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No evidence for a 'warning effect' of blue light in roe deer

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Physiological investigations of cervid eyes have revealed two different types of cones indicating high visual sensitivity in the 'blue' and 'green' spectral range (400–450 nm and 510–540 nm). Although detailed knowledge about light perception in large mammals is still missing, light reflecting devices such as wildlife warning reflectors are frequently used in animal-vehicle collision mitigation. Light of wavelengths in the range of 440–490 nm ('blue' light) has recently been advocated to evoke a warning effect in cervids due to its rare occurrence in the natural environment. We conducted a behavioural study with captive roe deer *Capreolus capreolus* to investigate whether roe deer exhibit a specific behavioural response to 'blue' light (wavelengths 440–490 nm). Compartmented feeders were pseudo-randomly illuminated with either 'blue' (colour: blue, 440–490 nm) or 'warm-white' light (colour: yellow-orange, 575–675 nm), or left unilluminated to assess changes in feeding time and feeder-compartment choice in dependence of illumination. Although feeding times were found to be generally shorter under illumination there was no difference between illumination types. Moreover, roe deer favoured the illuminated feeder compartment over non-illuminated ones. Our results highlight that roe deer differentiate between light and no light conditions while 'blue' light (440–490 nm) did not exert a 'warning effect' in roe deer.

The ability to distinguish light of different spectral characteristics is a key feature of contrast and object detection (Gegenfurtner and Kiper 2003) and depends on the existence of multiple receptor types (cones and rods) as well as a nervous system capable of comparing the absorption rates of the different types of receptor pigments (Goldsmith 1990, Birgersson et al. 2001, Jacobs 2009). Rods are characterised by a high sensitivity at wavelengths of about 500 ± 40 nm (Goldsmith 1990, Jacobs 2009) and play a major role for the detection of contrasts at low light levels (Yokoyama and Radlwimmer 1998). Cones differ according to the range of maximal absorption (Jacobs 2009). Most mammals have two different types of cone pigments, which show some extent of overlap in their absorbance level, with a maximum sensitivity near 430 nm (short-wavelength-sensitive cones; SWS) and around 540 to 560 nm (middle-to-long-wavelength-sensitive cones; MWS), respectively (Goldsmith 1990). In the context of deer (Cervidae)-vehicle collision mitigation, light of wavelengths between 440–490 nm ('blue' light) has recently been advocated as a 'warning colour' for deer in press due to its rare occurrence in the natural environment (Schilderwerk Beutha 2016). However, while light in general may affect deer behaviour (Blackwell and Seamans 2009), currently there is only little indication that light of any wavelength induces a specific behavioural response that could be utilized in the mitigation of deer-vehicle collisions.

Roe deer *Capreolus capreolus*, one of the most common European cervids (Apollonio et al. 2010), rely on vision mainly for browsing and vigilance. The eyes of roe deer contain cones with a maximal spectral sensitivity between 400–450 nm (SWS) and 510–540 nm (MWS), respectively (Ahnelt et al. 2006, Schiviz et al. 2008). The presence of two cone pigments entails the ability of colour vision while the distribution of SWS-cones is related to the detection of spectrally distinct objects (Jacobs et al. 1994, Yokoyama and Radlwimmer 1998, Sivic and Sielecki 2001). Jacobs et al. (1994) found evidence that the sensitivity of rod pigments in cervids peak at 497 nm. Thus, it appears possible that light of wavelengths between 400 and 500 nm might be especially well perceived by cervids, in turn enhancing the chance of object detection (Blackwell and Seamans 2009). However, whether roe deer or any other species exhibit elevated behavioural responses to specific light stimuli, especially to light of wavelengths within the spectrum of highest visual sensitivity, can only be answered by behavioural observation (Goldsmith 1990, Amann et al. 2012).

