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ORIGINAL PAPER

Light at night reduces digestive efficiency of developing birds: an experiment with king quail

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Abstract

Artificial light at night (ALAN) exposes animals to a novel environmental stimulus, one that is generally thought to be maladaptive. ALAN-related health problems have received little attention in non-model species, and we generally know little about the nutritional-physiological impacts of ALAN, especially in young animals. Here, we use a novel application of the acid steatocrit method to experimentally assess changes in digestive efficiency of growing king quail (*Excalfactoria chinensis*) in response to ALAN. Two weeks after hatching, quail were split into two groups ($n = 20–21$ per group): overnight-light-treated vs. overnight-dark-treated. When the chicks were 3 weeks old, the experimental group was exposed to weak blue light (ca. 0.3 lux) throughout the entire night for 6 consecutive weeks, until all the chicks had achieved sexual maturation. Fecal samples for assessing digestive efficiency were collected every week. We found that digestive efficiency of quail was reduced by ALAN at two time points from weeks 4 to 9 after hatching (quail reach adulthood by week 9). The negative effect of ALAN on digestion coincided with the period of fastest skeletal growth, which suggests that ALAN may reduce digestive efficiency when energetic demands of growth are at their highest. Interestingly, growth rate was not influenced by ALAN. This suggests that either the negative physiological impacts of ALAN may be concealed when food is provided *ad libitum*, the observed changes in digestive efficiency were too small to affect growth or condition, or that ALAN-exposed birds had reduced energy expenditure. Our results illustrate that the health impacts of ALAN on wild animals should not be restricted to traditional markers like body mass or growth rate, but instead on a wide array of integrated physiological traits.

Keywords Steatocrit · Light pollution · Development · Avian · Digestion · *Excalfactoria chinensis*

Introduction

As a result of anthropogenic activities, natural nighttime darkness has disappeared across much of the world (Falchi et al. 2016). Since organisms have evolved under a natural light-dark cycle with very low levels of night light, the addition of artificial light at night (ALAN) is generally thought to have harmful impacts. Evidence for the negative effects of ALAN on many species is accumulating (Rich and Longcore 2007; Alaasam et al. 2018; Svechkina et al. 2020). In humans, ALAN exposure has been linked to the global increase in the prevalence of obesity and metabolic disorders (Cho et al. 2015; Rybnikova et al. 2016), and similar changes have been shown in laboratory rodents (Fonken and Nelson 2014). However, to date, these ALAN-related health problems have received little attention outside model animal species (Dominoni et al. 2015, 2016). In wild animals, light pollution is associated with changes in circadian behavior, reproduction, and predator-prey interactions, but comparatively less is known about the physiological mechanisms underlying these changes (Dominoni et al. 2016).

Birds have become a popular study system for urban ecologists and are one of the most studied animal taxa in the context of ALAN (Dominoni et al. 2016). However, our

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understanding of the effects of light pollution on the biological rhythms of non-model avian species is mostly limited to behavioral responses (Dominoni et al. 2016, but see Masís-Vargas et al. 2019). Many bird species seem to prefer nesting places that are close to artificial light sources, either to simplify provisioning or to increase predator detection/evasion (Podkowa and Surmacki 2017; Welbers et al. 2017; Ulgezen et al. 2019). Impacts of ALAN may be especially relevant when the organism is exposed in early life, since the immature circadian system may be especially sensitive to rhythm disruptions through artificial light (Fonken and Nelson 2020). Increased light exposure during development has been suggested to accelerate embryo development for both wild species and the domestic hen (*Gallus gallus domesticus*), as well as increase nestling growth rate (reviewed in Podkowa and Surmacki 2017). In poultry, an extended photoperiod is generally associated with faster growth and weight gain (Ingram et al. 2000), possibly due to the increased overnight time when animals can feed.

Despite longer feeding times, studies in several species still indicate that ALAN can have adverse effects on growing birds (Raap et al. 2016a, b, 2018; Salmón et al. 2016). Faster growth carries the cost of metabolic disorders and increased morbidity (Baghbanzadeh and Decuypere 2008; Olanrewaju et al. 2015; Schwean-Lardner et al. 2013), both of which are related to increased food intake and lower nutrient digestibility (Yang et al. 2015). For example, in maturing broiler chickens, a very short or absent dark period resulted in decreased abdominal adipose tissue (Yang et al. 2015). This suggests that the time available for feeding is not the only factor that mediates relationships between growth and photoperiod and that physiological factors like digestive efficiency may also play a role. In wild birds, most studies on the health effects of ALAN on young animals have been conducted on great tits (*Parus major*), and these studies have indicated that even short-term ALAN exposure can affect weight gain (Raap et al. 2016a), increase physiological and oxidative stress levels, and reduce melatonin secretion (Raap et al. 2016b). In altricial birds, such as great tits, the relationship between nestling growth and ALAN exposure may be largely mediated by parental provisioning behavior (Welbers et al. 2017). In precocial birds, direct effects of ALAN on growing individuals can be assessed, as they feed on their own. More studies in a wider variety of bird species are therefore needed, targeting proximate mechanisms that might mediate links between ALAN exposure, growth, and nutritional physiology during development.

