

WIND TURBINES CAUSE CHRONIC STRESS IN BADGERS (*MELES MELES*) IN GREAT BRITAIN

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ABSTRACT: A paucity of data exists with which to assess the effects of wind turbines noise on terrestrial wildlife, despite growing concern about the impact of infrasound from wind farms on human health and well-being. In 2013, we assessed whether the presence of turbines in Great Britain impacted the stress levels of badgers (*Meles meles*) in nearby setts. Hair cortisol levels were used to determine if the badgers were physiologically stressed. Hair of badgers living >1 km of a wind farm had a 264% higher cortisol level than badgers <10 km from a wind farm. This demonstrates that affected badgers suffer from enhanced hypothalamo-pituitary-adrenal activity and are physiologically stressed. No differences were found between the cortisol levels of badgers living near wind farms operational since 2009 and 2012, indicating that the animals do not become habituated to turbine disturbance. Cortisol levels in the affected badgers did not vary in relation to the distance from turbines within 1 km, wind farm annual power output, or number of turbines. We suggest that the higher cortisol levels in affected badgers is caused by the turbines' sound and that these high levels may affect badgers' immune systems, which could result in increased risk of infection and disease in the badger population.

Key words: Badgers, cortisol, hair, *Meles*, stress, wildlife, wind turbines, WTS.

INTRODUCTION

Humans living within 2 km of a wind farm frequently report suffering from ill health (Shepherd et al. 2011), with symptoms ranging from headaches and sleep disturbance to increased stress (Pedersen 2009). Such symptoms are referred to as wind turbine syndrome (WTS; Colby et al. 2009), and it is widely attributed to audible or infrasound (sound with a frequency below 20 Hz; Salt and Kaltenbach 2011). Although the first UK public wind turbine became functional in 1951 (Price 2004), the effects of wind farms on human health remain poorly understood.

The impact of turbines on terrestrial wildlife is also not well understood. Research by Rabin et al. (2006) has demonstrated that wind turbines can have a negative impact on wildlife: squirrels living near turbines exhibit increased behavioral stress. Badgers (*Meles meles*) are suitable mammals to further assess physiologic changes as a result of wind farm developments because they often reside in habitats

in which turbines are constructed. Importantly, badgers also have a similar hearing range to humans (Heptner 2002).

To ascertain if “affected” badgers show physiologic stress (hereafter referred to as stress), cortisol levels in badgers chronically exposed to turbine disturbance were compared with the cortisol level of badgers in comparable areas without turbines. Cortisol is a steroid hormone assembled from cholesterol in the adrenal gland (Werbin and Chaikoff 1961), and this pathway is controlled by the hypothalamus in response to stress (Lundberg 2005). This same relationship exists for lower vertebrates: Kikuchi (2010) reported that fish develop raised cortisol levels when subjected to conditions of an offshore wind farm reconstructed in the laboratory.

The function of cortisol is to increase the sugar level in the blood through gluconeogenesis and to redirect energy (Kirschbaum et al. 1997) toward parts of the body, such as the brain and muscles, which would help the individual escape an immediate threat. In turn, this starves the immune and reproductive systems and may hinder their vital

TABLE 1. Key information relating to badger (*Meles meles*) setts sampled at six wind farms in Great Britain, 2013.

Wind farm (year functioning)	Sett	Distance to closest turbine (m)	Average distance to all turbines (m)	Number of turbines category	Annual megawatt production category
1 (2012)	1	100	1,021	<20	<40
	2	300	736	<20	<40
2 (2009)	1	40	720	>20	>40
	2	80	790	>20	>40
	3	350	1,113	>20	>40
3 (2012)	1	140	858	<20	<40
4 (2009)	1	990	1,189	>20	>40
5 (2009)	1	100	1,397	>20	>40
6 (2012)	1	100	862	<20	<40

function (Mostl and Palme 2002; Maeda and Tsukamura 2006). The effect of a short-term increase in cortisol is insignificant, but a prolonged increase in cortisol can lead to serious suppression of the immune system (Mostl and Palme 2002); in humans, it has been recorded to exacerbate an individual's susceptibility to infection (Agarwal et al. 2002; Cohen et al. 2012). Chronic raised cortisol may also affect reproduction (Tilbrook et al. 2000; Mostl and Palme 2002), as demonstrated in meerkats, where elevated stress increases abortion rates (Young et al. 2006).

