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RESEARCH ARTICLE

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Artificial light pollution: Shifting spectral wavelengths to mitigate physiological and health consequences in a nocturnal marsupial mammal

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Abstract

The focus of sustainable lighting tends to be on reduced CO_2 emissions and cost savings, but not on the wider environmental effects. Ironically, the introduction of energy-efficient lighting, such as light emitting diodes (LEDs), may be having a great impact on the health of wildlife. These white LEDs are generated with a high content of short-wavelength 'blue' light. While light of any kind can suppress melatonin and the physiological processes it regulates, these short wavelengths are potent suppressors of melatonin. Here, we manipulated the spectral composition of LED lights and tested their capacity to mitigate the physiological and health consequences associated with their use. We experimentally investigated the impact of white LEDs (peak wavelength 448 nm; mean irradiance 2.87 W/m^2), long-wavelength shifted amber LEDs (peak wavelength 605 nm; mean irradiance 2.00 W/m^2), and no lighting (irradiance from sky glow $< 0.37 \times 10^{-3}$ W/m^2), on melatonin production, lipid peroxidation, and circulating antioxidant capacity in the tammar wallaby (*Macropus eugenii*). Night-time melatonin and oxidative status were determined at baseline and again following 10 weeks exposure to light treatments. White LED exposed wallabies had significantly suppressed nocturnal melatonin compared to no light and amber LED exposed wallabies, while there was no difference in lipid peroxidation. Antioxidant capacity declined from baseline to week 10 under all treatments. These results provide further evidence that short-wavelength light at night is a potent suppressor of nocturnal melatonin. Importantly, we also illustrate that shifting the spectral output to longer wavelengths could mitigate these negative physiological impacts.

KEYWORDS

ALAN, anthropogenic disturbance, circadian disruption, *Macropus eugenii*, melatonin, oxidative stress

1 | INTRODUCTION

Worldwide human population growth has resulted in the rapid expansion of artificial light into previously unlit areas leading to a novel anthropogenic pressure on wildlife (Dominoni & Partecke, 2015; Lyytimäki, 2015; Swaddle et al., 2015). As artificial light encroaches into these untouched habitats, it leads to changes in the irradiance, direction, duration, and spectral composition of light (Blumstein & Berger-Tal, 2015; Davies, Bennie, Inger, & Gaston, 2013; Davies, Bennie, Inger, Ibarra, & Gaston, 2013; Gaston, Duffy, Gaston, Bennie, & Davies, 2014; Longcore & Rich, 2004). These changes mask natural photoperiods providing misleading cues and ultimately result in daily and seasonal desynchronization in photo-dependent animals (Duffy, Bennie, Durán, & Gaston, 2015; Gaston, Bennie, Davies, & Hopkins, 2013; Hölker, Wolter, Perkin, & Tockner, 2010; Le Tallec, Perret, & Théry, 2013; Pawson & Bader, 2014; Stone, Jones, & Harris, 2012).

A global need to reduce CO_2 emissions and achieve climate change targets has resulted in a push for the development of sustainable lighting technologies (Davies et al., 2013; Pawson & Bader, 2014; Stone, Wakefield, Harris, & Jones, 2015). Ironically, there has been no emphasis on the wider environmental impacts of these changes; for example, the introduction of energy-efficient light emitting diodes (LEDs) may have a greater impact on wildlife than previous lighting technologies. White LEDs emit broad spectrum light with a large peak in the short, blue wavelength region thereby increasing the potential for ecological and health consequences (Gaston et al., 2013; Gaston, Davies, Bennie, & Hopkins, 2012; Wright, Lack, & Partridge, 2001). However, one advantage of LED light sources compared to traditional lighting is the flexibility to control spectral composition to potentially reduce the

impacts on wildlife without significantly sacrificing energy efficiency (Spoelstra et al., 2015). The challenge presented now is characterizing what might constitute 'optimal' light spectrum for different species.