As 'blue' light is advocated to evoke aversive behaviour in wildlife we evaluated behavioural responses of roe deer to light of different wavelengths by assessing whether the animals exhibited changes in feeding behaviour, especially in response to light with a wavelength between 440–490 nm ('blue' light). We applied a controlled experimental setting in which roe deer were exposed to illuminated feeders among which animals could choose freely to assess the impact of illumination on both feeding time and the likelihood of choosing a specific feeder compartment. We tested two hypotheses based on the assumption that blue light represented an aversive stimulus. First, roe deer exhibit shorter feeding times in the presence of light, with 'blue' light having a stronger impact than light of another spectrum (H1). Second, roe deer avoid illuminated feeder compartments with avoidance of 'blue' light being stronger than that of other light (H2).

Methods

We conducted our experiment in semi-natural wildlife enclosures located at the Field Research Station of the Leibniz Institute for Zoo and Wildlife Research (IZW) in Niederfinow, Germany. We experimentally tested the effect of illumination on feeding time and feeder choice in four enclosures with an average size of 1386 m^2 housing a total of 19 roe deer (11 female, 4 male, 4 juveniles) in groups of three to four adult animals each. Roe deer were neither raised by hand nor tame and were individually marked by collars. All food was offered in a feeder with three separate compartments containing one food bowl each (Fig. 1A, Supplementary material Appendix 1 Fig. A1). The experiment was conducted over a period of eight consecutive weeks between June and August 2013. Individuals were fed ad libitum with a pelleted compound feed and pelleted hay in identical food bowls in all three feeder compartments. All compartments contained a LED clip light centred above each food bowl to guarantee full illumination and smooth rotational change of light bulbs during daytime.

We alternated treatment and control sessions, with the second and fourth week being treatment weeks for two enclosures at a time (Fig. 1B). The experimental setting was repeated once. During treatment, the feeder compartments were illuminated for a period of three hours around dawn and dusk of each day. The illumination regime consisted of an unpredictable rotational change of wavelength among the three compartments of the feeder to prevent habituation of the deer. We illuminated compartments either using 'blue' light (colour: blue) with a maximal intensity of wavelengths between 440–490 nm (LED light bulb 1W blue, GU10, 230V by Paulmann Licht GmbH, Springe, Germany; intensity $\geq$ 0.001 W m^{-2} nm^{-1}) or 'warm-white' light (colour: yellow-orange) with a maximal intensity of wavelengths between 575–675 nm (LED light bulb 1 W warm-white, GU10, 230V by Paulmann Licht GmbH, Springe, Germany; intensity $\geq$ 0.001 W m^{-2} nm^{-1}). One compartment always remained unilluminated. The illumination regime consisted of a daily pseudo-random change of the location of each light type among the three compartments (Fig. 1C). Feeders were left unilluminated at night and during the day to avoid habituation to the stimulus. During the control phase, all feeder compartments were likewise left unilluminated.

Deer behaviour was monitored using infrared video cameras in combination with infrared spotlights. We included only observations in which an animal entered the area around the feeder, fed and subsequently left the site (thus, observations where an animal used more than one feeder box were excluded). For each feeding bout, we recorded the time in seconds that an individual fed, the compartment choice (i.e. left, central, right), the illumination regime, the direction of approach to the feeder and the ID of the individual. For analysis, we only retained observations of adult individuals, since juveniles could not be individually identified. This resulted in a final dataset of 1962 independent observations of 15 study animals (4 male, 11 female) for control and illumination phases, of which 889 observations were obtained during phases of experimental illumination. In order to assess feeder compartment preferences, we extracted those periods with active illumination regime (n = 889) and linked this dataset to a dummy variable representing the three distinct options of choice in dependence of the respective illumination at each observation (i.e. representing in principle a case-control design with three distinct options of which one could be chosen, coded as 0,0,1 for each moment of choice).

