Wind turbines cause chronic stress in badgers

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Abstract

There is a paucity of data 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 wellbeing. The study was undertaken to assess whether the presence of turbines impact the stress levels of badgers in nearby setts. This was achieved by using their hair cortisol level to determine if the badgers are physiological stressed. The results reveal that the hair of badgers living within 1 km of a wind farm have a 264% higher cortisol level than control badgers. This demonstrates that affected badgers suffer from enhanced hypothalamo-pituitary-adrenal activity and thus 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 1km, wind farm annual power output or number of turbines. We propose that the rise in cortisol levels in affected badgers is caused by the turbines' sound. This could have detrimental impacts on the animals' immune system which could result in the increased risk of infection and disease and thus facilitating the spread of disease within the badger population.

Key words: badgers, cortisol, hair, *meles*, stress, wildlife, wind turbines, WTS

Introduction

Humans living within 2km 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 and/or infrasound (i.e. sound with a frequency below 20Hz (Salt and Kaltenbach 2011)). Despite the first UK public wind turbine becoming functional in 1951 (Price 2004) the negative effects of wind farms on human health remain poorly researched so are still not wholly accepted.

The impact of turbines on terrestrial wildlife is also not well understood however research by Rabin et al. (2006) has demonstrated that wind turbines can similarly have a negative impact on wildlife: squirrels living near turbines exhibit increased behavioural stress. Badgers (*Meles meles*) are suitable mammals to further assess physiological changes as a result of windfarm developments since they often reside in habitats in which turbines are constructed. Importantly, badgers also have a similar hearing range to humans (Roper 2010).

To ascertain if "affected” badgers do indeed show physiological stress (forthwith referred to as stress) cortisol levels in badgers chronically exposed to turbine disturbance were compared to the cortisol level of badgers in comparable areas without turbines. Cortisol is a steroid hormone that is assembled from cholesterol in the adrenal gland (Werbin and Chaikoff 1961) and this pathway is controlled by the hypothalamus in response to stress (Lungberg 2005). This is true even for lower vertebrates as 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 the energy of the body (Kirschbaun et al. 1997). This means that energy is directed towards parts of the body, such as the brain and muscles to help the individual escape an immediate threat. In turn, this starves the immune and reproductive systems hindering their vital function (Maeda and Tsukamura 2006, Mostl and Palme 2002). 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) thus exacerbating the transmission and virulence of a disease (Lundbeg 2005). It may also affect reproduction (Mostl and Palme 2002, Tilbrook et al. 2000) by preventing embryonic implantation and the maintenance of a foetus (Crocker et al. no date).

In mammals, cortisol levels are usually determined from blood, urine, saliva or faeces (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 non-invasively 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 faeces need to be fresh (Descovich et al. 2012, Washburn and Millspaugh 2002) which is impractical for the study of animals in remote sites.

Materials and Methods

Sampling

Twenty-five setts were selected, nine of which were < 1km from a turbine within a wind farm (affected setts) and 16 located at least 10km from any turbine or wind farm (control setts). Control setts were chosen, as far as possible, to be comparable to those at affected sites in terms of being situated in similar land types, at comparable distances from major roads and covering similar areas of Britain. In addition each sett was located at a sufficient distance from the others, such that they were deemed to belong to different badger clans, thus ensuring that the samples were independent. Where required, all 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, co-ordinates of the 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 & 3 describe in more detail the number of locations, setts and samples.

Badger hair was collected in 2013, either opportunistically by hand from the soil heaps outside the setts or by using hair traps (Balestrieri et al. 2010) placed on a badger path, from setts located throughout Britain. Samples were taken at least weekly for a six-week period from May to July to minimize the variation of nutrimental 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. The hair samples were wrapped in foil, double bagged and then stored in a domestic freezer until analysis. For each sample, the entire shaft of the guard hair was used and no distinction was made between the white or black coloured 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, 2-3mg of whole hair shafts were cut finely then immersed in 2ml of absolute methanol (18 h, 20 ^{o}C) before centrifugation (3000 g, 5 min, 4^{o}C). The resulting supernatants were dried (18 h, 60°C) before re-suspension in the assay buffer supplied in the kit and absorbance measurement at 450nm. The values were expressed as µg cortisol dL-1 normalised per mg hair sample. The inter- and intra-assay coefficients of variance were 11.15% and 8.7%, 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 above, but assayed undiluted, as well as at 1:3 and 1:6 dilutions. Known cortisol standards, prepared at the same dilutions (i.e. neat, 1:3, 1:6), were assayed in parallel. The results show that 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) (Figure 1), thereby validating the assay.

