What type of rigorous experiments are needed to investigate the impact of artificial light at night on individuals and populations?

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RESPONSE TO EDITOR

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In our recent paper on how artificial light at night (ALAN) affects within-individual changes in physiology, we used a unique experimental setup of colored LED lights to show effects on nighttime activity levels and physiology in free-living great tits, *Parus major* (Ouyang et al., 2017). Raap, Pinxten, and Eens (2017) response, entitled: "Rigorous field experiments are essential to understand the genuine severity of light pollution and to identify possible solutions" lists issues with our analyses. Rather than go into a detailed response, we use this forum to address the major critiques by answering the bigger question of what types of rigorous field experiments are needed to evaluate ALAN's impact.

In order to assess the impact of ALAN, the intensity and nature of the light source must be described. The photosensitivity of different species varies depending on whether the source is metal halide, LED, or fluorescent (Ouyang, Davies, & Dominoni, in review; Vorobyev, Osorio, Bennett, Marshall, & Cuthill, 1998). Experimental studies on different types of light are rare (Migaud, Cowan, Taylor, & Ferguson, 2007; Perkin et al., 2011). In addition to light type, authors do not always provide information about light intensity; and light levels are often reported in lux, a measure that is not meaningful for nonhuman beings. However, when the goal is to advise city planners and government agencies, light source yield is best reported in lux to facilitate visual tasks for humans.

Whether a study is in the field or in the laboratory has implications for research on ALAN. Controlling individual light exposure in the field is difficult. Workarounds, such as placing light sources inside of nest boxes (Raap, Pinxten, & Eens,, 2015), may result in unrealistic situations that are difficult to interpret. Currently, there is a paucity of true-to-life field studies, especially ones that measure direct light exposure for free-living animals (Ouyang et al., in review), but there are exceptions (Dominoni, Goymann, Helm, & Partecke, 2013; Knop et al., 2017; Robert, Lesku, Partecke, & Chambers, 2015). In our setup, we solve this problem by including the interaction between treatment and the distance from light source to roosting site. Often in field studies, separating the impact of ALAN from other factors, in this case the effects of urbanization, e.g., noise, heat, etc. is a challenge. Therefore, we are in need of experiments in natural settings where ALAN alone is experimentally manipulated, while all other environmental variables are constant, but such field experiments are difficult. The use of lampposts with relevant light intensities in previously unlit natural forests offers a more realistic scenario. We employed this approach using one of the only experimental field setups of its type and scale in the world (Spoelstra et al., 2015). Our treatment was randomized over sites, and we show that birds sleeping in sites with different light colors differed in their nighttime activity levels. The only systematic difference among our artificially lit areas is light color. While differences among treatment in sleeping location (e.g., height of roost) or amount of light the bird received may offer more precise explanations, we still found that treatment and distance interacted and ALAN affected nighttime activity levels.

Physiological traits are notoriously flexible (de Jong et al., 2016). We aimed to quantify within-individual changes to characterize phenotypic changes in relation to continuous presence of ALAN. Repeated measurements are important as sampling at a single time point may not characterize the fitness or health of an individual (Bonier, Martin, Moore, & Wingfield, 2009). Circulating concentration of oxalic acid is a physiological trait that may be affected by ALAN. Whether this effect is direct or indirect still needs to be tested, especially if it is used as a proxy for sleep deprivation (de Jong et al., 2016; Ouyang et al., 2017). Studies that link oxalic acid concentration directly to sleep in birds and other taxa are desperately needed and represent one frontier in this pioneering research field. Intraindividual changes in oxalic acid during a period which was preceded by increased nighttime activity is an interesting phenomenon, which we have discussed previously (Ouyang et al., 2017).

Lastly, we stress the potential importance of sex differences (Schoech et al., 2013). It is reasonable to assume that sex-related differences in behavior and reproductive physiology might cause response to ALAN to differ by sex, but this possibility currently has little support in our study system (Ouyang et al., 2017). In addition, a wider breadth of taxonomic diversity in response to "realistic" ALAN in natural habitats is necessary. Studies of amphibian and reptile physiological responses to ALAN are rare, even though photosensitivity and melatonin response of these taxonomic groups is notably different from other vertebrates (Underwood, 1986). Lastly, studies exploring latitudinal and photoperiod variation, which both regulate physiology, are likewise rare and needed (Da Silva & Kempenaers, 2017).

Artificial light at night is a global phenomenon, but the main ALAN studies involve temperate ecosystems and only a few taxa. As studies of ALAN on animal physiology, especially in relation to health and stress, become more common, nonmodel organisms in vulnerable but understudied locations must be targeted. To make significant strides in ecophysiology and impacts of ALAN, we need randomized field experiments with different treatments in lighting types and/or intensity (Ouyang et al., 2017; Spoelstra et al., 2015). Next to precise exposure measurements in the natural habitat, these are the best to tackle practical limitations of light experiments in field situations. Whether the observation of biological differences translates into evolutionarily meaningful fitness costs remains to be determined with long-term field studies. Experimental field setups, like the one we have used previously, are important and fundamental tools for providing rigorous data with clear policy implications.

CONFLICTS OF INTEREST

Authors declare that there are no conflicts of interest.

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