Light plays a key role in regulating the behavior and physiology of mammals (Bedrosian, Fonken, & Nelson, 2016). Endogenous circadian clocks require the oscillation of light and dark conditions to signal for daily and seasonal adaptation (Bedrosian et al., 2016; Brown, 2016). These photic cues are received by non-rod, non-cone photoreceptors (non-visual photoreceptors) which lie in the retina and communicate to the internal timekeeping system in the hypothalamic suprachiasmatic nuclei (SCN) (Freedman et al., 1999; Lockley, Brainard, & Czeisler, 2003; Thapan, Arendt, & Skene, 2001). The message from the SCN is primarily carried out by the endocrine hormone melatonin, which is produced and secreted from the pineal gland during the hours of darkness (Jones, Durrant, Michaelides, & Green, 2015; Pandi-Perumal et al., 2006; Wright et al., 2001). Regulation of melatonin is controlled by melanopsin, the photopigment protein responsible for responding to light levels with a peak sensitivity around 484 nm, corresponding to short-wavelength, blue light (Aubé, Roby, & Kocifaj, 2013; Newman, Walker, Brown, Cronin, & Robinson, 2003; Wright, Lack, & Kennaway, 2004). Exposure to high intensity light as brief as 1 min (Lerchl, 1995), or prolonged exposure to low intensity light during hours of natural darkness has been shown to substantially alter melatonin production in mammals (Bedrosian et al., 2016). Consequently, any changes to natural light regimes have the potential to provide misleading biological signals ultimately resulting in ecological and physiological damage (Bedrosian et al., 2016).

Melatonin plays a key role in in the immune system, acting as a potent antioxidant working with the antioxidant defense system to neutralize and remove excess reactive oxygen species to restore redox equilibrium (Schneeberger & Czirják, 2013; Tan et al., 2003). An imbalance of this system in favor of reactive species production increases the rate at which oxidative stress takes place, with the potential for oxidative damage to occur (Ghiselli, Serafini, Natella, & Scaccini, 2000; Schneeberger & Czirják, 2013). Exposure to constant illumination has been shown to diminish antioxidant capacity and increase oxidative stress in humans, rats, and hamsters, ultimately increasing the potential for oxidative damage to occur (Baydaş, Erçel, Canatan, Dönder, & Akyol, 2001; Benot et al., 1999; Tomás-Zapico, Coto-Montes, Martínez-Fraga, Rodríguez-Colunga, & Tolivia, 2003). However, these studies have been conducted under controlled laboratory conditions with restricted application to free-ranging mammals. To our knowledge, only four studies have examined the effect of artificial light on oxidative status using experimental field studies on the great tit (*Parus* major) (Casasole et al., 2017; Raap et al., 2016; Raap, Casasole, Pinxten, & Eens, 2016; Raap, Pinxten, Casasole, Dehnhard, & Eens, 2017) and no study has examined the impacts on a mammal under similar field conditions (Isaksson, 2015).

The tammar wallaby (*Macropus eugenii*) is a small nocturnal macropod marsupial, which exhibits a strict annual breeding cycle with most births occurring 6 weeks after the austral summer solstice (McConnell, 1986; McConnell & Tyndale-Biscoe, 1985). This highly synchronous breeding cycle is entrained to environmental light levels and is primarily controlled by pineal melatonin. Wild tammar wallabies exposed to high-pressure sodium lighting have been shown to experience suppressed nocturnal melatonin resulting in delayed reproduction (Robert, Lesku, Jesko, & Chambers, 2015). Tammar wallabies possess dichromatic color vision (Ebeling & Hemmi, 2014), with two spectrally distinct eye pigments (Hemmi, Maddess, & Mark, 2000). The peak sensitivity of wallaby vision occurs at 539 nm for the middle wavelength sensitive cone and 420 nm for the short-wavelength sensitive cone (Hemmi et al., 2000). Non-visual photopigments have not been specifically measured in wallabies but other studies on marsupials show light sensitive retinal ganglion cells in the retina that express melanopsin (OPN4m) (Bellingham et al., 2006; Pires et al., 2007). From a range of studies in eutherian mammals, melanopsin has a peak sensitivity of around 480 nm (Peirson, Halford, & Foster, 2009). This suggests that artificial light falling within this sensitive range will have the greatest impact on behavioral and physiological processes in this species.

The ecological effects of artificial light at night have been well studied across a wide range of species. Impacts on wild populations have largely focused on behavioral measures, while laboratory studies have concentrated on health implications. However, there has been relatively little overlap between the two fields (Dominoni, Borniger, & Nelson, 2016). In this study, we assess for the first time the impact of white LED night lighting on physiological measures of health by assessing nocturnal plasma melatonin, lipid peroxidation, and antioxidant capacity in the tammar wallaby under simulated field conditions.