Data analysis

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

Ethical 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, and licenses from the respective government environmental agencies, namely Natural England, Scottish Natural Heritage or the Countryside Council for Wales, are required for certain research procedures. However these bodies all confirmed that no licenses were required for the present research as the methodology was non-invasive. The procedures used in the present study also underwent formal ethical review and approval by independent expert scientists at the Zoological Society of London.

Results

The results of the assays on hair samples collected from traps or obtained opportunistically from the affected and control sites revealed that badger hair from the affected setts had a mean cortisol level of 3.16ug dL^{-1} mg^{-1} (SD= 2.42) whereas the mean level in control hair samples was 0.87ug dL^{-1} mg^{-1} (SD= 0.80) (Figure 2a displays the quartiles, highest and lowest values in the form of a box plot.). This represents an increase of 264% in cortisol levels in turbine-impacted animals. Statistical analysis established that the difference in cortisol is highly significant at P=0.001 (n=25, df =23). Importantly, a paired t test showed that there was no significant difference between the cortisol level in hair collected opportunistically or from traps (P=0.199, n=32, df=15). A second independent t-test, again with equal variance assumed, further showed that there were no differences in the mean cortisol levels of badgers whose setts were sited near wind farms operational since 2009 and those sited near turbines operational since 2012 (P=0.602, n=9, df=7) (Figure 2b). This indicates that badgers do not become habituated to the presence of turbines and that the stress is a result of functioning turbines and not only of the construction of the turbines. 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 relationship of cortisol with (i) the number of turbines in the array (ii) the distance from the sett to the closest turbine and (iii) the average distance of the sett to all the turbines within the wind farm. Surprisingly, no significant differences were found ((i)P=0.428, r^2=0.21, n=9; (ii)P=0.213, r^2=0.092, n=9; (iii) P=0.959, r^2=0.00, n=9). (Figure 3a, c-d). Finally the present study did not reveal a correlation between badger hair cortisol levels and the power output of the wind farms (P=0.407, r^2=0.1, n=9) (Figure 3b).

Discussion

The very high levels of cortisol detected in hair from badgers living near wind farms compared to that from the turbine-free sites strongly indicates that turbine-impacted badgers were suffering from a chronic increase in their hypothalamo-pituitary-adrenal (HPA) axis activity, and thus can be described as stressed (Mostl and Palme 2002). Crucially, cortisol is incorporated into hair throughout its growth (anagen) phase, which can last for 3 or 5 months in badgers (Maurel et al. 1986), thus these findings show that the badgers must have experienced stress for several months, and were therefore chronically affected. There has been conflicting research over the effect of hair colour on cortisol level. Tallo-Parra et al. 2015, Bennett and Hayssen 2010 and González-de-la-Vara et al. 2011 found hair colour to have an effect while in comparison Sauve et al. 2007 and Manenschijn et al. 2011 found no relationship between colour and cortisol concentrations. In this research it was reasonable to assume that hair colour had no bearing on the cortisol values. The hair trap design used for both affected and control groups will have removed guard hairs from the badgers' backs. Guard hairs have both black and white sections and there was no conspicuous bias to either colour within the affected or control groups.

It is well established that chronic stress with persistently high cortisol levels have serious detrimental physiological effects, and include the suppression of immunity and impaired reproduction (Mostl and Palme 2002). For badgers this would have implications for their health by rendering them more susceptible to infection and disease. Examples of research into the effect of raised cortisol levels on the immune system includes that by Baybutt and Holsboer (1990) who demonstrated that the prolonged presence of raised cortisol suppressed the differentiation and function of macrophages. This applies to other vertebrates as well. For instance, 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, therefore, reasonable to consider that badgers with raised cortisol levels living within close proximity to wind turbines, will have reduced immune function. This would likely increase their susceptibility to disease and facilitate the spread of pathogens amongst the affected population. As badgers are carriers of bovine TB (Wilson et al. 2011) an increase in disease susceptibility could have consequences in the spread of this disease in cattle, compounding the problem of TB for dairy farmers.

