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RESEARCH ARTICLE

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Dim artificial light at night affects mating, reproductive output, and reactive oxygen species in *Drosophila melanogaster*

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Abstract

Humans are lighting the night-time environment with ever increasing extent and intensity, resulting in a variety of negative ecological effects in individuals and populations. Effects of light at night on reproductive fitness traits are demonstrated across taxa however, the mechanisms underlying these effects are largely untested. One possible mechanism is that light at night may result in perturbed reactive oxygen species (ROS) and oxidative stress levels. Here, we reared *Drosophila melanogaster* under either dim (10 lx) light or no light (0 lx) at night for three generations and then compared mating and lifetime oviposition patterns. In a second experiment, we explored whether exposure to light at night treatments resulted in variation in ROS levels in the heads and ovaries of six, 23- and 36-day-old females. We demonstrate that dim light at night affects mating and reproductive output: 10 lx flies courted for longer prior to mating, and female oviposition patterns differed to 0 lx females. ROS levels were lower in the ovaries but not heads, of 10 lx compared with 0 lx females. We suggest that reduced ROS levels may reflect changes in ovarian physiology and cell signaling, which may be related to the differences observed in oviposition patterns. Taken together, our results indicate negative consequences for invertebrates under more stressful, urban, lit conditions and further investigation into the mechanisms driving these changes is warranted to manage invertebrate communities in a brighter future.

KEYWORDS

ALAN, *Drosophila melanogaster*, light pollution, mating, ovaries, oviposition, reactive oxygen species

1 | INTRODUCTION

The presence of artificial light at night (ALAN) is linked to species-wide shifts in behavioral and physiological traits, including courtship and mating, offspring production, growth, and survival (Longcore & Rich, 2004; Navara & Nelson, 2007). These effects are evident even at relatively low levels of ALAN (≤30 lx), typical of the light environment in some urban and peri-urban spaces. Field studies demonstrate a negative relationship between the presence of dim ALAN (≤5 lx) and reproductive success and juvenile growth in birds (Dominoni, Goymann, Helm, & Partecke, 2013; Dominoni, Quetting, & Partecke, 2013; Raap et al., 2016) and reproduction in mammals (LeTallec, Thery, & Perret, 2015; Robert, Lesku, Partecke, & Chambers, 2015), whereas laboratory experiments in hamsters and rats link exposure to dim ALAN (between 1 and 5 lx) with increases in tumor growth rates (Blask, Dauchy, Brainard, & Hanifin, 2009) and immune suppression (Bedrosian, Aubrecht, Kaugars, Weil, & Nelson, 2013). Similar responses to chronic exposure to dim ALAN (between 10 and 30 lx) are observed in invertebrates. Field experiments demonstrate lower mating success in moths (*Operophtera brumata*) (van Geffen, van Eck et al., 2015) and female aphids (*Megoura viciae*) were more likely to switch to an asexual mode of reproduction under dim ALAN conditions with potential implications for winter survival (Sanders et al., 2015). Furthermore, laboratory experiments exploring the effects of dim ALAN report decreased adult longevity and reduced oviposition and egg number of young *Drosophila melanogaster* females (McLay, Green, & Jones, 2017), a decline in the sexual attractiveness of female sex pheromones and accelerated juvenile development in the moth *Mammestra brassicae* (van Geffen, Groot et al., 2015).

A potential driver of the observed physiological effects of dim ALAN is the possibility that ALAN also affects levels of reactive oxygen species (ROS). ROS are a suite of highly reactive by-products of the oxidation–reduction (redox) reactions that occur as part of normal

cell metabolism (Dowling & Simmons, 2009). Low levels of ROS are essential for cell signaling (Droge, 2002; Poli, Leonarduzzi, Biasi, & Chiarpotto, 2004), however very high levels can cause oxidative stress, leading to damage to DNA, proteins, and lipids and consequent effects on organism fitness (Metcalfe & Alonso-Alvarez, 2010). ROS is so critical to normal metabolic function that it is proposed as a major mechanistic driver for the process of aging and a suite of life history constraints (Dowling & Simmons, 2009; Harman, 1956). Accordingly, male and female fertility is adversely affected by both high (for reviews see Agarwal, Aponte-Mellado, Premkumar, Shaman, & Gupta, 2012; Venkatesh, Deecaraman, Kumar, Shamsi, & Dada, 2009), and low levels of ROS and oxidative stress (de Lamirande, Jiang, Zini, Kodama, & Gagnon, 1997). Gametes are particularly vulnerable to the negative effects of high ROS and oxidative damage to DNA can lead to transgenerational effects for offspring viability (Metcalfe & Alonso-Alvarez, 2010). Together, these observations suggest that ROS may be important mediators in the trade-off between reproduction and longevity (Dowling & Simmons, 2009; Metcalfe & Alonso-Alvarez, 2010).