2 | MATERIALS AND METHODS

2.1 | Study population

All methods were approved by the La Trobe University Animal Ethics Committee (AEC 13–46). This study used adult female tammar wallabies (*Macropus eugenii*) (aged 1–8 years) either captured from the wild 7 years ago (Tutanning Nature Reserve, Western Australia) or bred in captivity from those captured. Animals were housed in large (900 m^2), naturalistic, outdoor enclosures at La Trobe University, Australia supplemented with ad libitum Kangaroo cubes (Glen Forrest Stockfeeders, Glen Forrest, Western Australia, Australia), hay cubes (MultiCube Stockfeeds, Yarrawonga, Victoria, Australia), mixed fruit and vegetables, and water. Females were group housed, with 10 to 13 animals in each experimental enclosure. Shade cloth (90%) was doubled over and attached to the fence lines as a visual barrier. A vacant enclosure also separated experimental enclosures to restrict flooding of artificial lighting from neighboring enclosures.

2.2 | Night-time illumination

Two experimental enclosures were fitted with artificial lighting while the third control enclosure remained unlit. Two lights, hung approximately 3 m above the ground in the center of each experimental enclosure provided night-time illumination (Figure 1a and b). Experimental lighting was comprised of 'optispan major MKII' housings (Aldridge Traffic Systems Pty Ltd, Eltham, Victoria, Australia), fitted with a HLTS LED modules (Hi-Lux Technical Services Pty Ltd,

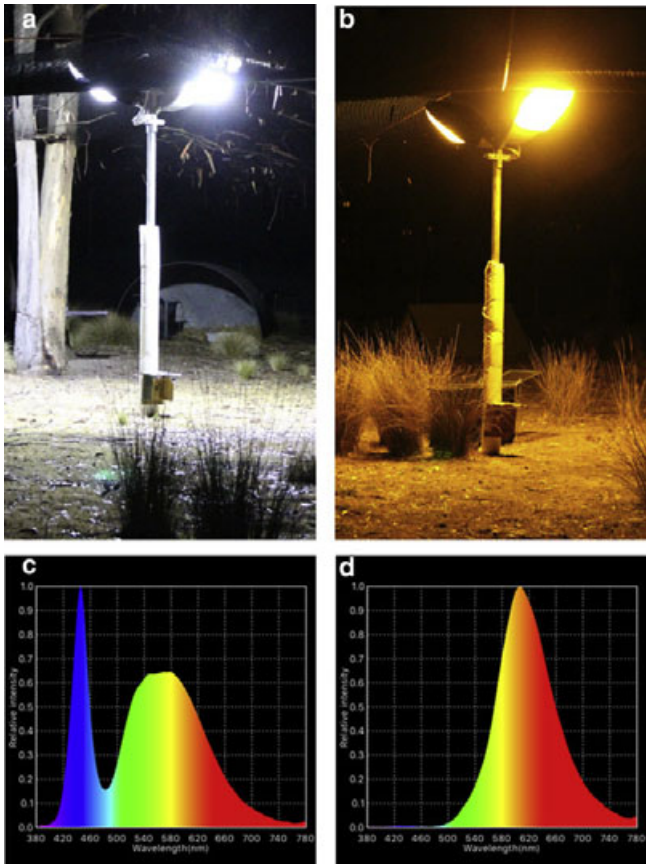

FIGURE 1 Experimental lighting—(a) White LEDs and (b) long-wavelength shifted amber LEDs mounted at a height of 3 m in the center of enclosures. Spectral composition of white LEDs (c) and long-wavelength shifted amber LEDs (d). The control enclosure consisted of light poles with no lights and no recordable light spectra. Peak wavelength for white LEDs was 448 nm and long-wavelength shifted amber LEDs was 605 nm [Color figure can be viewed at wileyonlinelibrary.com]

Thomastown, Victoria, Australia) so that lights were equivalent to a V class 150 W high-pressure sodium vapor street lamp. The HLTS LED module uses approximately 100 W of power and was comprised of Nichia LEDs (Nichia Corporation, Anan-Shi, Tokushima, Japan). The white lights used Nichia-NVSW219CT high power white LEDs (Nichia Corporation, Anan-Shi, Tokushima, Japan; CCT 5000 K) and the amber, 'wildlife friendly' prototype used unfiltered, high power amber Nichia-NVSA219BT LEDs (Nichia Corporation, Anan-Shi, Tokushima, Japan; CCT 1700 K). Lights were connected to analogue timers and set to turn on 1 hr before sunset and off 1 hr after sunrise (sunrise/sunset times obtained from Australian Bureau of Meteorology; available at https://www.bom.gov.au).

