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Artificial Light at Night Increases Growth and Impairs Reproductive Success in Budgerigars (*Melopsittacus undulatus*) in a Duration Dose-Dependent Manner

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† In the memory of the late Abraham Haim.

Simple Summary: Artificial light at night (ALAN) is increasingly being recognized as a new and rapidly growing form of environmental pollution that may impose severe health and ecological consequences. In our study, we examined the effects of bright ALAN exposure with increasing duration on growth and reproduction success in Budgerigars (*Melopsittacus undulatus*). Strong correlations have been detected between artificial light at night, melatonin sulfate levels, and both body mass and reproductive success. ALAN increased body mass gain and reduced reproduction efficiency in exposed birds compared with controls. Additionally, melatonin levels were also suppressed in an exposure dose-dependent manner, suggesting a role for the hormone suppression in mediating the effects of the environmental exposure on the growth and reproduction responses of Budgerigars.

Abstract: Short-wavelength artificial light at night is increasingly being associated with health and ecological risks. The negative impact of this relatively new source of pollution has been studied intensively in wild birds but to a much lesser extent in captive conspecifics. Using an avian model, our objective was to evaluate the effects of short-wavelength (200 lux at 460 nm) lighting on the body mass and reproductive success of Budgerigars (*Melopsittacus undulatus*) under captive conditions. Birds were maintained under a naturally increasing photoperiod from March to June, with one daily artificial light at night exposure of increasing duration (0, 30, 60, and 90 min) in the middle of the dark period. During the experiments, birds were monitored monthly for body mass, number of eggs laid, hatching success, and melatonin sulfate levels in droppings. Artificial light at night increased body mass and decreased melatonin sulfate levels as well as the number of eggs and hatching success in a duration dose-dependent manner. Our findings provide further evidence of the potential adverse impact of artificial light at night on captive birds and advocate the need for effective controlling measures for light pollution.

Keywords: body mass; light pollution; captive birds; melatonin; short wavelength

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1. Introduction

Light pollution emerging from artificial light at night (ALAN) is increasing worldwide [1]. Lighting technology has developed solutions offering energy-saving illumination of short-wavelength (SWL) light as light-emitting diodes (LEDs), among other technologies. However, regarding the health and ecological effects of such new ALAN sources, they have not tested widely or for extended periods and may have significant unexpected negative ecological and physiological effects when used extensively for outdoor or indoor lighting [2,3]. Virtually all living systems from single-celled to multicellular organisms, including birds, show distinct daily rhythms in their behavioral, physiological, and molecular activities [4,5]. In mammals and birds, the timing of daily and annual cycles in the photoperiod provides the most reliable and enduring signal for tracking changes in environmental conditions [6].

Diurnal activity and seasonal reproduction are common among avian species that are under photoperiodic control [7]. According to a study by Raap et al. [8], ALAN with the intensity of only 3 lux, applied either in December or February, decreased sleep duration and advanced activity onset by about 24 min in Great Tits (*Parus major*) compared with unexposed birds. Significant associations between ALAN exposure and early occurrence of seasonal dawn and dusk singing were also reported in different songbird species [9]. In addition, according to Zhang et al. [10], ALAN can potentially advance sexual hormone secretion and gonadal growth and, consequently, trigger earlier reproduction compared with regions unpolluted by ALAN [11]. The circadian system in birds comprises three major types of tissues that express multi-photoreceptors and multi-oscillators: the retina of the eye, the hypothalamic suprachiasmatic nucleus (SCN), and the pineal gland. Light signals for non-visual photoperiodic responses are detected by intrinsically photoreceptive retinal ganglion cells (ipRGCs) that innervate distinct brain centers responsible for entraining and coordinating circadian rhythms such as the SCN and the pineal gland [12]. The SCN regulates avian peripheral oscillators by both humoral signals and direct neural connections, of which the latter is of particular importance, as they innervate the pineal gland to modulate melatonin production and secretion [13]. The gland synthesizes and releases melatonin for circulation exclusively at night, but light exposure during the day or ALAN, especially of short wavelength (SWL), suppresses hormone synthesis [14].

ALAN exposures of different spectral compositions and irradiance have been found to affect sleep behavior, activity patterns, immune functions, metabolic responses, and reproductive status in birds [15–19].