Here, we investigate the potential of variation in ALAN (0 or 10 lx) to affect (i) reproductive output in the form of patterns of mating behavior, lifetime oviposition, and offspring development success and, in a second experiment, potential effects on physiology through (ii) differences in ROS levels of somatic (using the head) (Neretti et al., 2009) and reproductive tissue (ovaries) and accompanying morphological changes (measured through ovarian area). We use the model invertebrate *D. melanogaster*, a species with demonstrable reduction in early reproductive effort and adult survival in the presence of 10 lx ALAN (McLay et al., 2017). We predict that flies subjected to the presence of ALAN during juvenile development and the adult stage of the life cycle will have perturbed ROS levels compared to flies reared with no light at night (0 lx). Furthermore, we expect a concomitant reduction in mating success, oviposition rates, and offspring development.

2 | METHODS

2.1 | Fly stocks

A stock population of *D. melanogaster* was created from 100 adult females and 50 adult males collected in April 2015 from Oak Ridge Winery in the Yarra Valley, Victoria, Australia (−37.686908, 145.457438). Stock flies were maintained on Bloomington's cornmeal medium (Brent & Oster, 1974) under standard conditions, at a density of approximately 50 male and 50 female flies for 34 generations, in a retrofitted incubator at 28 ± 1°C, prior to the start of the experiment (for further details, see McLay et al. (2017)).

2.2 | Light treatments

We created two light treatments using Westinghouse incubators, as described in McLay et al. (2017). Both light treatments had an identical 12 hr daytime lx (2,600 lx—equivalent to an overcast day, 6,800 K), followed by one of two 12 hr night time lx (either 0 lx, 0 K, or 10 lx, 5,900 K) ALAN treatments. To ensure no incubator bias, flies and their corresponding ALAN environment were rotated between three incubators every 2–3 days and were randomly positioned within each incubator.

2.3 | Experimental flies

To create our experimental generation of flies, we allocated five stock bottles of approximately 50 recently emerged female and male flies to the light (10 lx) and dark (0 lx) ALAN treatments. We maintained flies in their designated light environment for a further two generations in the same manner as reported previously (McLay et al., 2017), after which time we collected newly emerged (< 6 hr old) virgin female and male flies under mild CO_2 anesthesia and transferred them to individual vials to mature for 2 days before pairing flies to mate. Measurement of reproductive output was conducted in two blocks ($N = 66$ male and female pairs from generation 35; $N = 70$ pairs from generation 36). We used data from this experiment and our previous study (McLay et al., 2017) to inform female age classes for the ROS study. The ROS assay and ovarian area study was also run over two blocks ($N = 267$ flies from generation 51; and $N = 259$ flies from generation 52).

2.4 | Effect of ALAN on reproductive output

2.4.1 | Mating behavior

To assess whether mating behavior varied under the two ALAN treatments, we paired 136 female and 136 male flies ($N = 66$ pairs in block 1, $N = 70$ pairs in block 2) in vials containing standard medium. Each pair was reared under the same light treatment but originated from different vials and thus were not siblings. A pair was permitted 30 min to commence mating. Flies that commenced copulation within the 30-min time interval were allowed to complete copulation after which time the male was discarded; pairs that did not commence copulation within 30 min were also discarded. For each trial, the time until male wing extension, defined here as time to courtship (Cobb & Jallon, 1990), the time to copulation and the duration of copulation were recorded. To explore whether the number of eggs laid and success of development from egg to adult varied between the two light environments, we transferred a random subset of the mated females from the mating behavior assay ($N = 38$ females, 31 from block 1; $N = 40$ from block 2) to individual vials containing fresh standard medium.

2.4.2 | Oviposition patterns

We counted the total number of eggs laid by each of the females over a maximum of eight 24-hr time points (at 3, 9, 13, 16, 20, 27, 30, and 34 days) over their adult lives. At the start of each time point, the female was transferred to a vial with standard medium, dyed blue (Queen Fine Foods, Alderley, Australia) to increase egg visibility. After 24 hr, the female was returned to a new vial containing standard medium until the next time point. The final time point (day 34) was chosen to cover the median lifespan of *D. melanogaster* under ALAN conditions: our

previous work demonstrates that approximately half of flies reared under 10 lx ALAN conditions are likely to have died by day 34 (McLay et al., 2017). After day 34, any surviving females were discarded, as by this time they would have almost certainly exhausted their store of sperm (Lefevre & Jonsson, 1962). The total number of eggs laid at each time point was counted twice by visual inspection under magnification.