Whilst it is true that certain intrinsic factors, such as sex, age and disease status, can influence cortisol levels, it is very unlikely that the 264% cortisol increase experienced by affected badgers is a result of these factors alone. Certainly, George et al. (2014) found no differences in badger serum or faecal cortisol values between sex, age classes or breeding status, so any gender difference, if any occurred at all, would be minimal. Neither can the difference be due to any differential degradation hair from different sites, as cortisol levels in hair appear to be very stable. Indeed, 100 year-old polar bear hair has been reported not to contain less cortisol then recent hair (Bechshoft et al. 2012). It could be argued that cortisol may vary with body condition or dominance status, but as the present study considered each sett as a single unit (which, in badger communities, usually consists of a dominant male and female with approximately 7 mixed sex adults who may be related or immigrants (Rogers et al. 1997)), variations in body condition, as a result of dominance, is removed as a confounding factor. Pre-existing disease is also unlikely to have influenced the results as all the setts were selected at random. Therefore the number of diseased badgers would not significantly diverge from the expected infection rate within a population, i.e. that the number of diseased setts, if there were any, should not significantly differ between affected and control setts.

Instead, as 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. Interestingly, Mikolajczak et al. (2013) has similarly reported an increase in cortisol of farmed (i.e. captive) geese in close proximity to wind farms and also attributed the cause to infrasound from turbines.

Of course, we cannot assert categorically that in the present study low frequency sound was directly responsible for our results. Both 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, the present investigation did not attempt to find a relationship between infrasound and cortisol level. Rather it looked at the overall effect of wind turbines on badgers' cortisol. However, even if there were no direct effect from noise, other more indirect consequences of the wind farm development could exert some influence on badger well being. For instance, the presence of turbine foundations and/or the 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 the cortisol values and the distance from the sett 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 in size from $0.2km^2$ to $1.5km^2$(Forestry Commission no date). 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 to simply a matter of distance. Noise is also not solely a function of the number of turbines, but rather their layout and concentration, which may account for the lack of relationship between the 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 (<2MW) (Moller and Pedersen 2011) a factor that could explain the lack of correlation between cortisol values and annual output.

The findings reported in the present paper, whilst preliminary, are important and have wider implications that relate to the wildlife and human WTS controversy. DEFRA has recognized that wind turbines generate low frequency noise and low frequency sound has negative implications for people (Casella Stanger 2001). Reduced immune functioning and reproductive success could have worrying impacts on wild animals already struggling to cope with habitat loss and other anthropogenic disturbances by significantly hampering conservation efforts.

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Author contributions and Competing financial interests

Conceived and designed the experiments: RCNA, RCF. Performed the experiments: RCNA, RCF. Analyzed the data: RCNA, VJS, RCF. Wrote the paper: RCNA, VJS, RCF.

The authors declare that there are no competing financial interests.

Literature Cited

Balestrieri A, Remonti L, Frantz AC, Capelli E, Zenato M, Dettori EE, Guidali F, Prigioni C. 2010. Efficacy of passive hair traps for the genetic sampling of a low density badger populations. *Hystrix,* 21(2), 137-146

Baybutt HN, Holsboer F. 1990. Inhibition of macrophages differentiation and function by cortisol, *Endocrinology*, 127(1):476

Bechshoft TO, Riget FF, Sonne C, Letcher RJ, Muir DCG, Novak MA, Henchey E, Meyer JS, Eulaers I, Jaspers VLB, Eens M, Covaci A, Dietz R. 2012. Measuring environmental stress in east Greenland polar bears, 1892-1927 and 1988-2009: What does hair cortisol tell us? *Environ. Int.,* 45, 15-21

Bennett A, Hayssen V. 2010. Measuring cortisol in hair and saliva from dogs: coat color and pigment differences 39(3) 171–180

Casella Stanger. 2001. Low frequency noise, DEFRA. Accessed 5th August 2013: http://www.google.co.uk/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=1&ved=0CDwQFjAA&url=http%3A%2F%2Fwww.scotland.gov.uk%2FResource%2FDoc%2F158512%2F0042973.pdf&ei=c7UQUo7bFIaV0AXCyIGICQ&usg=AFQjCNERdRQVCaZc-eU43eNjDK4DDpvqvA&sig2=unE5Ilgwj5nfOx3TUskGvg&bvm=bv.50768961,d.d2k