2.3 | Measuring light at night

Micro-light loggers (custom made by the University of Konstanz, Konstanz, Germany; spectral range 300–1100 nm) were used to quantify irradiance (W/m^2) in the center of each pen, approximately 3 m directly below lights and at a distance approximately 10 m from the light pole. The spectral composition of each light source was quantified using a hand-held spectrometer (Lighting passport, Asensetek, New Taioei, Taiwan; spectral range 380–780 nm; 5–50,000 lux). Measurements of light characteristics were made with each instrument 4 hr after sunset at week zero (over five consecutive nights), prior to the operation of night lighting and again following 10 weeks exposure to artificial night lighting (over two consecutive nights).

2.4 | Trapping and blood collection

Wallabies were trapped at two time points, week zero (baseline) and following 10 weeks of night lighting using "Thomas traps" (soft-sided traps constructed from shade cloth suspended from a wire frame, 450 × 450 × 800 mm; Wiretainers Pty Ltd, Preston, Victoria, Australia). Traps were set just before sunset and checked every 3 hr between astronomical sunset and astronomical sunrise (times obtained from Australian Bureau of Meteorology; available at https://www.bom.gov.au). At capture, we recorded individual identity, weight, pes (foot) length, and collected 2–5 ml of blood from the lateral caudal vein by venepuncture using a 22 × ½ gauge hypodermic needle. Blood was transferred to heparin vials (BD Vacutainer; Becton, Dickinson & Company, New Jersey, USA) and centrifuged for 15 min at 3000 rpm, plasma was pipetted into Eppendorf tubes and stored frozen at −80°C until assayed.

2.5 | Melatonin analysis

Plasma samples were assayed for melatonin using a commercial enzyme-linked immunoassay (ELISA) kit (Wallaby Melatonin Kit, Cat no. KT-61019, Kamiya, Biomedical Company, Seattle, USA), validated previously (Robert et al., 2015). Plates were read at a wavelength of 450 nm on an Anthos 2010 plate reader (Anthos Labtec Instruments, Salzburg, Austria). All samples were assayed in duplicate and the average absorbance was calculated. Melatonin concentration of unknown samples was calculated from the standard curve produced from provided standard calibrators. The sensitivity of the assay was 1.0 pg/ml. Intra- and inter-assay coefficients of variation were 9.84% and 11.99%, respectively.

2.6 | Lipid peroxidation analysis

Plasma samples were assayed for malondialdehyde (MDA), a biomarker of lipid peroxidation, using TBARS (TCA method) assay kit (Cat. number 700870, Cayman Chemical, Ann Arbor, Michigan, USA). Plates were read on the Anthos 2010 plate reader to measure colorimetric changes at 540 nm. Plasma samples and standards were assayed in duplicate and the average absorbance was calculated. MDA concentrations of unknown samples were calculated from the standard curve produced using provided calibrators. Intra- and inter-assay coefficients of variation were 9.15% and 14.8%, respectively.

2.7 | Antioxidant capacity analysis

Plasma samples were assayed for total antioxidant capacity using Trolox equivalent antioxidant capacity (TEAC) assay kit (Antioxidant

Assay Kit, Cat. number 709001, Cayman Chemical, Ann Arbor, Michigan, USA). Samples were diluted 1:5 with assay buffer. Plates were read on the Anthos 2010 plate reader and measured colorimetrically at 405 nm. Plasma samples and standards were assayed in duplicate and the average absorbance was calculated. Total antioxidant concentrations were determined following calculation of the standard curve using the provided calibrators accounting for dilution of samples. Intra- and inter-assay coefficients of variation were 8.96% and 11.1%, respectively.

2.8 | Statistics

All analyses were run in JMP 13.0.0 (SAS Institute, Cary, USA). Measures of light irradiance were not normally distributed, consequently, nonparametric Wilcoxon signed rank tests were used to analyze variation between baseline (week 0) and week 10 measures in the center and edge of experimental pens. To analyze variation in melatonin concentration, lipid peroxidation (MDA concentration) and antioxidant capacity (TEAC concentration) at baseline and following 10 weeks of night lighting, we built generalized linear models with an identity function. Mean concentration was the response variable, time and treatments were fixed effects. Linear models were produced including an interaction between week and treatment. Post-hoc contrasts were run to examine differences at each level. Model assumptions were tested using Shapiro–Wilk test (normality) and Levene's test (homogeneity of variance). Melatonin and lipid peroxidation data was log-transformed to meet the assumptions of the model.