2.4.3 | Egg to adult success

To assess variation in the number of emerging adult offspring, we allowed eggs from the Day 3, 20, and 34 egg counts to develop to the adult stage. These time intervals represented different slopes in the survival curve of flies under these ALAN conditions (little to no mortality at Day 3, both 0 and 10 lx = 98% survival; a slow decline in survival to Day 20, 0 lx = 96% survival, 10 lx = 86% survival; and a sharp decline in survival to Day 34, 0 lx = 77% survival, 10 lx = 57% survival) (McLay et al., 2017). Vials were checked every 1–2 days until the first adult eclosed and then for a further 7 days, after which time the vial was discarded. The number and sex of all emerging adults was recorded and the egg to adult success for each vial was determined as the number of emerging adults divided by the total number of eggs laid.

2.5 | Effect of ALAN on ovarian area and comparative ROS levels in the head and ovary

In a second experiment, we investigated the effects of the different light regimens on ovarian area (as a proxy for the number of eggs remaining) and on ROS levels in heads and ovaries. A total of 526 single-mated females (N = 267 in block 1; N = 259 in block 2) were randomly allocated to one of three age cohorts (young = 6 days; medium-age = 23 days; old = 36 days post eclosion). We used heads for somatic tissue ROS levels (Neretti et al., 2009) and ovaries to determine ROS levels in female germ cells. Once a female had reached her designated age, we lightly anesthetized her with CO_2, removed the head and dissected out the ovaries.

2.5.1 | Ovarian area

To explore whether exposure to ALAN resulted in differences in developing gametes, we used total ovarian area as a proxy for the number of eggs remaining when the female was assessed. This assumption is justified, as in our population ovarian area correlates with the number of visible eggs (stages 9–14) (Bate & Martinez Arias, 1993) (mean ± SE; total eggs: 26.65 ± 2.45, N = 20 females; F = 111.1, R^2 = 0.86, $P < 0.0001$) and mature eggs (stages 13 and 14) (5.65 ± 2.33, N = 20 females; F = 49.97, R^2 = 0.74, $P < 0.0001$). Ovaries were photographed at × 64 magnification with a dissecting microscope (Olympus SZX16, Tokyo, Japan), with attached camera (SONY ILCE-QX1, Tokyo, Japan). We calculated total ovarian area of each fly (mm^2) by drawing a line around the perimeter of each ovary using Image J software (NIH, Rockville, MD).

2.5.2 | Comparative levels of ROS

To assess whether total ROS levels varied under the different light treatments at different female ages, we measured total ROS using a 2′7′-dichlorohydrofluorescin diacetate (H2DCFDA) assay, modified from Wang and Joseph (1999), in which the nonfluorescent DCFH is oxidized by ROS to form fluorescent DCF and can be detected by fluorometry (Wang & Joseph, 1999). From each light treatment and age cohort, samples of ovaries from single-mated flies (N = 50 pools of five pairs of ovaries per pool) and individual heads (N = 108 females) were assayed. Briefly, individual samples were placed into 50 μl of cold lysis buffer (TPER; Thermo Fisher Scientific, Rockford, IL) in a 96 well PCR plate (Axygen Scientific, Union City, CA) on ice. We then homogenized each sample for 45 sec using a micropestle made from a modified sterile 1,000 μl pipette tip (Eppendorf, Hamburg, Germany). The plate was centrifuged at 400g for 5 min at 4°C and 10 μl of supernatant transferred to a 96-well black assay plate (Greiner Bio-one, Neuberg, Germany). We added 40 μl of cold 10 mM solution of 2′7′-H2DCFDA (Sigma–Aldrich, Castle Hill, Australia) dissolved in dimethyl sulfoxide (Sigma–Aldrich, St Louis, MO). The plate was incubated in darkness for 45 min at 37°C and then read for fluorescence in a microplate reader (PerkinElmer, EnSpire Multimode, Waltham, MA) at 485 nm excitation and 535 nm emission (Wang & Joseph, 1999). We commenced all assays between 2.5 and 3 hr after incubator lights on; average time between fly death and the start of the assay was 2.0 ± 0.5 hr. All samples were kept on ice until dissections and photographs were complete. For all ROS assay plates, we used positive/quality control and negative control samples. The positive/quality control was prepared separately and consisted of the supernatant from 200 whole flies from the stock population, homogenized in 3,000 μl TPER, and stored at −80°C in sub-aliquots until required. The negative control contained all reagents except the tissue extract for each plate. Samples and controls were run in triplicate across plates and the mean fluorescence reading (subtracting the negative control) was used for analysis, where the coefficient of variation (CV) for the triplicate reading was < 15%. Interplate (CV of positive/quality controls) and intra-plate (CV of the mean CV of the first two and last two ovarian and head readings per plate) specific analyses were undertaken to assess the consistency of readings between plates.