Colby WD, Dobie R, Leventhall G, Lipscomb DM, McCunney RJ, Seilo MT, Sondergaard B. 2009. Wind turbine sound and health effects. An expert panel review, http://www.google.co.uk/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=2&ved=0CD0QFjAB&url=http%3A%2F%2Fwww.canwea.ca%2Fpdf%2Ftalkwind%2FWind_Turbine_Sound_and_Health_Effects.pdf&ei=DL0QUq3ZKqqj0QXDzIHICA&usg=AFQjCNGToo8TsZ-rzZofcTLnLcdZTCiPsw&sig2=kPc1hBupmRhaynCLrxYxHA&bvm=bv.50768961,d.d2k Accessed 31st October 2015

Creel S, Fox JE, Hardy A, Sands J, Garrott B, Peterson RO. 2002. Snowmobile activity and glucocorticoid stress response in wolves and Elk. *Conserv. Biol.* 16(3), 809-814

Crocker D, Champayne C, Fowler M, Hassrick J, Robinson P, Simmons S, Costa D. no date. Long term foraging success impacts cortisol levels and natality in northern elephant seals, Society of Marine Mammalogy

Davenport MD, Tefenbacher S, Lutz CK, Novak MA, Meyer JS. 2006. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *Gen. Comp. Endocr.,* 147, 255-261

Descovich KA, Lisle AT, Johnston S, Keeley T, Phillips CJC. 2012. Intrasample variation and the effect of storage delay on faecal metabolite concentrations in the southern hairy-nosed wombat (*Lasiorhinus latifrons*). *Aust. Mammal.,* 34(2) 217-222

Edwards S, Clow A, Evans P, Hucklebridge F. 2001. Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity, *Life Sci.,* 68:2093-2103

Espelid S, Lokken GB, Steiro K, Bogwald J. 1996. Effects of cortisol and stress on the immune system in atlantic salmon (*Salmo salar L.*), *Fish Shellfish Immun*., 6:95-110

Forestry Commission, no date, *Badger*, http://www.forestry.gov.uk/forestry/Badger - accessed 31 October 2015

Fowkes RC, Moradi-Bidhendi N, Branceleone V, Zariwala MG, Brady D, Jessops DS, Perretti M, Renshaw D. 2013 Annexin-A1 protein and its relationship to cortisol in human saliva. *Psychoneuroendocrino*., 38(5): 722-727

George SC, Smith TE, Mac Cana PSS, Coleman R, Montgomery WI. 2014. Physiological stress in the Eurasian badger (*Meles meles*): Effects of host, disease and environment. *Gen. Comp. Endocr.,* 200, 54-60

González-de-la-Vara MDR, Valdez RA, Lemus-Ramirez V, Vázquez-Chagoyán JC, Villa-Godoy A, and Romano MC. 2011. Effects of adrenocorticotropic hormone challenge and age on hair cortisol concentrations in dairy cattle, *Can. J. Vet. Res.,* 75(3): 216–221

Kikuchi R. 2010. Risk formulation for the sonic effects of offshore wind farms on fish in the EU region, *Mar. Pollut. Bull*., 60(2):172-177

Kirschbaum C, Bono E G, Rohleder N, Gessner C, Pirke KM, Salvadoe A, Hellhammer DH. 1997. Effects of fasting and glucose load on free cortisol responses to stress and nicotine, *J. Clin. Endocr. Metab.,* 82(4):1101-1105

Kowalczyk, R., Zalewski, A., Jedrzejewska, B., 2004 Seasonal and spatial pattern of shelter use by badgers *Meles meles* in Bialowieza Primeval Forest (Poland), *Acta Theriol.,* 49(1) 75-92

Lundberg U. 2005. Stress hormones in health and illness: The roles of work and gender, *Psychoneuroendocrino*, 30(10):1017-1021

Maeda K, Tsukamura H. 2006. The impact of stress on reproduction: Are glucocorticoids inhibiting or protective to gonadotrophin secretion, *Endocrinology*, 147(3):1085-1086

Manenschijn L, Koper JW, Lamberts SWJ, van Rossum EFC. 2011. Evaluation of a method to measure long term cortisol levels. *Steroids*, 76 (10-11) 1032-1036

Maule AG, Tripps RA, Kaattari SL, Schreck CB. 1989. Stress alters immune function and disease resistance in chinook salmon (Omcorhynchus tshawytscha), *J. Endocrinol*, 120(1):135-42