Budgerigars (*Melopsittacus undulatus*) are a social parrot, living in flocks in Australia. Under natural conditions, an increasing photoperiod predicts the beginning of the rainy season, which corresponds to the Budgerigar's breeding season. Today, Budgerigar is a common pet all over the world and reproduces easily in captivity [20]. While the impacts of light pollution on behavioral and ecological responses of birds have been extensively studied in recent years, less is known about the physiological signaling mechanism, particularly in captive birds [21]. Pet birds share a common challenge in coping with irregular captivity conditions imposed by the unpredicted schedules of their owners, particularly in regard to lighting conditions during the scotophase [22]. In most cases, the physiology and behavior of these animals are compromised by quite different environmental conditions, particularly acute excessive artificial light at night of higher irradiance and shorter wavelength compared with wild-type conspecifics [23]. Therefore, ecological exposure with continuous dim light is relevant for pet birds.

The main objective of this study was to evaluate the effects of ALAN on Budgerigars growth and reproductive success as well as explore the potential underlying physiological mechanism involved. To this end, birds were maintained on a naturally increasing photoperiod from March to June, with one ALAN exposure per night, at the middle of the dark period and of increasing exposure duration (ED). Birds were monitored monthly for body-mass (W_b) gain, reproductive success, and melatonin levels in droppings as measured as melatonin sulfate (MLTS). Our hypothesis is that ALAN for increasing duration will disrupt the growth and reproduction activity in a dose-dependent manner.

2. Materials and Methods

2.1. Birds and Housing

For this study, we used a total of 24 female and 24 male sexually mature Budgerigars (age 12 months; W_b 41 ± 0.48 g). Birds were hand-reared (Ya'ad, Israel; 32°52′44″ N, 35°14′36″ E) and housed in a specially designed chamber (360 × 100 × 200 cm). The chamber was divided by opaque wooden boards into four identical rectangle compartments. The front of the cage was opened during the daytime to the external light and closed during the nighttime by an opaque cloth curtain to avoid light pollution from external sources. Six identical wire breeding cages (60 × 40 × 28 cm) were placed in each compartment. Each breeding cage had an outside mount nest wooden box (17 × 14 × 14 cm) with an opening of 4 cm in diameter as well as a 50 mL water tube and food bowl. In each compartment, illumination was provided using a high-efficiency fluorescent lamp connected to a timer circuit that produces 200 lux (60 µW/cm^2, photon flux 1.65 × 1014 photon cm^{-2} sec^{-1}) at λDominant = 460 nm (AvaSpec-2048-FT-SDU, Avantes, Eerbeek, The Netherlands). Each lamp was installed horizontally 50 cm directly in front of the breeding cages on the middle of the rear wall of the rectangle compartment. Birds were provided with ad libitum access to water and commercial grain diet. All experiment procedures were conducted with approval from the Ethics and Animal Care Committee of the University of Haifa (Protocol number: 548/18).

2.2. Experimental Design

Birds from all experimental groups were paired for breeding and after a one-month retention period, birds were randomly allocated to one of four ED (0 (control group), 30, 60, and 90 min) groups of 12 birds (2 × 6 birds × cages). For all ED groups, ALAN onset was at the middle of the dark period using the same light source, irradiance, and spectral composition as during the day. We used these levels because generally under captivity, companion animals such as Budgerigars are housed under similar ALAN levels [22,23], which may affect the birds' circadian regulation, resulting in adverse responses including compromising the immune system [24] of the birds and consequently exposing the fanciers to potential infectious diseases [25]. In our study, the birds were exposed to 200 lux because this level is frequently used in light at night studies in mammals and birds [26,27]. Additionally, ALAN effects have been poorly studied in Budgerigars; therefore, as a first step, we sought to evaluate whether the species responds to ALAN regarding growth and reproduction regulations. We used high SWL lighting levels to make sure that the exposure signal was sufficient to be detected by the photoreception system of the bird.

2.3. W_b, Egg Production, and Hatchability

The nests were regularly examined every other day, and the number of eggs laid and hatching success were recorded. Individual W_b was measured at the start of the first month (W_b-Initial) and at the end of each following month until June (W_b-Final). Percentage change in W_b at the end of April, May, and June was calculated by dividing the mass differences between W_b-Final and W_b-Initial by W_b-Initial. W_b was determined by transferring the bird to a cotton bag and attaching it to a spring scale (100 ± 0.1 g, 3B Scientific, Hamburg, Germany).

2.4. Droppings Collection

In each ED group, droppings were collected at the end of both April and June. Pairs in the breeding cages were separated by placing a removable divider 24 h before sampling. All individual fresh droppings that were free of food and feathers were collected in the early morning (06:00–70:00 h) and transferred to 1.5 mL tared Eppendorf tubes, weighted wet, and frozen at −20 °C until later analysis for MLTS concentration [28].