2.6 | Statistical analyses

Analyses were performed in R (R core team 2016) using the *lme4* software package (Bates, Machler, Bolker, & Walker, 2015). Prior to analysis, data were assessed for normality and transformed where appropriate (time to commencement of courtship and time from commencement of courtship to onset of copulation were log transformed, and time for copulation was cube root transformed). We used standard least squares linear models to explore differences in mating behaviors, total ovarian area, and ROS levels; and a generalized linear model (GLM) with a binomial error distribution and logit link function to explore differences in the proportion of pairs commencing copulation in 30 min and numbers of females surviving to Day 34. Generalized linear mixed models (GLMM) fitted by maximum likelihood (ML) assuming a binomial error distribution and logit link function were used for all other data for reproductive output except for cumulative number of eggs, which did not include a binomial function and was fitted with restricted maximum likelihood (REML). We included light

TABLE 1 Statistical models exploring the effect of artificial light at night (ALAN) on reproductive output in *D. Melanogaster*

Model parameters	Mean ± SE or proportions		Statistic, *P* value
	0 lx	10 lx	
Mating behavior			
(a) Number of pairs commencing copulation in 30 minutes			
Light treatment	62/68	58/68	$\chi^2 = 1.14$, $P = 0.28$
(b) Time to commencement of courtship (log secs)			
Light treatment	4.60 ± 0.11	4.86 ± 0.14	$F_{1, 111} = 2.10$, $P = 0.15$
(c) Time from commencement of courting to onset of copulation (log secs)			
Light treatment	4.51 ± 0.16	5.02 ± 0.17	$F_{1, 120} = 4.65$, $\mathbf{P = 0.03}$
(d) Time for copulation (cube root secs)			
Light treatment	9.60 ± 0.07	9.63 ± 0.07	$F_{1, 116} = 0.08$, $P = 0.78$
Oviposition and offspring development			
(e) Number of females laying at least one egg at any egg count			
Light treatment	185/249	132/199	$\chi^2 = 0.62$, $P = 0.43$
Maternal age			$\chi^2 = 28.40$, $\mathbf{P < 0.005}$
Maternal age × maternal age			$\chi^2 = 17.45$, $\mathbf{P < 0.005}$
Light treatment × (maternal age × maternal age)			$\chi^2 = 6.89$, $\mathbf{P = 0.01}$
(f) Cumulative number of eggs laid per female			
Light treatment	81.25 ± 2.79	72.88 ± 3.12	$\chi^2 = 2.15$, $P = 0.14$
Maternal age			$\chi^2 = 127.31$, $\mathbf{P < 0.005}$
Maternal age × maternal age			$\chi^2 = 15.78$, $\mathbf{P < 0.005}$
Light treatment × (maternal age × maternal age)			$\chi^2 = 12.79$, $\mathbf{P < 0.005}$
(g) Egg to adult success – proportion of vials where eggs were laid that had any adults emerge			
Light treatment	38/65	31/54	$\chi^2 = 0.01$, $P = 0.91$
Maternal age			$\chi^2 = 39.90$, $\mathbf{P < 0.005}$
(h) Egg to adult success – proportion of eggs to emerge as adults where any adults emerged			
Light treatment	0.51 ± 0.03	0.48 ± 0.03	$\chi^2 = 0.81$, $P = 0.37$
(i) Sex ratio of offspring (Days 3 and 20)			
Light treatment	0.50 ± 0.03	0.48 ± 0.03	$\chi^2 = 0.001$, $P = 0.97$

Besides the main variable of light treatment only those variables contributing to the minimal adequate model are reported. All statistics are mean ± standard error except where indicated.

treatment and block as categorical factors in all models and other factors as appropriate: age cohort as a categorical factor in total ovarian area and ROS levels; maternal age as a continuous and polynomial variable in number of females laying at least one egg at any egg count and cumulative number of eggs laid per female; female identity as a random effect for all oviposition and offspring development models; number of days survived as a continuous variable for number of females laying at least one egg at any egg count; number of eggs laid per female at an egg count as a continuous variable for the proportion of eggs to emerge as adults where some adults emerged and for the sex ratio of offspring. Interactions between all of these variables were included. The significance of parameters was assessed using hierarchical backwards stepwise deletion, dropping terms from the model (except the main parameter of interest, light treatment) where $P > 0.10$. Post hoc Tukey's tests were used to determine differences between groups. Unless otherwise stated all data presented are means ± standard errors and the level of statistical significance was taken as $P < 0.05$.

3 | RESULTS

3.1 | Effect of ALAN on reproductive output

3.1.1 | Mating behavior

Flies in the 10 lx treatment took longer from the onset of courtship to commence copulation than 0 lx flies ($P = 0.03$; Table 1c). In contrast, the probability of copulation commencing within 30 min; the time taken between first introduction into the vial and the onset of male courtship behavior; and, the total duration of copulation, were comparable for the two light treatments (all $P > 0.15$; Table 1a, b, and d).