Maurel D, Coutant C, Booissin-Agasse L, Boissin J. 1986. Seasonal moulting patterns in three fur bearing mammals: the European badger (*Meles meles L.),* the red fox (*Vulpes vulpes L.),* the mink (*Mustela vison*). A morphological and histological study, *Can. J. Zool.,* 64(8) 1757-1764

Meyer J, Novak M, Hamel A, Rosenberg K. 2014. Extraction and Analysis of Cortisol from Human and Monkey Hair. *J. Vis. Exp.,* (83), e50882, doi:10.3791/50882

Mikolajczak J, Boowski S, Marc-Pienkowska J, Odowaz-Sypniewska G, Bernacki Z, Siodmiak J, Szterk P. 2013. Preliminary studies on the reaction of growing geese (*Anseranser F. domestica*) to the proximity of wind turbines. *Pol. J. Vet. Sci.,* 16(4), 79-686

Moller H, Pedersen CS. 2011. Low-frequency noise from large wind turbines. *J. Acoust. Soc. Am.,* 129(6), 3727-3744

Morton DJ, Anderson E, Foggin CM, Kock MD, Tiran EP. 1995. Plasma cortisol as an indicator of stress due to capture and translocation in wildlife species. *Vet. Rec.,* 136, 60-63

Mostl E, Palme R. 2002. Hormones as indicators of stress. *Domest. Anim. Endocrin.,* 23(1-2):67-74

Pedersen E. 2009. Effects of wind turbine noise on humans, third international meeting on wind turbine noise, http://hh.diva-portal.org/smash/record.jsf?pid=diva2:240110 Accessed 5th August 2013

Price TJ. 2004. *Blyth, James (1839-1906),* Oxford Dictionary of National Biography, Oxford University Press

Rabin LA, Coss RG, Owings DH. 2006. The effects of wind turbines on anti-predator behaviour in California ground squirrels (*Spermophilus beecheyl*). *Biol. Conserv.,* 131(3), 410-420

Rogers LM, Cheeseman CL, Mallinson PJ, Clifton-Hadley R. 1997. The demography of a high-density badger (*Meles meles*) population in the west of England. *J. Zool.,* 242(4)705-728

Roper T. 2010. Badger, *Collins*, London

Rosca M, Forcada Y, Solcan G, Church DB, Niessen SJ. 2014. Screening diabetic cats for hypersomatotropism: performance of an enzyme-linked immunosorbent assay for insulin-like growth factor 1. *J. Feline Med. Surg.,* 16(2):82-8. doi:

Salt AN, Kaltenbach JA. 2011. Infrasound from wind turbines could affect humans. *B. Sci, Technol. Soc.,* 31(4), 296-302

Sauve B, Koren G, Walsh G, Tokmakejian S, Van Uum SHM. 2007. Measurement of cortisol in human hair as biomarker of systemic exposure, *Clin. Invest. Med*., 30(5):183-190

Shepherd D, McBride D, Welch D, Dirks KN, Hill E M. 2011. Evaluating the impact of wind turbine noise on health-related quality of life*. Noise Health*, 13(54), 333-339

Tallo-Parra O, Manteca X, Sabes-Alsina M, Carbajal A,Lopez-Bejar A. 2015. Hair cortisol detection in dairy cattle by using EIA: protocol validation and correlation with faecal cortisol metabolites, *Animal,* DOI: 10.1017/S1751731115000294

Tilbrook AJ, Turner A I, Clarke IJ. 2000. Effects of stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences, *Rev. Reprod.,* 5:105-113

Washburn BE, Millspaugh JJ. 2002. Effects of simulated environmental conditions on glucocorticoid metabolite measurements in white-tailed deer feces, *Gen. and Comp. Endocr*., 127 (3) 217-222

Werbin H, Chaikoff IL. 1961. Utilization of adrenal gland cholesterol for synthesis of cortisol by the intact normal and the ACTH-treated guinea pig, *Arch. Biochem. Biophys.*, 93(3):476-482

Wilson GJ, Carter SP, Delahay RJ. 2011. Advances and prospects for management of TB transmission between badgers and cattle, *Vet. Microbiol*., 151(1-2):43-50

Tables

Table 1: Key information relating to the 6 wind farms included in the study.

