitats and feeds on the green parts of grasses and herbaceous plants (Zima, 1999). In Poland, the species inhabits mainly farmlands, pastures, meadows, and gardens. It avoids storehouses and human dwellings and rarely occurs in urban areas, as it is known as urban-sensitive species (Łopucki and Kitowski, 2017). Hence, potentially, the striped field mouse should be more resistant to such anthropogenic disturbances as wind farms or they habituate faster to this stressor over time than the common vole. In consequence, lower differences in the corticosterone level should be observed in mice than in vole species, which would explain our results.

#### 4.2.2. Home ranges and family social groups

The striped field mouse does not form family social groups, whereas the common vole lives in colonies consisting of several breeding females and their offspring. Within their home ranges, voles maintain aboveground runways, which facilitate their movements and orientation. The home ranges of the common vole are small and their size was estimated similarly by various authors: 125–145 m<sup>2</sup> (Briner et al., 2005), 145–350 m<sup>2</sup> (Mackin-Rogalska, 1981), 200 m<sup>2</sup> (up to 450 m<sup>2</sup>) (Jacob and Hempel, 2003). The home range size of the striped field mouse is usually one order of magnitude larger: 1800–2400 m<sup>2</sup> (Yang and Zhuge 1989) or even 5600 m<sup>2</sup> (Wierzbowska and Chelkowska, 1970). Additionally, a tendency for long distance movements was found for the striped field mouse, where 60% of studied individuals were observed to move at a distance over 100 m (Liro and Szacki, 1987). In contrast, the opposite spatial behaviour was observed for the common vole – radio-tracked female voles spent 99% of the time within 2 m radius around the nests (Boyce and Boyce, 1988) and a 95% spatial use of voles was estimated at 31 m<sup>2</sup> (Briner et al., 2005).

The small home ranges of voles suggest that those trapped near the wind turbine lived within its proximity and spent there all the time under its influence. The substantially larger home ranges of the striped field mouse indicate that at least part of the time they could occupy habitats at a greater distance from the turbine. As a consequence, the common vole is more exposed to the effects of wind turbines, which may explain the higher corticosterone levels found for this species in our study.

#### 4.2.3. Daily activity and predation pressure

According to Monarca et al. (2015), daily activity of rodents may affect their response toward stressors. In their study, wood mice *A. sylvaticus*, reacted physiologically to the stress-inducing sound stronger during the active than the resting period. In our study, both species had different daily activity patterns: voles represent an ultradian rhythm, whereas mice are mostly active during the night (Gerkema and Verhulst, 1990; Gliwicz and Kryštufek, 1999). Consequently, voles are more exposed to anthropogenic stressors such as noise described above.

The ultradian rhythm exposes voles to a broader group of predators (day and night time predators) and, in fact, the common vole is one of the main food sources for a considerable number of predators in Central Europe (Jacob and Brown, 2000). Due to the high predatory pressure, voles may depend on social synchrony of ultradian feeding rhythms by exchanging warning signals when a predator appears (Gerkema and Verhulst, 1990). The aerodynamic noise of wind turbines, which masks acoustic signals from the environment, may be an important factor influencing the corticosterone level in this social species.

## 5. Conclusions

Wind turbines may have a stressful impact on some species of small mammals living in their proximity. We observed such an effect on the

common vole – a social species, with a narrow ecological niche, low spatial mobility, and living under high predatory pressure. The main factors that may increase corticosterone levels in this species include permanent exposure to the aerodynamic noise of wind turbines and episodes of mechanical noise. These factors may increase the general vigilance of animals by masking the acoustic warning signals from the environment most of the time and by exposing animals to sudden, unexpected mechanical sounds repeated many times throughout the day. The other studied species, the striped field mouse, did not show a stress response on the area near wind turbines. We hypothesise that this is an effect of high ecological plasticity of the species and its capability of adjustment or habituation to various anthropogenic disturbances. This is the first study showing evidence of the impact of wind farms on physiological stress reactions in wild rodent populations. It shows that more attention should be given to the effects of wind farms upon non-flying wildlife. Investors and responsible authorities should always consider various impacts of wind farms on wildlife during environmental impact assessments and try to reduce these negative effects. One possible solution is to avoid formation of new attractive habitats in the vicinity of turbines, because animals colonizing them after completing the construction can be exposed to the negative impact of the operating machinery.

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