In mammals, cortisol levels are usually determined from blood, urine, saliva, or feces (Morton et al. 1995; Creel et al. 2002) but obtaining such samples from badgers poses significant problems of capture, restraint, and handling, all of which cause stress. Hair samples can be collected noninvasively, and cortisol from hair has been shown to give a reliable measure of chronic stress in animals, including wildlife (Davenport et al. 2006). The use of hair further avoids the problem of diurnal fluctuations of cortisol level in body fluids (Edwards et al. 2001), as it gives a measure of the average cortisol level over a prolonged period (Davenport et al. 2006). Assaying hair is further justified in that saliva and feces need to be fresh (Washburn and Millsaugh 2002; Descovich et al. 2012), which is challenging with wild animals in remote sites.

MATERIALS AND METHODS

Sampling

Twenty-five badger setts were selected, nine of which were <1 km from a turbine within a wind farm (affected setts), and 16 located >10 km from any turbine or wind farm (control setts), throughout Great Britain (from the north of Scotland, to Cornwall and into Wales). Control setts were chosen to be as comparable as possible to those at affected sites in terms of habitat type, distance from major roads, and covering a similar geographic spread throughout Britain. Six wind farms were included in the study, with multiple badger setts being used in proximity to wind farm 1 and 2. Setts were sufficiently distant from each other so that they were deemed to belong to different badger clans, thus ensuring that the samples were independent. Where required, samples were taken with permission of the landowners, although an important condition for gaining access was that the locations remained confidential. In Britain, badgers are legally protected against persecution by badger baiters, farmers, and others. Therefore, it is normal practice and is required legally under the terms of the access consents that their locations are withheld from the public domain. It is not even possible to identify the local authorities without compromising confidentiality. However, coordinates of field study locations can be made available to researchers upon the appropriate consents being obtained. Table 1 lists in further detail the wind farms included in this study. Tables 2 and 3 describe in more detail the number of locations, setts, and samples.

Badger hair was collected in 2013, either opportunistically by hand from soil heaps outside the setts or by using hair traps (Balestrieri et al. 2010) placed on badger paths, from setts located throughout Britain. Samples were taken at least

TABLE 2. Number of badger (*Meles meles*) setts located at each of the six wind farms and the number of samples (opportunistic and trap) collected at each sett, Great Britain, 2013.

Wind farm (year operational)	Sett name	Samples collected opportunistically	Trap samples produced	Total samples produced
1 (2012)	1	1	2	3
	2	1	2	3
2 (2009)	1	2	1	3
	2	2	3	5
	3	1	2	3
3 (2012)	1	2	3	5
4 (2009)	1	1	2	3
5 (2009)	1	3	2	5
6 (2012)	1	1	0	1
Total	9	14	17	31

weekly for a 6-wk period from May to July to minimize the variation of nutritional and environmental stresses the badgers in different setts may experience. Capturing individuals for independent sample collections raises animal welfare concerns; thus, it was decided to treat individual setts as one sample. Hair samples were wrapped in foil, double bagged, and stored in a domestic freezer at -20°C until analysis. For each sample, the entire shaft of the guard hair was used, and no distinction was made between the white or black sections of the hairs.

Cortisol assay

Cortisol in the samples was determined with a commercial salivary cortisol assay kit (Salimetrics, Newmarket, UK; Fowkes et al. 2013; Meyer et al. 2014). Briefly, 2–3 mg of whole hair shafts were finely cut and immersed in 2 mL of absolute methanol (18 h at 20°C) before centrifugation ($3,000 \times G$ for 5 min at 4°C). The supernatants were dried (18 h at 60°C) before resuspension in the assay buffer supplied in the kit and absorbance measurement at 450 nm. Values were expressed as micrograms of cortisol per deciliter normalized per milligram hair sample. The inter-

TABLE 3. Number of control setts located in each of the six counties included and the number of hair samples (opportunistic and trap) collected at each control sett, Great Britain, 2013.

County	Sett	Samples collected opportunistically	Trap samples produced	Total samples produced
1	1	1	1	2
	2	1	0	1
	3	1	0	1
2	1	0	3	3
	2	0	2	2
	3	3	3	6
	4	1	1	2
	5	2	1	3
3	1	1	1	2
	2	1	1	2
4	1	0	1	1
	2	0	1	1
	3	1	2	3
	4	0	2	2
5	1	2	1	3
6	1	2	0	2
Total	16	16	20	36