2.5. MLTS Assay

Before assay, samples were thawed at room temperature, and a 0.3 g aliquot of homogenized individual samples was placed in 1.5 mL microcentrifuge tube, mixed with 1 mL 75% ethanol followed by vigorous vortexing for 5 min, and centrifuged for 10 min at 12,000 rpm at 4 °C, and then, the supernatant was aspirated into new vials. The assay was run in duplicates according to manufacturer's protocol (ELISA; IBL, Hamburg, Germany; Cat. No. RE54031). The optical density was measured at 450 nm with 650 nm as a reference wavelength using a microtiter plate ELISA reader (PowerWave HT, Biotek, Winooski, VT, USA). The calculated intra- and inter-assay coefficients for bird droppings were

0.92–3.09 ng/mL (CV: 0.58–13.7%; Mean (n = 96 samples) = 5.53%) and 4.56–197 ng/mL (9.45–19.66%; Mean (n = 5 plates) = 14.6%), respectively. Thus, the calculated intra-assay was below 10%, and the inter-assay was below 15%, which is acceptable [29].

2.6. Statistical Analysis

The statistical effect of increasing ED on W_b and MLTS was assessed using split-plot analysis of variance (SPANOVA) where ED (4 levels) was incorporated as the between-subject factor and time (W_b: 3 levels, MLTS: 2 levels) as the within-subject factor. If SPANOVA detected a significant effect of time and/or interaction, one-way ANOVA (ANOVA) and ANOVA repeated measures (ANOVAR) with Tukey and Bonferroni, respectively, as well as post hoc comparisons were used for testing mean differences between and within groups at different time points. Paired t-test was used to evaluate the differences in mean levels of MLTS over time within each ED. The Pearson correlation test was used to evaluate the relationship between ED, MLTS, and both egg number and percentage of eggs that hatched. Results are presented as mean ± s.e.m. unless otherwise stated. All statistical analyses were set at $p < 0.05$ and conducted using IBM© SPSS© Statistics for Windows, version 21.0.0.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Body Mass Changes

The SPANOVA revealed no significant gender difference in W_b under each ED group (F1,46 = 0.72, p = 0.40). Therefore, data were pooled by gender. ED had a significant effect on W_b (F1,44 = 93.47, p = 0.0001). Significant time (F2,88 = 3.39, p = 0.04) and time × ED (F6,88 = 85.27, p = 0.0001) effects were also detected in response to ED. In comparison with controls (ED = 0 min), birds gained mass with increasing ED with more prevalent effects in June compared with April and May (Figure 1). Under control conditions, birds lost mass with increasing photoperiod (April: −6.77 ± 2.27%, May −12.97 ± 2.28%, and June: −19.63 ± 2.05%), whereas a dose-dependent increase in mass gain was observed with increasing ED and increasing photoperiod (ED = 90 min: April 8.51 ± 2.28%, May 13.40 ± 3.32%, and June 18.57 ± 3.58%).

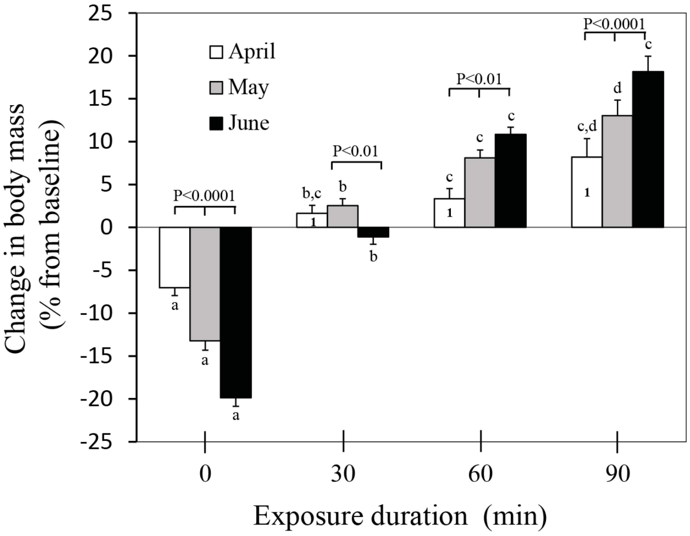

Figure 1. Percentage change in body mass (W_b) of Budgerigars exposed to artificial light at night (ALAN) of four increasing exposure durations (ED) on three successive months. Birds were acclimated to naturally increasing photoperiod from March to June. Each ALAN-ED was administered once daily at the middle of the dark period using bright fluorescent light (60 µW/cm^2 and 460 nm). Results are mean ± s.e.m. of n = 48. Different letters indicate significant statistical differences between ALAN-ED at the same month (Tukey post hoc, p < 0.05). p-values for significant time-related differences within each ED are also depicted (Bonferroni post hoc).