All analyses were performed using R (<www.r-project.org>). We employed linear mixed models (LMMs, package nlme, Pinheiro et al. 2016) on the complete final dataset to

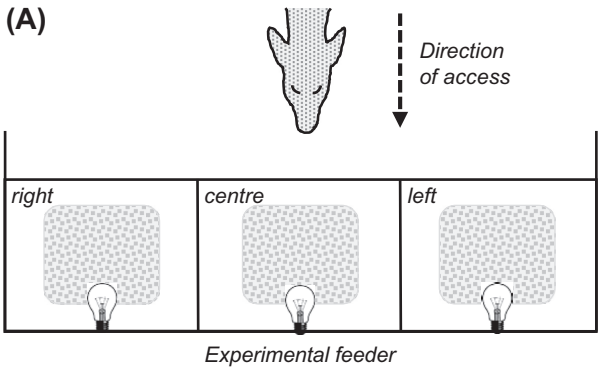

Figure 1. Experimental feeder with separated feeder compartments (A). Pelleted feed was provided ad libitum in each compartment. Each compartment received either no illumination or illumination with 'blue' (colour: blue, wavelengths between 440–490 nm) or 'warm-white' light (colour: yellow-orange, wavelengths between 575–675 nm) respectively during darkness hours. The experimental setup for control and illumination phases (B) was identical for all enclosures, each phase six days in duration. Compartments were illuminated based on a rotational regime. The order of light conditions in each feeder compartment differed between each enclosure and each illumination phase (cf. (C) for one example).

assess the effect of illumination regime on feeding time (H1). Feeding times were log-transformed (log10(x)) after initially failing to meet parametric assumptions regarding normality. Log-transformation outperformed alternative transformations (e.g. square-root). Individual differences between animals (Supplementary material Appendix 1 Fig. A2) were accounted for by fitting random intercepts for study animal ID nested within enclosure ID and by incorporating heterogeneous variances for each of the study animals. We specified the fixed effects in our model to reflect the experimental design around our main target variable illumination. Our global model thus included the illumination regime (factor: no illumination, 'blue' light 440–490 nm, 'warm-white' light 575–675 nm) and confounders as follows: experimental phase (factor: coded as control 1 and 2 versus experimental (light) 1 and 2) to control for differences in behaviour between illuminated and non-illuminated phases and the feeder compartment (factor: inner versus outer (i.e. left and right) compartment) to control for animal preferences of the outer compartments in our experiment. We modelled feeding times as:

$$\begin{aligned} \text{resp} \sim\ & \text{illumination regime} + \text{experimental phase} \\ & + \text{feeder compartment} \\ & + \left(\text{random} = 1 \,|\, \text{enclosure} / \text{ID}\right) \end{aligned} \quad (1)$$

Moreover, we employed generalized linear mixed models (GLMM, package lme4, Bates et al. 2015) to assess preferences in feeder compartment choice in relation to the illumination regime (H2). We fitted a binomial response model with logit-link using the illumination regime (factor: no illumination, 'blue' light 440–490 nm, 'warm-white' light 575–675 nm) as a predictor. As above, we also included the feeder compartment (factor: inner versus outer (i.e. left and right) compartment) as confounder to control for bias stemming from compartment preferences that were independent of illumination. We also included the experimental phase (factor: observations recorded during the illuminated phases 1 and 2) to account for potential habituation to the light stimulus in the second experimental phase. As for feeding times, we fitted random intercepts for study animal ID nested within enclosure ID, but included a random intercept for each discrete choice made by the animal nested within animal ID to satisfy the case-control design of our experiment. We modelled feeder compartment choice in dependence of illumination as:

$$\begin{aligned} \text{resp} \sim\ & \text{illumination regime} + \text{feeder compartment} \\ & + \text{experimental phase} + \left(1 \,|\, \text{enclosure} / \text{ID} / \text{choice}\right) \end{aligned} \quad (2)$$

For both models, we obtained confidence intervals for coefficient estimates and effect plots using a multi-level non-parametric bootstrap with 10 000 iterations assuring that each sample reflected the properties of the process that generated the data.

Results

We found feeding times to be shorter on average at illuminated feeder compartments compared to non-illuminated ones ('blue' light, wavelengths 440–490 nm; $p = 0.002$; 'warm-white' light, wavelengths 575–675 nm; $p = 0.007$; Fig. 2, Table 1). Mean feeding time (re-transformed) was reduced under illumination relative to no-light by 24.7% for 'blue' light (wavelength 440–490 nm) and 22.3% for 'warm-white' light (wavelength 575–675 nm). Mean feeding times, however, did not differ between illumination types of different wavelength. Variance of the random intercepts for individual feeding time was estimated at 0.025 around the intercept of 1.506.