3.1.2 | Oviposition patterns and survival

Female survival to Day 34 ($\chi^2 = 0.42$, $P = 0.52$) and the number of females laying at least one egg at a given egg count ($P = 0.43$; Table 1e) were comparable for the two light treatment groups. Overall, the likelihood of an egg being laid varied with age ($P < 0.005$; Table 1e), but

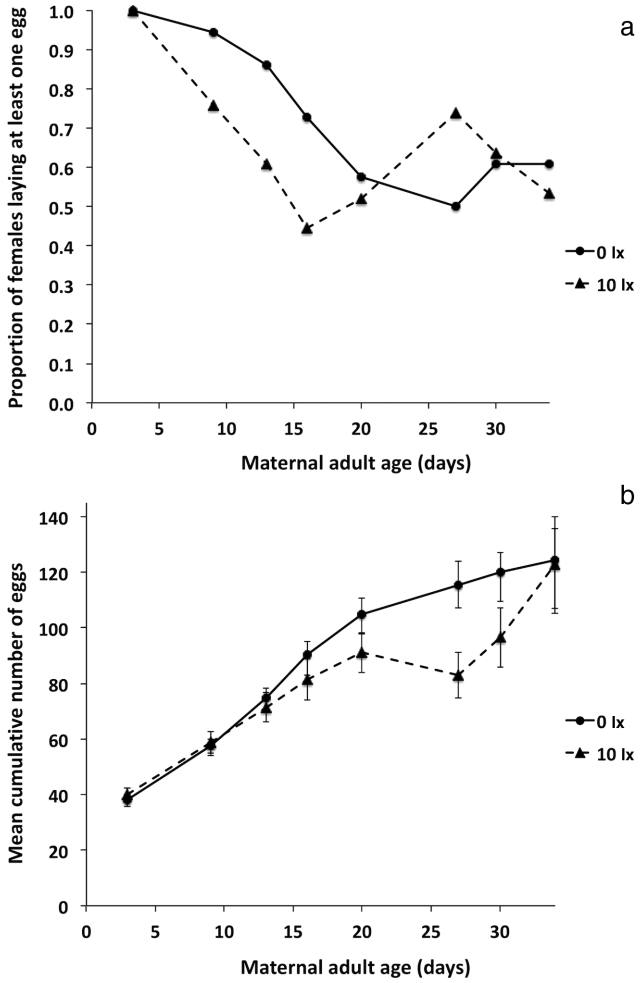

FIGURE 1 a) Proportion females ovipositing varying with maternal age (significant interaction term of treatment x maternal age x maternal age, P = 0.01 N = 348); and b) Mean cumulative number of eggs varying with maternal age (significant interaction term of treatment x maternal age x maternal age, P < 0.005, N = 305)

the relationship between maternal age and the probability of laying an egg was curvilinear ($P < 0.005$; Table 1e) and this pattern varied across the two ALAN treatments ($P = 0.01$; Table 1e and Figure 1a). A similar pattern was observed for the cumulative number of eggs laid per female. The cumulative number of eggs laid was comparable for 0 and 10 lx females ($P = 0.14$; Table 1f), but the relationship over time was nonlinear ($P < 0.005$; Table 1f) and varied across the two ALAN treatments ($P < 0.005$; Table 1f and Figure 1b).

3.1.3 | Egg to adult success

The probability that a vial with eggs present produced adult flies was the same for both light treatments ($P = 0.91$; Table 1g). However, no adults emerged from the Day 34 vials (number of vials with adults emerging for 0 lx Day 3 females = 33/36, Day 20 females = 5/22, Day 34 females = 0/0; 10 lx—Day 3 females = 28/32, Day 20 females = 3/19, Day 34 females = 0/0; $P < 0.005$; Table 1h). The proportion of eggs emerging as adults ($P = 0.37$; Table 1h) and adult sex ratio ($P = 0.97$, Table 1i) were comparable between the 0 and 10 lx treatments.

3.2 | Effect of ALAN on ovarian area and comparative ROS levels in the head and ovary

3.2.1 | Ovarian area and survival

There was no difference in ovarian area between the ALAN treatments ($P = 0.75$; Table 2a). The proportion of flies that survived to Day 34 was also comparable ($\chi^2 = 0.27$, $P = 0.61$).

3.2.2 | Comparative levels of ROS

The level of ROS in female's heads did not differ between the ALAN treatments ($P = 0.33$; Table 2b and Figure 2a), but 10 lx females had lower ovarian ROS levels compared with 0 lx females ($P = 0.04$; Table 2c and Figure 2a). ROS levels of both heads and ovaries varied with female age (heads: $P < 0.005$, and ovaries: $P = 0.01$, respectively; Table 2b and c and Figure 2b and c). Post hoc Tukey's tests revealed higher ROS levels in the heads of Day 23 compared with Day 36 flies, but no differences between Day 6 flies and either Day 23 or Day 36 flies. Additionally, ROS levels were higher in the ovaries of Day 6 compared with Day 36 flies but no different to flies of Day 23. The intra and interspecific plate CVs were 14.91% and 10.50%, respectively.