3.2. Reproductive Success

The ANOVA model detected a significant effect of increasing ED on the total number of eggs laid (F3,44 = 26.21, p = 0.0001). The highest number of eggs was laid under control conditions (n = 4 ± 0.39 eggs). The number of eggs laid decreased with increasing ED, showing 2 ± 0.469 eggs and 0.67 ± 0.33 eggs at 30 min and 60 min of ED, respectively. The longest ED (90 min) had the most deleterious effect on the number of eggs laid, as birds did not lay eggs at all (Figure 2). Similarly, hatchability percentage was also affected by increasing ED (F3,44 = 65.45, p = 0.0001). The highest hatching percentage (93.83 ± 2.64%) was obtained at control conditions, while the lowest percentage (0%) was obtained at the longest ED (90 min, Figure 2).

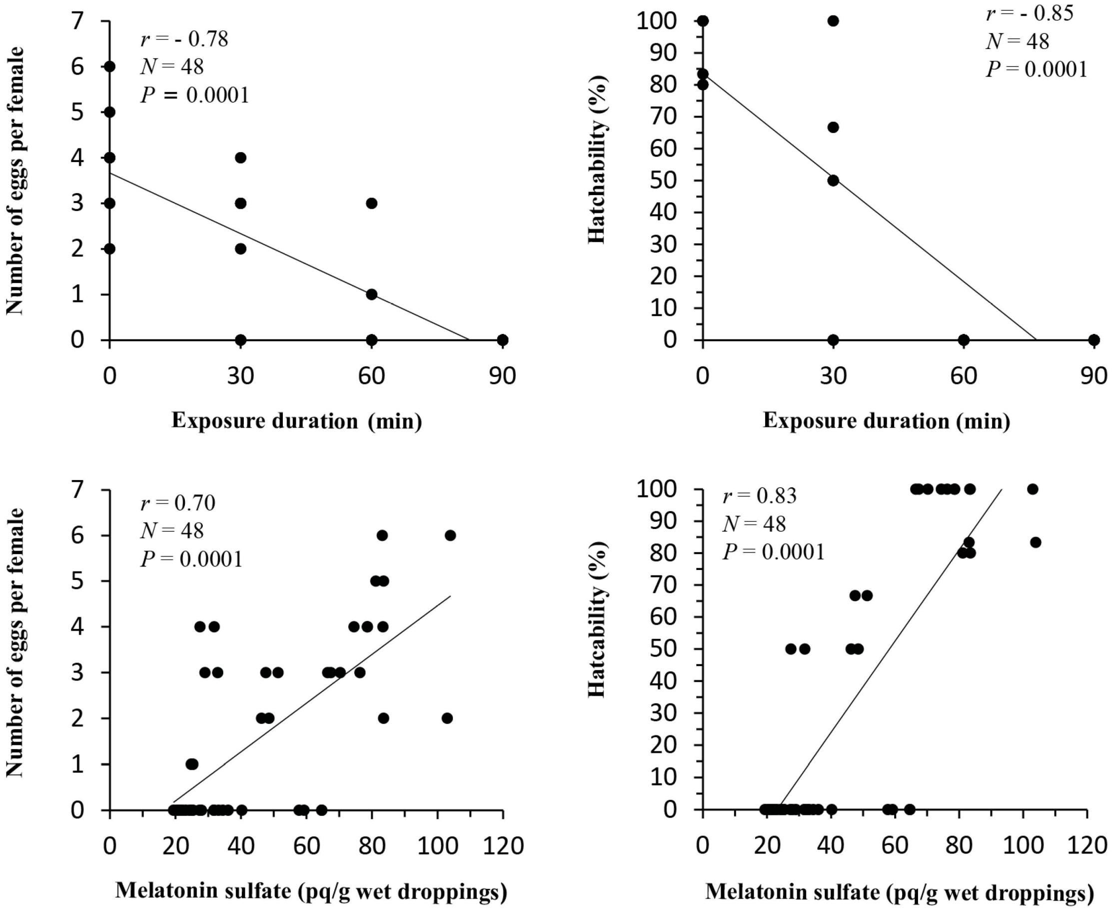

Figure 2. Correlation data showing association between either exposure duration (ED) (upper panel) of artificial light at night or melatonin sulfate (MLTS) (lower panel) and both number of eggs and percentage of eggs that hatched in Budgerigars. Data (n = 48) for each variable at the different ALAN ED were pooled. The Pearson correlation results (r, n, and p-value) are presented for each analysis.