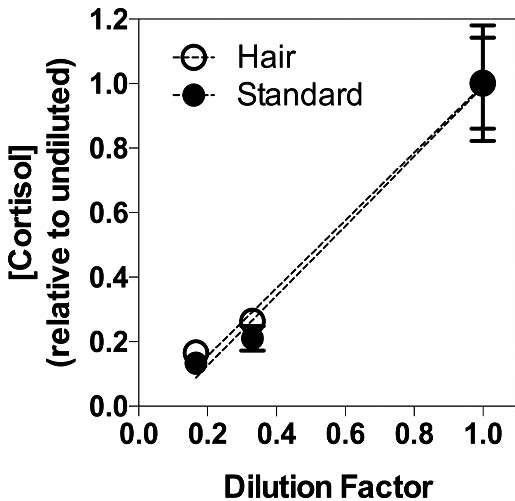


FIGURE 1. Demonstration of linearity under dilution of pooled badger (*Meles meles*) hair samples, compared with cortisol standards, with significant correlation coefficients (0.99, hair and 0.98, cortisol standard).

and intraassay coefficients of variance were 11 and 9%, respectively. To ascertain the performance of the cortisol assay for hair, as opposed to saliva, for which the kit is marketed, linearity under dilution of a pooled hair sample and cortisol standard was assessed as described previously (Rosca et al. 2014). For these, badger hair samples, collected from multiple setts, were pooled and extracted, as described previously but assayed undiluted and at 1:3 and 1:6 dilutions. Known cortisol standards, prepared at the same dilutions, were assayed in parallel. The hair sample and cortisol standard performed identically under dilution, with a correlation coefficient (r^2) of 0.99 (hair) and 0.98 (cortisol standard; Fig. 1).

Data analysis

As the data were not normally distributed, transformation to natural log was performed before independent sample t -tests and linear regression analyses with GraphPad Prism software. The statistical significance level was $P \leq 0.05$. Linearity under dilution of the cortisol assay was analyzed by ordinary linear regression analysis by using built-in equations within GraphPad Prism 6.0a (GraphPad, La Jolla, California, USA).

Ethics statement

In the UK, it is illegal to injure, kill, or cruelly treat a badger or to disturb badger setts under the Protection of Badgers Act 1992. Licenses from government environmental agencies, including

Natural England, Scottish Natural Heritage, or the Countryside Council for Wales, are required for certain research procedures. However, these bodies confirmed that no licenses were required for our noninvasive methods. Our methods also underwent formal ethical review and approval by independent experts at the Zoological Society of London.

RESULTS

Badger hair from the affected and control setts had a mean cortisol level of 3.16 $\mu\text{g/dL}$ per milligram ($\text{SD}=2.41$) and 0.87 $\mu\text{g/dL}$ per milligram ($\text{SD}=0.79$), respectively (Fig. 2a), an increase of 264% in cortisol levels in turbine-impacted animals ($P=0.001$; $n=25$, $\text{df}=23$). There was no significant difference between cortisol levels in hair collected opportunistically or from traps (paired t -test; $P=0.236$, $n=32$, $\text{df}=15$). A second independent t -test, again with equal variance assumed, showed no differences between mean cortisol levels of badgers with setts were near wind farms operational since 2009 and those near turbines operational since 2012 ($P=0.583$, $n=9$, $\text{df}=7$; Fig. 2b). This indicates that badgers do not become habituated to the turbines and that the stress is a result of functioning turbines and not only turbine construction. To test if cortisol levels were affected by the wind farm size or the proximity of setts to turbines, additional regression analyses were performed to assess the relationship of cortisol with 1) the number of turbines in the array 2) the distance from the sett to the closest turbine, and 3) the average distance of the sett to all the turbines within the wind farm. We found no significant differences: 1) $P=0.472$, $r^2=0.076$, $n=9$; 2) $P=0.217$, $r^2=0.208$, $n=9$; 3) $P=0.976$, $r^2=0.00$, $n=9$; Fig. 3a, c, d. There was no significant correlation between badger hair cortisol levels and power output of the wind farms ($P=0.460$, $r^2=0.08$, $n=9$; Fig. 3b).

