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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 ( ± 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 $ml^{-1}$. 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 ± 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