3.3. MLTS Levels

There were no significant gender differences in MLTS concentrations for any ED group in April and June (F1,46 = 0.31, p = 0.58); therefore, data were pooled by gender. The SPANOVA model detected significant time (F1,44 = 10.59, p = 0.002), ED (F1,44 = 158.9.12, p = 0.0001), and time x ED interaction effects (F3,44 = 22.44, p = 0.0001). Similarly, significant time effects were also observed for each ED group.

Increasing ED elicited a significant effect on MLTS concentrations that was manifested in a dose-dependent decrement manner with time-related changes (Figure 3).

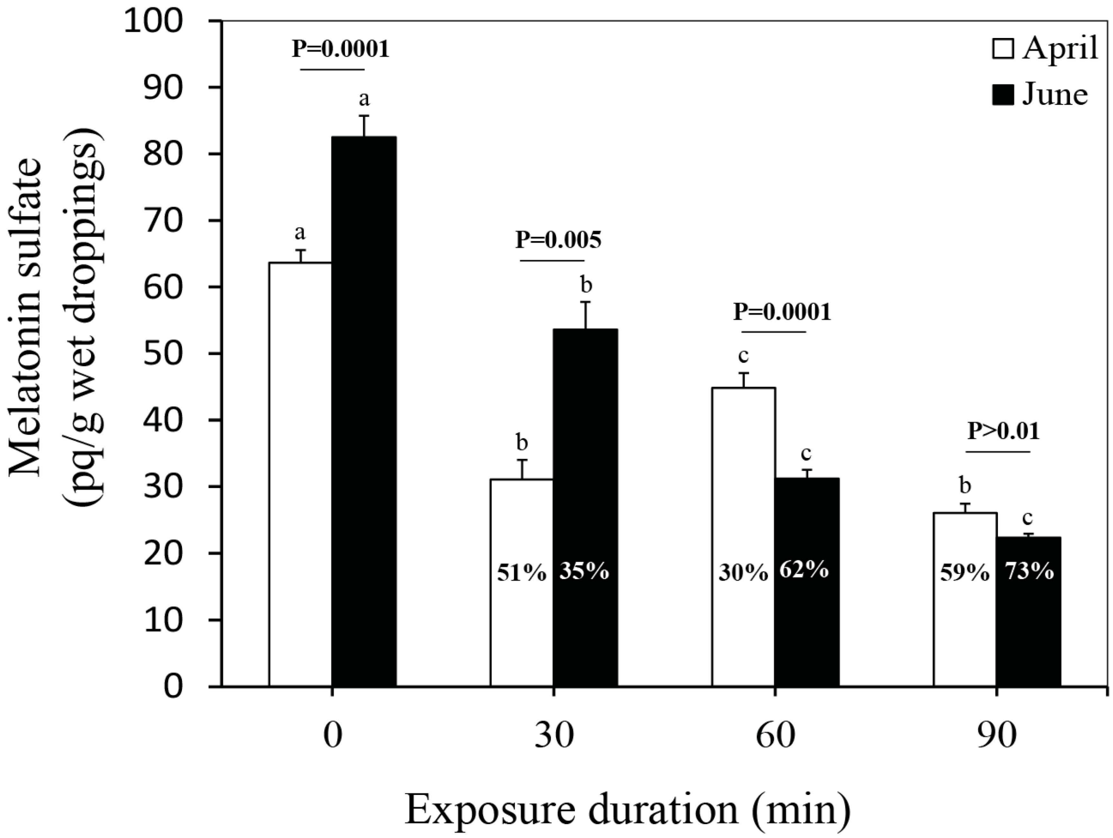

Figure 3. Melatonin sulfate (MLTS) levels of Budgerigars exposed to artificial light at night (ALAN) in four increasing exposure durations (ED) during the two months of April and June. Numbers in column represent the percentage of decrease in MLTS from control levels (ED = 0 min) in the same month. Different letters indicate significant statistical differences between ALAN-ED in the same month (Tukey post hoc, $p < 0.05$). p-values for significant time-related differences within each ED are also depicted (paired t-test).