3 | RESULTS

3.1 | Light irradiance and spectral wavelength

Animals in the control treatment were only exposed to astronomical sources of light at night. Mean irradiance remained low (< 0.00037 ± 0.0001 W/m^2) across all trap nights between baseline measures and week 10 measures (Figure 2a) and there was no measurable light spectra. Both experimental light pens consisted of a heterogeneous light environment with significantly higher mean irradiance in the center than at the edge of the pen (Wilcoxon rank sums test; week 10 white: $z = -39.83$, $p < 0.0001$; week 10 amber: $z = 29.44$, $p < 0.0001$). Mean irradiance under white LEDs remained high (2.87 ± 0.02 W/m^2) across week 10 trap nights, these measures were significantly higher than baseline (week 0) irradiance at both the center and edge of the pen (Wilcoxon signed rank test; center: $S = 281696$, $p < 0.0001$; edge: $S = -176610$, $p < 0.0001$; Figure 2b). White LEDs emitted light across a wide color spectrum encompassing the entire 380–780 nm measurable spectral range (Figure 1c), with a peak irradiance of 448 nm. Mean irradiance under long-wavelength shifted amber LEDs remained high (2.00 ± 0.02 W/m^2) across week 10 trap nights, these measures were significantly higher than baseline (week 0) irradiance at both the center and edge of the pen (Wilcoxon signed rank test; center: $S = 498552$, $p < 0.0001$; edge: $S = 278981$, $p < 0.0001$; Figure 2c). Amber LEDs emitted light in a narrow region of the color spectrum consisting of longer wavelengths (Figure 1d), with a peak irradiance of 605 nm.

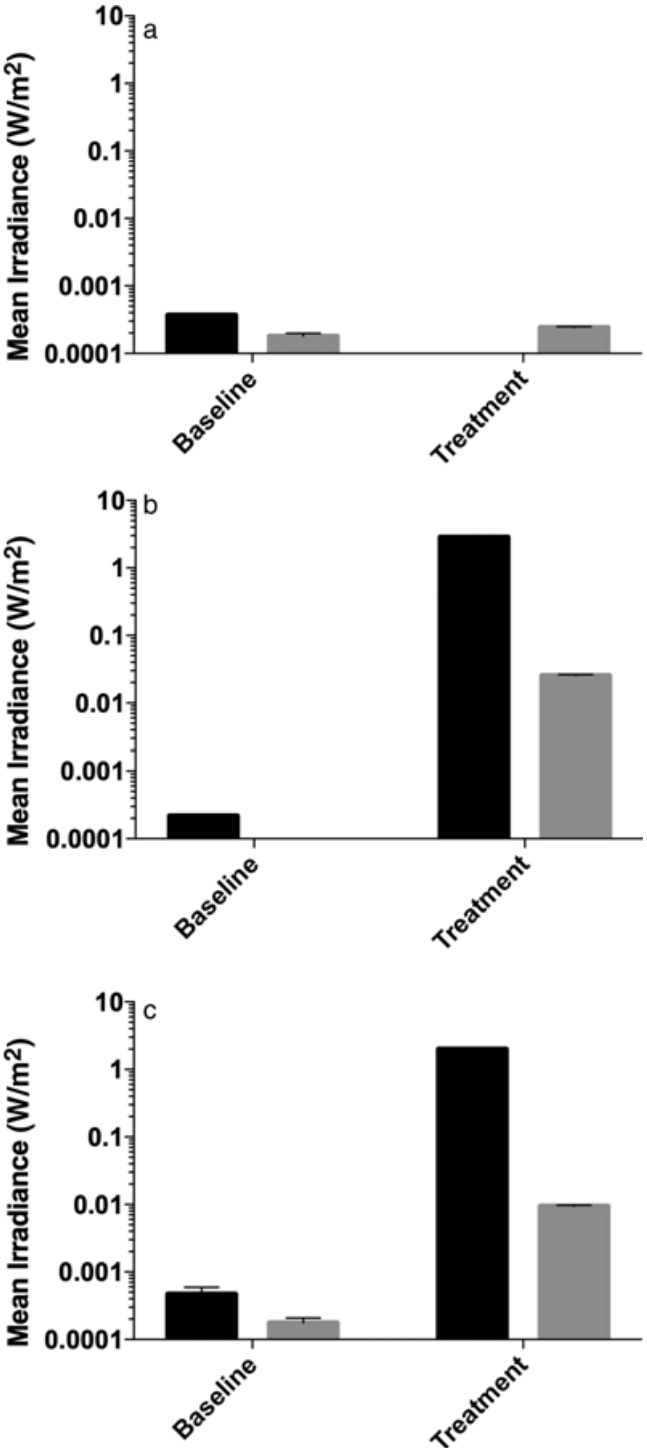

FIGURE 2 Light irradiance—Mean (± SE) night-time light irradiance (W/m^2) under (a) control, (b) white LED, and (c) long-wavelength shifted amber LED treatments. Measures taken on all traps nights in February (8–12 February, baseline measures) and in April (25–26 April, treatment measures) following 10 weeks light at night treatment. Black bars represent measurements recorded from the center of the pen directly below light poles. While shaded bars represent measurements recorded 10 m from the light pole. Due to logger failure we were unable to obtain measures of light irradiance in the center of the pen during week 10 trap nights for the control treatment