DISCUSSION

The very high levels of cortisol detected in hair from badgers living near wind farms compared with turbine-free sites strongly

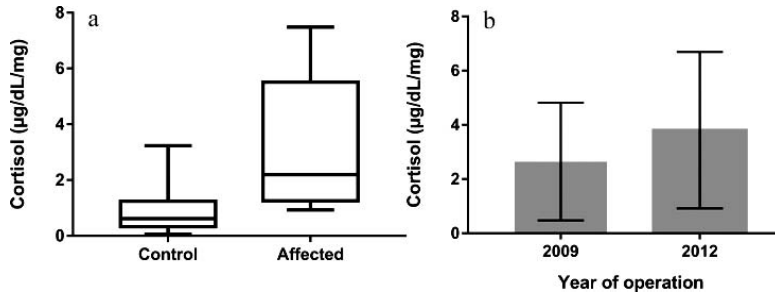


FIGURE 2. Cortisol levels (micrograms per deciliter per milligram) in badger (*Meles meles*) hair collected from various locations in Great Britain, close to or remote from a wind farm. (a) Three quartiles, the highest and the lowest values for the hair cortisol from control badger sets (>10 km from the closest turbine within a wind farm) and affected badger sets (<1 km from a turbine within a wind farm), control $n=6$ and affected $n=9$. (b) Mean cortisol levels in badger hair from sets at established and new wind farm sites, with ± 1 SD bars. All sets were within 1 km of a turbine within a wind farm. Established wind farms are those that have been operational since 2009 (sets $n=5$; wind farms, $n=3$). New wind farms are those that have been operational from 2012 (sets $n=4$; wind farms, $n=3$).

indicates that turbine-impacted badgers were suffering from a chronic increase in their hypothalamo-pituitary-adrenal axis activity and thus can be described as stressed (Mostl and Palme 2002). Cortisol is incorporated into hair throughout its growth (anagen) phase, which can last 3–5 mo in badgers (Maurel et al. 1986); thus, the badgers had experienced stress for several months and were chronically

affected. There have been conflicting results concerning the effect of hair color on cortisol level. Although Bennett and Hayssen (2010), González-de-la-Vara et al. (2011), and Tallo-Parra et al. (2015) found hair color to have an effect, Sauve et al. (2007) and Manenschijn et al. (2011) found no relationship between color and cortisol concentrations. In our study, we assumed that hair color had no effect on

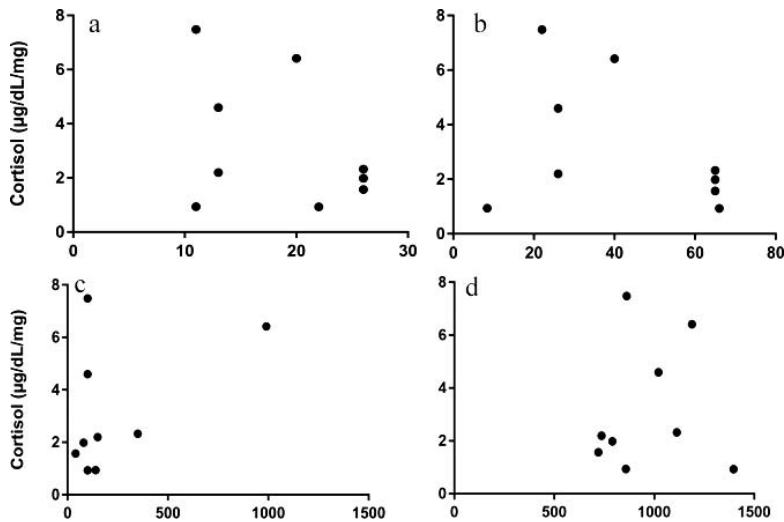


FIGURE 3. Relationship between cortisol levels (micrograms per deciliter per milligram) in badger (*Meles meles*) hair and characteristics of wind farms. Scatter plots depicting no relationship (when linear regression was performed) between hair cortisol values. (a) Number of turbines in the wind farm (10–30 turbines). (b) Number of megawatts produced annually by the wind farms (range: 5–70 MW). (c) Distance from the sett to the closest wind turbine (range: 40–990 m) and (d) average distance of badger setts to all the wind turbines on the farm (range: 720–1,397 m). In all cases, $n=9$.

cortisol values. The hair trap design used for both affected and control groups would have removed guard hairs from the badgers' backs. Guard hairs have both black and white sections, and there was no conspicuous bias to either color within the affected or control groups.