Under control conditions, the main MLTS concentrations detected at the end of April (63.65 ± 1.87 pg/g wet droppings) were significantly ($t = -6.4$, df = 11, $p = 0.0001$) lower compared with those measured at the end of June (82.50 ± 3.24 pg/g wet droppings). The ED 90 min group showed the largest percent (April 59% and June 73% from control levels under ED 0 min exposure) of MLTS inhibition compared with 30 min (April 51% and June 35%) and 60 min (April 30% and June 62%) conspecifics exposure groups.

3.4. Correlation Analysis

Correlation analyses between increasing ED, MLTS, and reproductive success are presented in Figure 2. A significant negative correlation was detected between increasing ED and both number of eggs laid ($r = -0.78$, $n = 48$, $p = 0.0001$) and hatching percentage ($r = -0.85$, $n = 48$, $p = 0.0001$). Conversely, reproductive success correlated positively with MLTS levels with $r = 0.7$ for the number of eggs laid and $r = 0.83$ for hatching percentage ($n = 48$, $p = 0.0001$). There was a significant positive correlation between increasing ED and percentage change in W_b at the end of April ($R^2 = 0.55$, $p = 0.0001$) and June ($R^2 = 0.89$, $p = 0.0001$, Figure 4). Furthermore, a significant negative correlation was detected between ED and MLTS concentrations at the end of April ($R^2 = 0.47$, $p = 0.0001$) and June ($R^2 = 0.83$, $p = 0.0001$). Finally, the correlation analysis showed a significant negative correlation (Figure 5) between MLTS concentrations and percentage change in W_b at the end of April ($R^2 = -0.45$, $p = 0.0001$) and June ($R^2 = -0.82$, $p = 0.0001$).