Wind farm (The year they became functioning)	Sett	Distance to closest turbine (metres)	Average distance to all turbines (metres)	Number of turbines category	Annual megawatt production category
1 (2012)	1	100	1021	<20	<40
	2	300	736	<20	<40
2 (2009)	1	40	720	>20	>40
	2	80	790	>20	>40
	3	350	1113	>20	>40
3 (2012)	1	140	858	<20	<40
4 (2009)	1	990	1189	>20	>40
5 (2009)	1	100	1397	>20	>40
6 (2012)	1	100	862	<20	<40

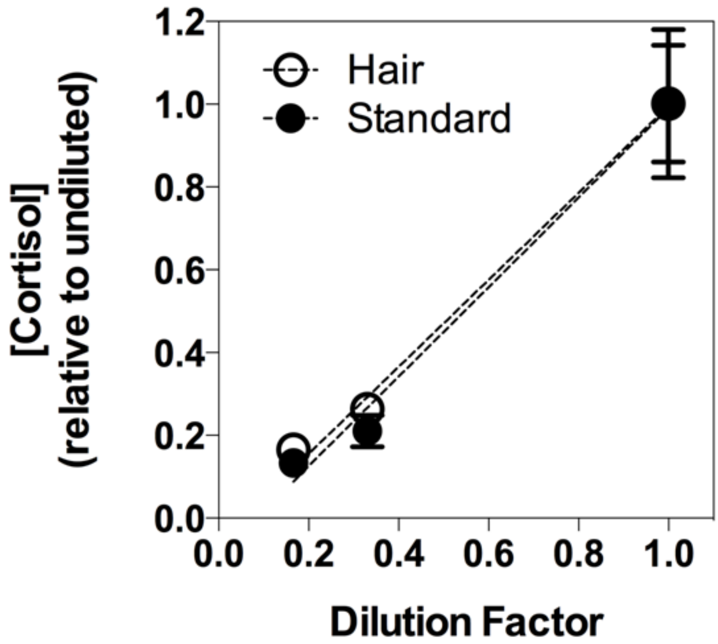
Table 2: Number of setts located at each of the 6 wind warms included in the study and the number of samples, opportunistic and trap, that were collected at each sett.

	Wind farm (The year they became operational)	Sett	No of samples collected through opportunistic collection	No of trap samples produced	Total no of 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	6	9	14	17	31

Table 3: Number of setts located in each of the 6 British counties included in the study and the number of hair samples, opportunistic and trapped, that were collected at each sett.

	County	Sett	No of samples collected through opportunistic collection	No. of trap samples produced	Total no. of 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	6	16	16	20	36

Figure 1:


Hair
Standard
[Cortisol]
(relative to undiluted)
Dilution Factor
1.2
1.0
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0.2
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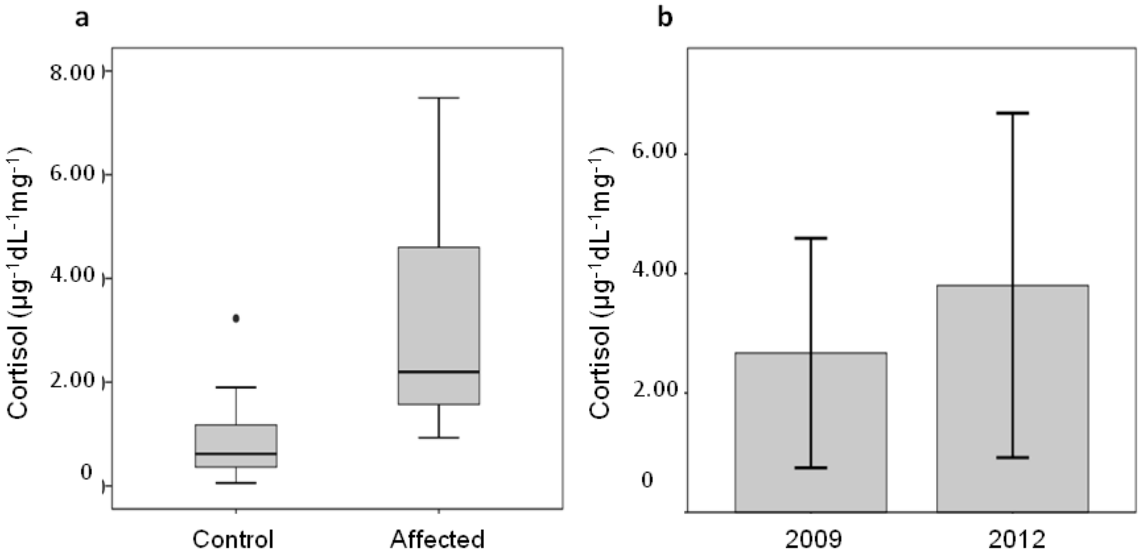
0.6
0.8
1.0