3.2 | Nocturnal melatonin

Wallabies exposed to 10 weeks of white LED night lighting showed significant melatonin suppression, while wallabies exposed to long-wavelength shifted amber LED or control treatment (no light) showed no significant reduction in night-time melatonin (post-hoc following Generalized linear model (GLM): control $\chi^2 = 0.66$, $p = 0.42$; amber $\chi^2 = 0.00004$, p = 0.99; white $\chi^2 = 5.42$, p = 0.02; Figure 3a).

3.3 | Lipid peroxidation

Light treatment did not have a significant effect on lipid peroxidation levels with MDA concentrations declining under all treatments from baseline measures to 10 weeks post treatment, however, these changes were not significant (post-hoc following GLM: control: $\chi^2 = 3.62$, $p = 0.06$; amber: $\chi^2 = 3.19$, $p = 0.07$; white: $\chi^2 = 1.72$, $p = 0.19$; Figure 3b). Total lipid peroxidation concentrations following 10 weeks of light exposure were not significantly different across all treatments (post-hoc following GLM: control–amber: $\chi^2 = 0.49$, $p = 0.49$; control–white: $\chi^2 = 0.3$, $p = 0.58$; amber–white: $\chi^2 = 1.45$, $p = 0.23$).

3.4 | Antioxidant capacity

Change in antioxidant capacity showed a significant decline under all treatments following 10 weeks of night lighting (post-hoc following GLM: control: $\chi^2 = 36.93$, $p < 0.0001$; amber: $\chi^2 = 39.52$, $p < 0.0001$; white: $\chi^2 = 52.42$, $p < 0.0001$; Figure 3c). Total antioxidant concentrations following 10 weeks of light exposure were significantly higher under amber LEDs and marginally significant under white LEDs compared to control treatment (no light) (post-hoc following GLM: control–amber: $\chi^2 = 4.44$, $p = 0.04$; control–white: $\chi^2 = 3.7$, $p = 0.05$; amber–white; $\chi^2 = 0.04$, $p = 0.85$).

4 | DISCUSSION

A global push toward energy efficiency has resulted in the development of energy efficient, white LED lighting, the use of which is growing at a rate of approximately 30% per year (Mottier, 2009). However, these bright white lights are known to suppress melatonin in humans at a rate 4–5 times greater than traditional lighting (Aubé et al., 2013; Falchi, Cinzano, Elvidge, Keith, & Haim, 2011), thereby increasing the potential for ecological and health consequences in wildlife. Despite this, few studies have assessed the impacts of LED lighting on measures of health in wildlife. Here, we provide further evidence that short-wavelength light is a potent suppressor of nocturnal melatonin in a marsupial mammal. Importantly, our results provide evidence that long-wavelength shifted LEDs can be used to mitigate these negative impacts.

Diel rhythms of melatonin follow light and dark cycles, with peak production during the dark phase (Jones et al., 2015; Pandi-Perumal et al., 2006; Wright et al., 2001). Exposure to artificial light has