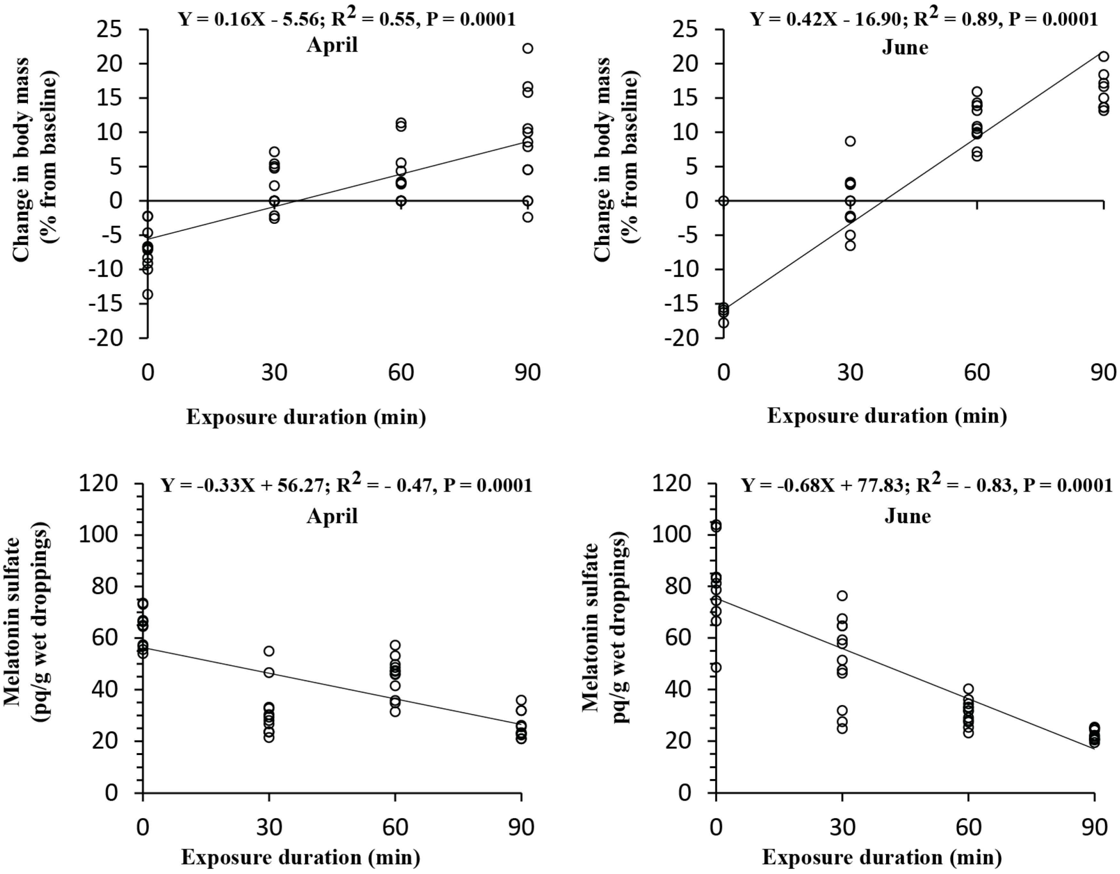

Figure 4. Correlation data showing the association between increasing artificial light at night (ALAN) exposure duration (ED) and both change in body mass W_b (upper panel) and melatonin sulfate (MLTS) levels (lower panel) in Budgerigars. Correlations were imposed on the raw data of $n = 48$ during the corresponding month of measurement. The equation, R^2, and p-value of the simple linear regression are also presented for each analysis.

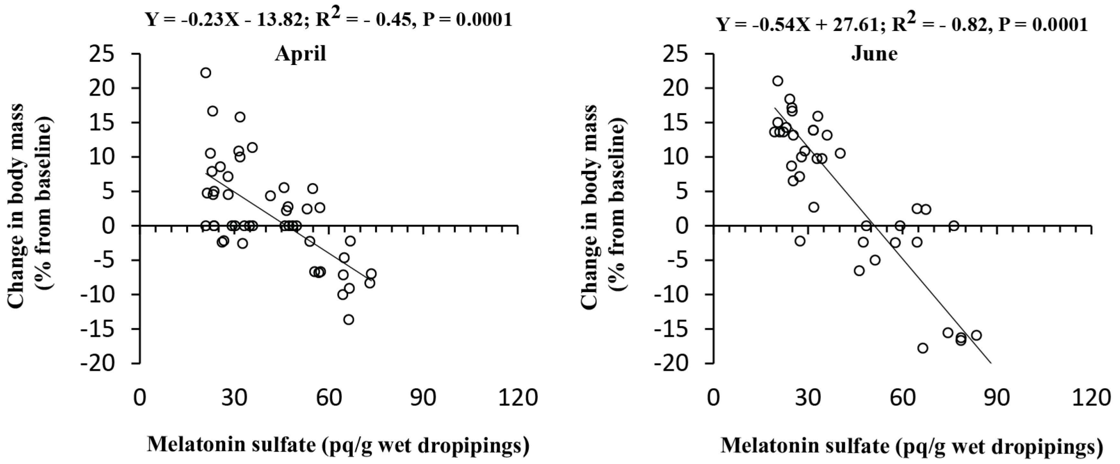

Figure 5. Linear regression modeling the association between melatonin sulfate (MLTS) levels and change in body mass (W_b) in Budgerigars exposed to artificial light at night (ALAN) in four increasing exposure durations (ED) during the two months of April and June. Data ($n = 48$) for each variable at a given month were pooled. The equation, R^2, and p-value of the simple linear regression are also presented for each analysis. There was confusion in Figure legends, I corrected it. There was confusion in Figure legends, I corrected it.

4. Discussion

Using the Budgerigar as a bird model, we showed that ALAN affected the development and reproduction status in an ED dose-dependent manner. Our results demonstrated that ALAN markedly increased W_b, particularly at the longest ED (90 min), compared with control birds who showed marked mass loss. Additionally, the ALAN-induced mass gain was also associated with photoperiod length, with the greatest effect during June measurements compared with April. A similar mass gain in response to ALAN exposure was also demonstrated in mice showing a wavelength dependent with the strongest effect at the SWL of the spectrum [30]. In mice, ALAN exposure can induce mass gain by altering the animal feeding behavior, e.g., shifting the typical timing of food intake [1,17]. In birds, ALAN can extend the bird's activity by advancing activity onset and delaying activity offset in an intensity direct dose-dependent manner [15]. Indeed, ALAN was demonstrated to increase feeding rates in female nesting Great Tits (*Parus major*), particularly when the nestlings were between 9 and 16 days [31]. Accordingly, ALAN may have extended the activity of Budgerigars, leading to increased food intake and substantial mass gain compared with control conspecifics. Conversely, in Great Tits, prolonged ALAN exposure had no effect on W_b after two days compared with unexposed birds [19]. The discrepancy between our results and those of the latter study may possibly have emerged from differences in both methodology and taxa responses to ALAN.