Figure 2: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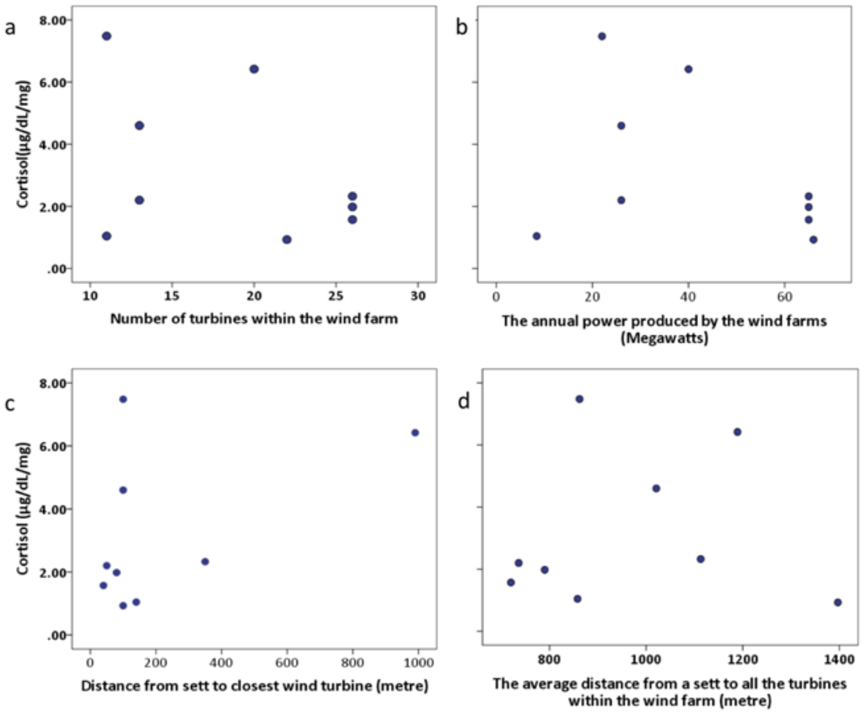
2.00
0
Cortisol (µg-1dL-1mg-1)
Control
Affected
b
6.00
4.00
2.00
0
Cortisol (µg-1dL-1mg-1)
2009
2012

Figure 3:



a
b
c
d
Cortisol(µg/dL/mg)
Cortisol (µg/dL/mg)
8.00
6.00
4.00
2.00
.00
10
15
20
25
30
Number of turbines within the wind farm
0
20
40
60
The annual power produced by the wind farms (Megawatts)
0
200
400
600
800
1000
Distance from sett to closest wind turbine (metre)
800
1000
1200
1400
The average distance from a sett to all the turbines within the wind farm (metre)

Figure Legends

Figure 1: Demonstration of linearity under dilution of pooled badger hair samples, compared with cortisol standards, with significant correlation coefficients (0.99 (hair) and 0.98 (cortisol standard)

Figure 2: Cortisol levels in badger (*Meles meles*) hair collected from various locations close to or remote from a wind farm. a: Box plot showing the 3 quartiles, the highest and the lowest values for the hair cortisol from control badger setts (over 10km from the closest turbine within a wind farm) and affected badger setts (less than 1km from a turbine within a wind farm), Control n=16 and affected n=9; b: Bar chart depicting the mean cortisol levels in badger hair from setts at established and new wind farm sites, with +/- 2 standard error bars. All setts were within 1km from a turbine within a wind farm. Established wind farms are those that have been operational since 2009 (setts n=5; wind farms, n=3). New wind farms are those that have been operational from 2012 (setts n=4; wind farms, n= 3).

Figure 3: Relationship between cortisol levels 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: The number of turbines in the wind farm (10-30 turbines); b: The number of megawatts produced annually by the wind farms (range: 5MW-70MW); c: The distance from the sett to the closest wind turbine (range: 40m-990m): d: The average distance of badger setts to all the wind turbines on the farm (range: 720m-1,397m). In all cases n=9.