Chronic stress with persistently high cortisol levels can have serious detrimental physiologic effects, including suppression of immunity and impaired reproduction (Mostl and Palme 2002). This could render badgers more susceptible to infection and disease. The prolonged presence of elevated cortisol levels suppresses the differentiation and function of macrophages (Baybutt and Holsboer 1990). This may apply to other vertebrates as well. For example, Maule et al. (1989) demonstrated that stress reduced the ability of lymphocytes from Chinook salmon to produce specific antibodies, while Espelid et al. (1996) similarly found that cortisol suppresses B and T lymphocytes in fish. It is possible that badgers with raised cortisol levels living within close proximity to wind turbines could potentially suffer from reduced immune function. This could result in increased susceptibility to disease and potentially facilitate the spread of pathogens among the affected population. As badgers are carriers of bovine tuberculosis (bTB; Wilson et al. 2011), an increase in disease susceptibility could have consequences for the spread of this disease in cattle, compounding the problem of bTB for farmers. However, establishing a relationship between elevated cortisol levels and disease susceptibility in badgers requires further investigation.

Although certain intrinsic factors, such as sex, age, and disease status, have been thought to influence cortisol levels, it is very unlikely that the 264% cortisol increase experienced by affected badgers is a result of these factors alone. George et al. (2014) found no differences in badger serum or fecal cortisol values between sexes, age classes, or breeding status categories, so a sex difference, if present, would presumably be minimal. Differences in cortisol levels between affected and control animals are likely not due to differential

degradation of hair from different sites, as cortisol levels in hair appear to be very stable indicated by research by Bennett et al. (2010) who found no significant difference in cortisol between the distal and proximal end of the hairs. In addition, this hypothesis is supported by work by Bechshoft et al. (2012) that showed that 100-yr-old polar bear hair did not contain less cortisol than recent hair, and the work of Webb et al. (2010) found similar results: higher cortisol concentration in archaeologic hair than modern hair.

Cortisol may vary with body condition (George et al. 2015) or dominance status (Abbott et al. 2003), but because we considered each sett as a single unit, which in badger communities, usually consists of a dominant male and female with approximately seven adults of both sexes who may be related or immigrants (Rogers et al. 1997), variations in body condition, as a result of dominance status, is likely not a confounding factor. Preexisting disease is also unlikely to have influenced the results because setts were selected in an arbitrary manner. Therefore, the number of diseased badgers would not significantly diverge from the expected infection rate within a population (i.e., the number of diseased setts, if there were any, should not significantly differ between affected and control setts).

Because the major factor distinguishing the badgers from the two categories of sampling sites was the presence of wind turbines, it is reasonable to suggest that the increased cortisol levels in the hair is the result of disturbance from these installations, with vibration, noise, and especially infrasound, the most likely reasons. Mikolajczak et al. (2013) similarly reported an increase in cortisol of farmed (captive) geese near wind farms and attributed the cause to infrasound from turbines.

We cannot assert categorically that low-frequency sound was directly responsible for our results. Noise and infrasound are problematic to measure and understand in terms of their perception by badgers. Complicating factors, such as vegetation cover and temperature, are highly variable (Colby et al.

2009) and may attenuate infrasound in or around the setts. Furthermore, badgers spend a large proportion of their life underground in setts throughout their territories (Roper 2010). Setts may consist of numerous chambers at different depths and soil compositions, all of which could attenuate infrasound to different extents. Accordingly, we did not attempt to find a relationship between infrasound and cortisol level. Rather, we looked at the overall effect of wind turbines on cortisol in badgers. However, even if there were no direct effect from noise, other more indirect consequences of the wind farm development could influence badger well-being. For example, the turbine foundations or vibration created might have altered the population of earthworms upon which badgers rely for food (Kowalczyk et al. 2004). To the best of our knowledge, there is no information available about this.

The absence of a correlation between cortisol values and distance to the closest turbine or the average distance from the sett to all the turbines could be explained by the fact that badgers move around their territories, which range from 0.2 km² to 1.5 km² (Forestry Commission England 2016). In addition, the degree to which sound carries is largely governed by terrain, vegetation, and weather (Colby et al. 2009), making it difficult to equate noise level simply to distance. Also, noise is not solely a function of the number of turbines, but rather their layout and concentration, which may account for the lack of relationship between cortisol values and the number of turbines reported here. Furthermore, turbines vary in size and power, thus, also the level of sound they produce. Large turbines (2.3–3.6 MW) tend to create higher levels of infrasound than smaller ones (<2 MW; Moller and Pedersen 2011) a factor that could explain the lack of correlation between cortisol values and annual output.

Our findings are preliminary, but may have wider implications that relate to the wildlife and human WTS controversy. A report written for the Department for Environment,

Food and Rural Affairs recognized that wind turbines generate low-frequency noise, and low-frequency sound has negative implications for humans, such as nausea (Casella Stanger 2001). Reduced immune functioning and reproductive success could have impacts on wild animals already challenged with habitat loss and other anthropogenic disturbances.

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