4 | DISCUSSION

This study provides three key findings suggesting that exposure to dim ALAN has behavioral and physiological consequences. First, chronic exposure to dim ALAN of 10 lx was associated with an increase in the time taken between the onset of courtship and the commencement of copulation. Second, the pattern of oviposition over a female's life was different between light treatments, with both the likelihood of a female laying eggs within a 24-hr period and cumulative eggs laid over a female's life varying with the interaction between light treatment and maternal age. Finally, ROS levels of ovaries were comparatively lower in flies under 10 lx compared with the 0 lx treatment.

Our result that flies reared under 10 lx spent significantly longer courting but were equally likely to eventually copulate is one of the first experimental studies to record a difference in courting and mating behavior under dim (10 lx) conditions. To our knowledge, these behaviors have yet to be assessed in vertebrates under dim ALAN. However, Botha, Jones, and Hopkins (2017) demonstrated interactions between light treatment and other variables in the duration of mating and number of mating bouts in crickets *(Teleogryllus commodus*). Additionally, a field study in moths (*O. brumata*) demonstrated reduced mating success for females under 10 lx ALAN conditions (van Geffen, van Eck et al., 2015). Given the peak time for mating in *D. melanogaster* is during daylight hours (Sakai & Ishida, 2001), which is when our mating assays were conducted, it is unlikely that the presence of light during a trial is responsible for the behavioral differences observed here. Our study did not aim to identify possible mechanisms for this change in behavior, and we note that van Geffen, van Eck et al. (2015) did not investigate courtship behavior. However, the fact that a key aspect of mating behavior shifted following lifetime exposure to ALAN suggests that, in both species, its presence has the potential to interfere with

TABLE 2 Statistical models exploring the effect of artificial light at night (ALAN) on reactive oxygen species (ROS) levels and ovarian area

Model parameters	Mean ± SE		Statistic, *P* value
	0 lx	10 lx	
Ovarian area			
(a) Total ovarian area			
Light treatment	1.24 ± 0.06	1.27 ± 0.06	$F_{1, 110} = 0.10, P = 0.75$
ROS			
(b) ROS levels - female head			
Light treatment	234.76 ± 8.78	222.22 ± 9.93	$F_{1, 108} = 0.97, P = 0.33$
Female age			$F_{2, 108} = 5.64, \mathbf{P < 0.005}$
(c) ROS levels - ovaries			
Light treatment	111.07 ± 11.14	87.25 ± 6.88	$F_{1, 50} = 4.49, \mathbf{P = 0.04}$
Female age			$F_{2, 50} = 5.05, \mathbf{P = 0.01}$

(a) Total ovarian area; (b) ROS levels in female heads; and (c) ROS levels of ovaries. Besides the main variable of light treatment, only those variables contributing to the minimal adequate model are reported. All statistics are mean ± standard error.

mating per se. In another species of moth (*Mamestra brassicae*), ALAN is related to reduction in the attractiveness of female sex pheromones, which is likely to disrupt mating cues (van Geffen, Groot et al., 2015). *D. melanogaster* have cuticular hydrocarbons that act as sex- and species-specific pheromone cues (Howard & Blomquist, 2005) and are subject to environmental perturbation (Higgie, Chenoweth, & Blows, 2000), but whether they exhibit similar variation under ALAN is currently untested and merits investigation. Regardless of the underlying mechanism, the increased time spent courting represents a fitness cost for ALAN, due to increased competition and vulnerability to predators (Endler, 1987; Magnhagen, 1991).

Age-related declines in fecundity and oviposition rates are common across taxa (Clutton-Brock, 1988) including insects such as Coleoptera (Tanaka, 1990), Lepidoptera (Braby & Jones, 1995), Orthoptera (Carrière & Roff, 1995), Diptera (Jann & Ward, 1999), as well as *D. melanogaster* (Partridge, Fowler, Trevitt, & Sharp, 1986) and such declines in propensity to lay and egg number over time were paralleled here. In contrast, while a reduction in the number of eggs produced under ALAN conditions in young adult females was previously reported for *D. melanogaster* (McLay et al., 2017), this is the first evidence for variation in patterns of lifetime egg production and variation in dim ALAN. The interaction we observed between female age and light treatment suggests that such an investigation was warranted, as the relationship was nonlinear and thus, assessing variation within a single age-class may not be representative of lifetime oviposition. It is unlikely the observed pattern of oviposition was driven by differences in survival between the two light treatments, as the likelihood of survival to Day 34 was the same for both treatments and thus it seems that this represents a real difference in the pattern of offspring production. Maternal age effects aside, our study did not aim to disentangle male and female contributions to overall egg production. Male *D. melanogaster* transfer seminal fluids during mating, which stimulate egg production, ovulation and sperm retention (Gillott, 2003; Wolfner, 2002). Female *D. melanogaster* typically retain sperm for approximately 14 days following mating (Qazi, Heifetz, & Wolfner, 2003) and offspring production is positively related to sperm storage (Qazi et al., 2003). There is the potential that the amount or quality of sperm and/or seminal fluids transferred during mating or subsequently stored, differed between the light treatments. Such a mechanism may explain why the oviposition curves initially diverge at approximately 15 days in the current study. Therefore, further investigation into the relative contributions of females and males to egg production is warranted.