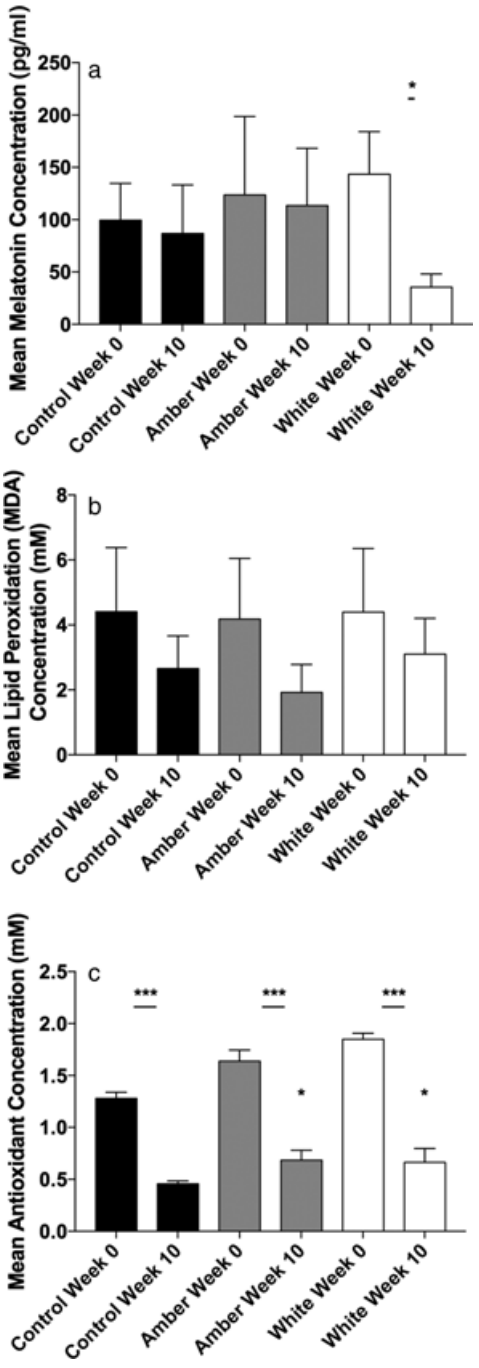

FIGURE 3 (a) Melatonin—Mean (± s.e.) night-time melatonin levels in control (black bars; week 0, $n = 4$; week 10, $n = 5$), long-wavelength shifted amber LED treatment (grey bars; week 0, $n = 4$; week 10, $n = 5$), and white LED treatment (white bars; week 0, $n = 4$; week 10, $n = 5$), (b) Lipid peroxidation—Mean (± SE) night-time lipid peroxidation levels in control (black bars; week 0, $n = 5$; week 10, $n = 7$), long-wavelength shifted amber LED treatment (grey bars; week 0, $n = 5$; week 10, $n = 5$), and white LED treatment (white bars; week 0, $n = 5$; week 10, $n = 8$), (c) Antioxidants—Mean (± SE) night-time circulating antioxidant levels in control (black bars; week 0, $n = 6$; week 10, $n = 6$), long-wavelength shifted amber LED treatment (grey bars; week 0, $n = 5$; week 10, $n = 5$), and white LED treatment (white bars; week 0, $n = 6$; week 10, $n = 5$). Samples were taken in February (week 0) and April (week 10), 2016. Underlined asterisks represent a significant difference at week 10 from baseline (week 0) levels and asterisks represent a significant difference to control treatment measures $^*p < 0.05$, $^{***}p < 0.0001$

been show to suppress melatonin in a range of animals from fish to humans (Franke, Brüning, Hölker, & Kloas, 2013; Lewy, Wehr, Goodwin, Newsome, & Markey, 1980). Here, we have demonstrated further support for the suppressive effects of short-wavelength light in a nocturnal marsupial. The peak spectral wavelength of our white LEDs (448 nm) falls within the visual spectral sensitivity range of the tammar wallaby (Hemmi et al., 2000), and the non-visual spectral sensitivity range for marsupial mammals (Pires et al., 2007), and consequently resulted in significant suppression of melatonin. Nocturnal concentrations were consistent with previously reported diurnal melatonin profiles (McConnell, 1986). Moreover, our white LEDs suppressed melatonin 1.5 times more than both captive and wild free-ranging tammar wallabies exposed to high pressure sodium lighting (McConnell, Tyndale-Biscoe, & Hinds, 1986; Robert et al., 2015). This type of night lighting significantly reduces the difference between the brightest and darkest part of the day, suppressing nocturnal melatonin production, and ultimately weakening this crucial chronobiological cue (Dominoni, Goymann, Helm, & Partecke, 2013). This disturbance is known to alter activity, foraging, orientation, and migration ultimately resulting in desynchronization of biological rhythms and reduced fitness (Bird, Branch, & Miller, 2004; Le Tallec et al., 2013; Poot et al., 2008). Furthermore, for seasonal breeders such as the tammar wallaby, the annual cycle of changing photoperiod, and subsequent changes in nocturnal melatonin profiles provide the cue for reproductive activation (McConnell & Tyndale-Biscoe, 1985; Robert et al., 2015; Sadleir & Tyndale-Biscoe, 1977). This ensures that young are born at a time of year that is favorable for maternal peak lactation demands (Cork & Dove, 1989) and offspring survival (Bronson, 1985). This close relationship between light and reproduction means seasonal breeders exposed to artificial light are extremely vulnerable to receiving misleading chronobiological signals. Field studies in the tammar wallaby have shown exposure to high-pressure sodium lighting eliminates this fundamental light cue, resulting in a desynchronization of births with a long-term potential for a trophic mismatch with resources, potentially leading to increased offspring mortality and decreased offspring production (Post & Forchhammer, 2008; Robert et al., 2015).