In our study, we discovered that ALAN impaired Budgerigar reproduction success in an ED dose-dependent manner. A significant negative correlation was detected between ALAN, ED, and both number of eggs laid and hatchability percentage, in which birds that were exposed to the longest ED (90 min) failed to commence egg production. Alternatively, ALAN exposures may have affected male vocalization, which is an important factor that may interact synergistically with the photoperiod to modulate breeding behavior in birds including Budgerigars [32]. In female Budgerigars, it was shown the male vocalization may advance ovarian maturation and female nesting behavior [33]. More recently, pineal melatonin was suggested to mediate photoperiodic signals to the high vocal center (HVC) nucleus that is responsible for regulating singing behavior [34]. In the present study, the involvement of male singing behavior was not measured; therefore, future studies should clarify this issue by recording the effect of ALAN exposures on the male vocal behavior and the activity pattern of both genders.

Melatonin suppression by ALAN is a fundamental response across all vertebrates that display an irradiance and wavelength dependence [35,36]. Our results showed that increasing the ED of SWL ALAN (460 nm) elicited a dose-dependent suppression of melatonin, showing 59% and 73% suppression from control levels measured at the end of April and June, respectively. In Great Tits, white LED ALAN provoked an intense dose-dependent suppression of melatonin [15]. ALAN of different intensities decreased summer and winter melatonin levels in a dose-dependent manner in urban and rural European blackbirds (*Turdus merula*), with comparable responses between regions [11]. Melatonin levels in our study demonstrated a close and positive correlation with reproductive success, suggesting a role for the pineal hormone in mediating the adverse effects of ALAN exposure in Budgerigars. Accordingly, future studies are encouraged to examine whether exogenous melatonin during the ALAN-interrupted scotophase can return the modified biological functions to control levels.

Indeed, ALAN exposures of different ED, intensity, and spectral composition have been demonstrated to elicit robust stress responses in the HPA axis in both wild and laboratory rodents [3,30]. In Zebra Finches (*Taeniopygia guttata*), 5000 K LED ALAN illumination increased nocturnal activity and glucocorticoid levels relative to control birds [37]. Additionally, ALAN can inhibit the release of reproductive hormones in European blackbirds by triggering stress responses that excrete negative feedback on the HPA axis [38]. Stress responses play a pivotal role in preparing the animal to adapt its behavior and physiology in response to environmental changes [39]. While acute stress may coordinate enhanced physiological responses, chronic activation (e.g., by ALAN) of this survival axis is known to elicit maladaptive responses, including metabolic and reproductive disorders [40]. The impact of ALAN on stress responses was not evaluated for Budgerigars; therefore, this issue needs to be confirmed by a further study.

5. Conclusions

Exposure to ALAN is increasingly concurrent with urbanization as well as ecological function and public health risks. In this study, we demonstrated a robust correlation between SWL ALAN of increasing ED and impaired growth regulation and reproductive success in Budgerigars. ED suppressed melatonin levels in a dose-dependent manner, which in turn showed a strong correlation with the observed impaired functions. Therefore, our results suggest that the ALAN-induced adverse effects in Budgerigars are at least partly mediated by melatonin suppression. However, considering the complexity of the avian photoperiodic system comprised of multi-photoreceptors and multi-oscillators, it is possible that ALAN impacts are mediated by other components and not exclusively by the melatonin transduction pathway. Further direct studies are warranted for eliciting the differential role of these components in mediating ALAN effects, of which the chronic stress-related axis is of particular interest, as ALAN has become a defining feature affecting natural ecological systems and public health. Accordingly, effective measures should be considered to mitigate light pollution, including public behavioral change, reducing lights use and expansion in ecosystems, enhancing lightening standards, and above all encouraging the development of new light technologies that do not emit SWL under 500 nm [41]. Nonetheless, the observed adverse effects of ALAN in our study may be superlative in natural habitats since ALAN exposures are of much lower irradiance and frequency compared with those used in our study. The irradiance and spectral composition threshold sensitivity of the circadian system in Budgerigars will be evaluated in future studies.

Author Contributions: The author A.H. has made a significant contribution to this manuscript in planning the study, collecting the data sources, analyzing the results, and assisting in the writing of the manuscript; the author M.I. has made a significant contribution to this manuscript in planning the research, setting up the research system, performing the experiments in the laboratory, collecting the sources of information, analyzing the results, and writing the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki. All experiment procedures have been conducted with approval from the Ethics and Animal Care Committee of the University of Haifa (Protocol number: 548/18).

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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