A potential underlying mechanism for the observed physiological and behavioral effects of dim ALAN is its potential to suppress endogenous melatonin production (Blask et al., 2005; Blask et al., 2009; Brainard, Richardson, Petterborg, & Reiter, 1982). The photosensitive indolamine melatonin is a key driver of circadian rhythm, as well as a powerful antioxidant (for review see Reiter, Tan, & Fuentes-Broto, 2010). The primary site of its nocturnal production across taxa is in the head (the location of primary photoreceptors) (Helfrich-Forster, Winter, Hofbauer, Hall, & Stanewsky, 2001; Vivien-Roels & Pevet, 1993) including in *D. melanogaster* (Callebert, Jaunay, & Jallon, 1991; Finocchiaro, Callebert, Launay, & Jallon, 1988) and its reduction can lead to circadian disruption and a change in levels of ROS (Pandi-Perumal et al., 2006) although this has not yet been demonstrated in *D. melanogaster.*

Our data did not support the oft-cited prediction that in the presence of ALAN, levels of ROS are likely to be increased (Jones, Durrant, Michaelides, & Green, 2015; Navara & Nelson, 2007; Pandi-Perumal et al., 2006; Reiter et al., 2003; Tan et al., 2010). On the contrary, we found no difference in ROS levels in female heads between light treatments and comparatively lower levels of ROS in the ovaries of 10 lx females compared with their 0 lx counterparts. In the absence of an effect of ALAN on total ovarian area or on the cumulative number of eggs laid, it is unlikely that the difference between the two light treatments arises due to reduction in the quantity of material assayed and thus a reduced signal. Instead, we suggest that the observed variation reflects ALAN-induced changes in ovarian physiology that may explain differences in the pattern of eggs laid over a female's lifespan. A certain level of ROS is required for signaling pathways in cells (Droge, 2002). In *D. melanogaster*, ROS generated from nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) is required for normal ovulation (Ritsick, Edens, Finnerty, & Lambeth, 2007) and