Roe deer chose the illuminated feeder compartments over the non-illuminated ones in our experiment ('blue' light, 440–490 nm: $p < 0.001$; 'warm-white' light, 575–675 nm: $p = 0.035$; Table 1), with the highest preference exhibited for the compartments illuminated by 'blue' light, followed by 'warm-white' light (Fig. 2, Table 1). Again, there was no difference between illumination types. The probability of choosing a compartment according to illumination was as follows (note that probabilities are conditional on the combination of parameter levels represented in the model intercept): 'blue' light, 440–490 nm: $p_b = 0.367$; 'warm-white' light, 575–675 nm: $p_w = 0.334$; no light: $p_{no} = 0.288$ ($\sum p \neq 1$ due to multiple regression). The ratio of odds of choosing 'blue' light of 440–490 nm wavelength over no light were 1.437 and 1.241 for choosing 'warm-white' light of 575–675 nm wavelength over no light (Fig. 2).

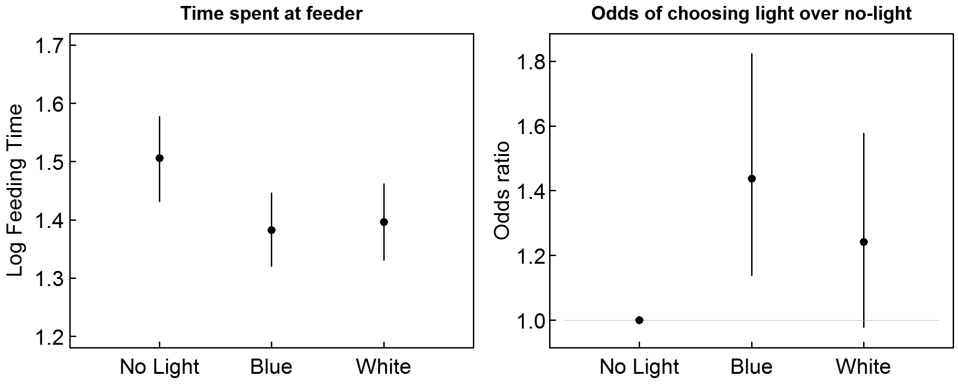

Figure 2. Predicted differences in feeding time (left) and the ratio of odds for choosing an illuminated feeder compartment (right) over a non-illuminated one. Bars indicate bootstrap confidence intervals for the model predictions.

Table 1. Final model results for feeding time (top) and feeder compartment choice (bottom) in dependence of illumination. Model coefficients, default model coefficient standard errors (SE), p-value (p) and bootstrap 95 % confidence intervals of all predictors are provided. Feeding times were log-transformed as log10(×). Variable codes and predictor reference classes are specified below. Independent variables for which confidence intervals not overlapping zero are highlighted with asterisk.

		IC*	BlueL*	WhiteL*	BoxC*	PL2	PN1	PN2
Feeding	β	1.506	–0.123	–0.109	–0.155	–0.060	0.005	–0.063
time	SE	0.106	0.040	0.040	0.024	0.032	0.039	0.041
log10(×)	p	0.000	0.002	0.007	0.000	0.063	0.892	0.127
	lower	1.4318	–0.2013	–0.1865	–0.2028	–0.1216	–0.0708	–0.1439
	upper	1.5775	–0.0406	–0.0287	–0.1084	0.0029	0.0829	0.0200
		IC*	BlueL*	WhiteL	BoxC	PL2		
Feeder	β	–0.907	0.363	0.216	0.026	0.000		
choice	SE	0.105	0.101	0.103	0.088	0.082		
	p	0.000	0.000	0.035	0.768	1.000		
	lower	–1.1057	0.1300	–0.0223	–0.1612	–0.0004		
	upper	–0.7218	0.6006	0.4561	0.2123	0.0004		

BlueL/WhiteL: 'blue' (colour: blue, wavelengths between 440-490 nm) or 'warm-white' (colour: yellow-orange, wavelengths between 575–675 nm) illumination compared to no-light; BoxC: central feeder box as compared to outer (i.e. left and right) boxes; PL2: experimental phase 2 compared to phase 1; PN1/PN2: control phase 1 and 2; β: model parameter estimate; SE: standard error of the model beta.