Through the use of long-wavelength shifted amber LEDs, we were able to eliminate short, blue wavelengths. The peak of these long-wavelength shifted amber lights occurs at 605 nm, falling outside the upper-limit of the visual sensitivity range for tammar wallabies (Hemmi et al., 2000). Importantly, 10 weeks exposure to these lights had no effect on nocturnal melatonin production. Nocturnal concentrations were consistent with wallabies exposed only to astronomical sources of light, as well as previously reported nocturnal melatonin profiles of wallabies during autumn and winter (McConnell, 1986). These findings support previous research showing wavelength dependent effects on melatonin suppression in humans, however, until now the impacts in wildlife were unknown (Brainard et al., 1985; Wright & Lack, 2001). These findings provide support for the use of long-wavelength shifted LEDs to provide high intensity, energy efficient lighting for humans, while minimizing the physiological and behavioral effects on wildlife.

Light at night is also known to have deleterious consequences at the cellular level, affecting important mechanisms of cellular defense and redox homeostasis. Nocturnal melatonin increases antioxidant activity and attenuates a stress response in order to restore equilibrium (Ashkenazi & Haim, 2013). Consequently, exposure to short-wavelength artificial light can lead to serious health consequences, including increased oxidative stress with the potential for oxidative damage through peroxidation of membranes, fatty acids, and carbonylation of proteins (Ghiselli et al., 2000; Navara & Nelson, 2007; Schneeberger & Czirják, 2013). Despite observing suppressed nocturnal melatonin under white LEDs, we found no evidence to support increased oxidative stress in tammar wallabies maintained in natural outdoor enclosures. Mean lipid peroxidation levels declined under all treatments following 10 weeks exposure to experimental lighting. These measures were accompanied by a decline in circulating antioxidant levels. Higher antioxidant levels can often be misinterpreted as a sign of a beneficial redox state (Hõrak & Cohen, 2010), although this is often not the case (Chuang et al., 2006). The pro-oxidant/antioxidant system exists as a feedback-based, homeostatic equilibrium (Ghiselli et al., 2000; Palmieri & Sblendorio, 2007). This suggests our initial measures of antioxidant capacity may be an adaptive response to high oxidative stress, with levels subsiding once the redox equilibrium was restored. However, due to high individual variation and the absence of repeated measures of individuals, we are unable to identify individual changes and must limit our assessment to population measures. To our knowledge, no studies have reported seasonal changes in lipid peroxidation or antioxidant concentrations in tammar wallabies under natural conditions. Consequently, we are unable to quantitatively assess our measures of redox status to determine if our observed levels are considered high. However, our results are strictly limited to lipid peroxidation as a biomarker of oxidative stress. Previous studies have shown heightened oxidative damage to proteins in Syrian hamsters, while lipid damage was minimally affected under continuous light (Tomás-Zapico et al., 2003). Hence, it is likely that we have underestimated the total in vivo redox state, consequently, the pro-oxidant effect of continuous night lighting may be even higher.

While the cost benefits of LED lighting for humans is clear, our findings suggest that these short-wavelengths are increasing the potential for misleading photic cues, disrupting physiological processes, and ultimately increasing the potential for long-term ecological, physiological, and health consequences in natural systems (Davies et al., 2013; Gaston, Visser, & Hölker, 2015). With over 400,000 LEDs already installed in Australia, it is clear there is an urgent need for an improved understanding of the physiological costs associated with their use. Here, we have provided preliminary evidence that changing the spectral composition of lights can reduce these physiological impacts. The challenge now is quantitatively assessing the long-term impacts and further exploring changes to the duration, direction, and spectral composition of night lighting to mitigate these impacts on a variety of taxa with differing visual sensitivities to ensure species persistence in in disturbed habitats.

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AUTHOR CONTRIBUTIONS

Both authors (A.M.D. and K.A.R.) conceived the study, performed the data collection and data analysis, and wrote the manuscript with assistance from those mentioned in the acknowledgments.

ETHICS

All work was conducted according to relevant national and international guidelines. The project was approved by La Trobe University's Animal Ethics Committee (AEC 13–46) and the Department of Environment, Land, Water, and Planning Research Permit No: 10007502.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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