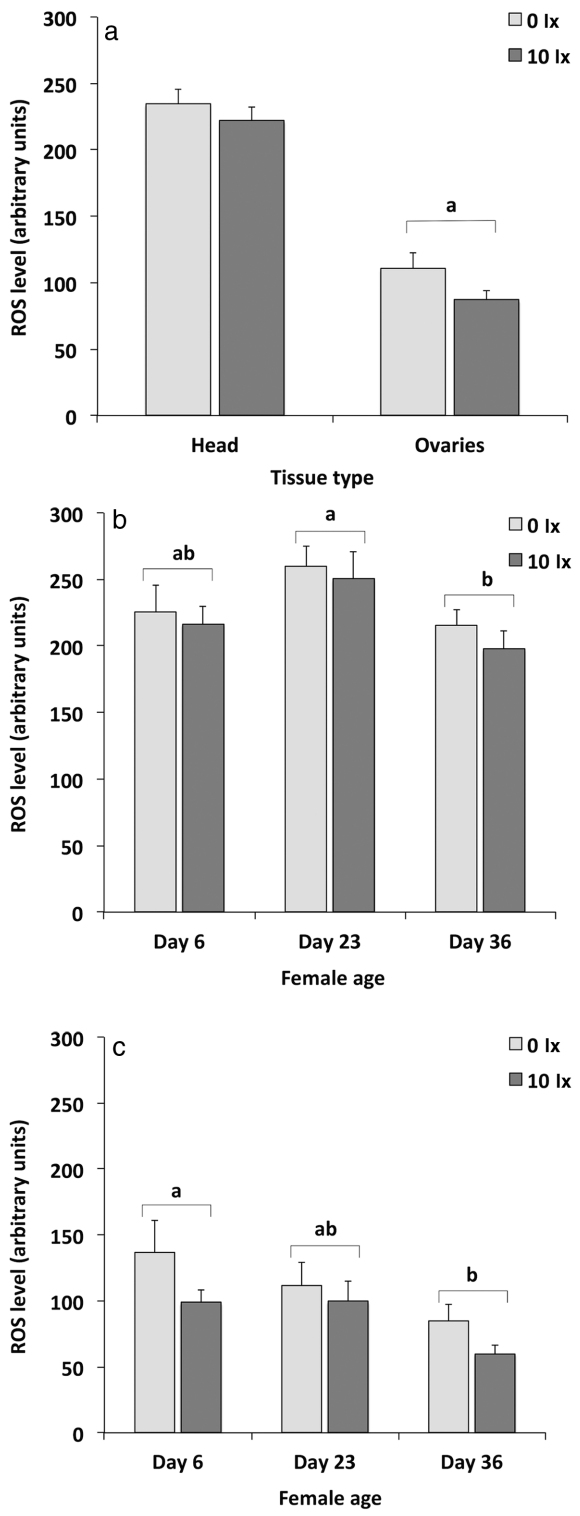

FIGURE 2 Comparative mean ROS levels (arbitrary relative units) in a) Heads and pooled ovarian samples (five pairs of ovaries per pool) combined for all ages for each light treatment (heads $P = 0.33$, $N = 108$); ovaries $P = 0.04$, $N = 50$); b) Heads varying with female age for each light treatment ($P < 0.005$, $N = 108$; and c) Pooled ovarian samples varying with female age for each light treatment ($P = 0.01$, $N = 50$). Different superscripts denote differences ($P < 0.05$) between; a) light treatment for ovarian samples; and b) & c) between female ages

it is conceivable that these pathways are modulated in the presence of ALAN. The implications of this effect on fitness is currently unclear, as aside from changes for the ALAN exposed females in the oviposition pattern and cumulative egg number over time, the egg to adult success rates of offspring were unchanged in the current laboratory study.

Intriguingly, despite detectable differences in the ovaries between the two light treatments, ROS levels in the heads did not differ. This highlights the possibility of tissue-specific responses to ALAN arising through variation in the levels and pattern of ROS generation between the two tissue types. Ovaries contain rapidly dividing cells that typically contain higher numbers of mitochondria than somatic cells (Cree et al., 2015) and as ROS generation occurs primarily in mitochondria (Finkel & Holbrook, 2000), we may expect ovaries to generate greater amounts of ROS than somatic tissue. A direct link between metabolic rate and ROS levels remains controversial (Alonso-Alvarez, Canelo, & Romero-Haro, 2017; Salin et al., 2015), nonetheless, extramitochondrial ROS production, for example from NOX in reproductive tissues (Alonso-Alvarez et al., 2017; Ritsick et al., 2007) could also lead to discernible differences between tissues under ALAN conditions. Variation in these two ROS generators could also explain the age-specific differences in ROS levels in both heads and ovaries between age cohorts (higher ROS levels in the Day 6 cohort compared to the Day 36 cohort). Higher metabolic activity and investment in reproduction is associated with young organisms, while older individuals undergo senescence (Frisard et al., 2007; Sohal & Weindruch, 1996), so we may expect to see shifts in ROS levels between the beginning and end of adult lifespan.

Our current study measured overall ovarian ROS at a specific timepoint (during the daylight and not during the dark period) and was not designed to identify specific signaling pathways. We note that ROS levels vary in different tissues over a 24 hr period due to rhythms in circadian activity and antioxidant production (Hardeland, Coto-Montes, & Poeggeler, 2003). Therefore, we do not know whether the observed ROS levels reflect the overall diurnal changes in redox status of the ovary. Future experimentation with more sampling time points is needed to assess relative temporal, tissue-specific differences in ROS. Additionally, our experiment was undertaken in flies that had been under light treatment for three generations, it is possible (albeit not tested) that only flies with inherently lower levels of ROS progressed to the third generation. Further multigenerational studies would elucidate whether selection is occurring between generations under ALAN. Moreover, this experiment was conducted in a benign laboratory environment, with constant temperature and food ad libitum. While the imposed light stress was strong enough to generate detectable differences within the ovaries, it may not reflect the more extreme ecological stresses imposed in nature.

In conclusion, we have demonstrated that dim anthropogenic light at night has a detrimental impact on two traits related to reproductive fitness in *D. melanogaster* (mating behavior and oviposition patterns over a female's life). Additionally, dim ALAN lowers ovarian ROS levels compared with a no light at night treatment, which may reflect altered ovarian physiology, that is, cell signaling. Further studies on effects of ALAN on mating cues and nocturnal/diurnal ROS loading are required to understand the underlying mechanism(s) behind and consequences

of our results. Given that we are lighting the world with increasing extent and intensity (Kyba et al., 2017), understanding the mechanisms behind and the consequences of this anthropogenically induced pollutant to populations and ecosystems is critical for the effective management of our urban ecosystems and their future diversity.

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CONFLICT OF INTEREST

None.

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