Discussion

This is the first behavioural study that has investigated the potential of light with different wavelengths to alter the behaviour of European roe deer. Even though we monitored feeding times and feeder choice of only 19 roe deer individuals (11 female, 4 male, 4 juveniles) our results clearly indicate that light is capable of inducing a behavioural response in the roe deer. We demonstrated that the probability to choose a compartment was higher for illuminated feeder compartments but there were only slight, non-significant differences between 'blue' (440–490 nm) and 'warm-white' (575–675 nm) light. At the same time, feeding time was clearly shorter at illuminated compartments than at unilluminated ones, with no difference between 'blue' (440–490 nm) and 'warm-white' (575–675 nm) illumination. We thus find no evidence of an elevated behavioural response specifically to 'blue' light of wavelengths between 440–490 nm.

Our primary interest in conducting this study arose from traffic safety concerns. For 50 years wildlife warning reflectors have been used in collision mitigation (Brieger et al. 2016). Reflectors scatter the beam of a car's headlights into the roadside environment with the goal to alert cervids of approaching vehicles (Gladfelter 1984, Schafer and Penland 1985, Sivic and Sielecki 2001) in order to induce a flight reaction or increase awareness (cf. Schafer and Penland 1985, Zacks 1986, Grenier 2002, D'Angelo et al. 2006). In the last decade, wildlife warning reflectors with blue-coloured retroreflection foil have become the standard in Europe. Manufacturers claim that this type of colour is uncommon in the natural environment and that wildlife will thus be frightened by the unfamiliar stimulus (Schilderwerk Beutha 2016).

So far, there is no evidence that light of any wavelength will evoke a specific response in cervids (Zacks 1986, VerCauteren et al. 2003, 2006; but see Blackwell and Seamans 2009). Results from studies into colour perception indicate that the absorption capability of the retina in roe deer has the highest sensitivity within the blue light spectrum (Ahnelt et al. 2006, Schiviz et al. 2008, Amann et al. 2012). This is regarded as an evolutionary adaptation to crepuscular and nocturnal activity patterns (Schiviz et al. 2008, Khokhlova 2013). We observed a preference for illuminated feeder compartments compared to non-illuminated ones, but no difference between illumination types, thus dispelling our hypothesis of an elevated behavioural response that was specific to 'blue' light (wavelengths 440–490 nm). We presume that roe deer perceived the illuminated feeder compartments during low light conditions better than non-illuminated ones, reflected by the feeder choice. However, while illuminated feeder compartments were preferentially selected, the intense illumination during feeding may have led to shorter feeding times at illuminated compartments due to the intensity of the light stimulus under otherwise low light conditions at dusk and dawn.

Our results support the findings of Blackwell and Seamans (2009) that light of wavelengths between 400 and 500 nm might increase the potential of perception and in turn increase the chance of object detection and thus provide an animal with the required time to initialise a successful evasive response. However, in the context of traffic safety it is not a reasonable interpretation that the perception of an object as a 'threat' depends on the wavelength of the emitted light. In the light of our results, we doubt, that 'blue' light or any other specific wavelength will be perceived by wildlife species as a 'warning colour' per se. This finding is supported by previous conclusions regarding white tailed deer (Zacks 1986, VerCauteren et al. 2003, 2006). Although our results indicate that light in general may possess the capability to impact upon deer behaviour, the effectiveness of 'blue' light in collision mitigation appears questionable. In the context of traffic safety, we suggest that the intensity or wavelength of light might improve object detection by cervids, but that the type of behavioural reaction exhibited by the animal does not depend on the spectral characteristics of the light.

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Supplementary material (available online as Appendix wlb-00331 at <www.wildlifebiology.org/appendix/wlb-00331>). Appendix 1.