A better understanding of the effect of ALAN on digestive efficiency could lead to an improved understanding of why growing birds that have more time for feeding still often show signs of poor nutritional state and health. Digestive efficiency affects an animal's ability to absorb energy and nutrients from food, and poor digestion poses a major physiological constraint that affects the amount of resources available for allocation to maintenance, growth, signaling, and reproduction (Meitern et al. 2016). Recently, a non-invasive method for quantifying digestive efficiency by measuring the percentage of fat in fecal samples (i.e., acid steatocrit method; Phuapradit et al. 1981) has been adapted for use in birds (Meitern et al. 2016). This method has been successfully applied in wild birds for assessing the link between digestive efficiency and plumage coloration (Madonia et al. 2017), as well as intestinal parasite infection (Meitern et al. 2016). Here, we apply this method for the first time to study the effect of ALAN on the digestive efficiency of growing birds. While the effects of ALAN on food intake have been studied before (e.g. Ingram et al. 2000, Fonken et al. 2010), digestive efficiency is an important dimension that has not been examined. We experimentally exposed young king quail (*Excalfactoria chinensis*) to nighttime lighting and, in comparison to a control group, assessed weekly changes in digestive efficiency in relation to metrics of growth and body condition. We hypothesized that the adverse effects of ALAN on growing birds shown in studies of wild birds and poultry (e.g., Yang et al. 2015; Raap et al. 2016a) would be, in part, mediated by lower digestive efficiency. We thereby predicted that ALAN would reduce the digestive efficiency of growing king quail. Additionally, we predicted that lower digestive efficiency might be related to slower growth and weight gain and to lower body condition. Alternatively, growth might be faster in ALAN-exposed birds, if the negative physiological effects of ALAN are compensated by the longer time available for feeding, as has been shown in studies in poultry (Ingram et al. 2000), or by reduced energy expenditure in ALAN-exposed birds (Welbers et al. 2017).

Methods

We artificially incubated and hatched 41 king quail eggs (details of the hatching procedure and housing are described in Saini et al. 2019). Quail were given *ad libitum* access to water and food (Gamebird Starter Crumble, Purina, St. Louis, MO), which consisted of a minimum of 2.5% crude fat. Two weeks after hatching, quail were split into two groups ($n = 20$–21 per group): overnight-light-treated vs. overnight-dark-treated. Within each group, quail were housed in random-sex pairs (because we did not know hatchling sex at two weeks of age) in large cages (dimensions: 38 cm L × 46 cm W × 46 cm H). Steatocrit values were not affected by cage identity (Table S1 in Online resource 1). Quail were split between treatment groups evenly from our two brooders and alternated based on hatch order to mitigate any possible confounds of group size, brooder, or hatch order. The experimental group was exposed to weak blue light (ca. 0.3 lux, measured with a light meter inside the cage with probe facing the light; Traceable Products, Galveston, TX, USA, spectral data

in Figure S1) throughout the entire night (18 h light/6 h dark; chosen for optimal growth and survival of hatchlings, see Landry 2015). We chose this weak-intensity night-lighting based on estimates of ALAN exposure in wild and free-flying birds (Dominoni et al. 2013). We used blue light because of its dominance in natural moonlight, its increasing incorporation into artificial nightlight sources (Gaston et al. 2013), and its specific neurophysiological effects (i.e., absorption by non-visual opsins in retina and brain; Ouyang et al. 2018).

The nightlight manipulation began 19–23 days (depending on individual hatch date) after hatch for the full group of birds (Figure S2). The blue light treatment shone directionally at the cages (distance approximately 0.3 m) for the overnight-light-treated group, though reflection on walls and other surfaces provided diffuse, omnidirectional exposure. The control group had the same night-lighting structure setup in their housing room, but the lights were not turned on. Day lighting was solely supplied in the form of bright overhead white fluorescent bulbs (~ 100 lux; GE Landing, Ashland, OH). Housing rooms were window-less, so there was never any solar illumination. At the end of the study, all birds were euthanized (anesthesia with CO_2 followed by decapitation, following the protocol approved by the Arizona State University's Institutional Animal Care and Use Committee) to confirm sex based on gonads and collect tissues for other ongoing projects.

The experimental treatment lasted for 6 weeks (subsequently noted as week 4 to week 9 of the birds' life), which was sufficient for all birds to complete skeletal growth and reach sexual maturity (Landry 2015). Each week, we measured body mass (in grams, using a digital scale) and tarsus length (in millimeters, using calipers) to assess growth rate, and we took fecal samples for assessing digestive efficiency. Body condition index was calculated as residuals from a least-squares linear regression analysis between weekly values of body mass (dependent variable) and tarsus length (Schulte-Hostedde et al. 2005). Analyses for body condition were run separately for males and females due to the larger body mass of females at the end of the growth period.

Fecal samples were collected every week at the same time in early mornings, while handling the birds during the weekly measuring and blood sampling (for another study) procedure. Mass of collected feces differed for birds. Samples were collected in small closed plastic tubes and stored at 4 °C until we analyzed them within a month after collection. Steatocrit was measured according to Meitern et al. (2016). Briefly, we diluted bird droppings with deionized water (1:3) and homogenized them. Perchloric acid (5 M) was added in volume 1:5 to the homogenate, which was subsequently vortexed, collected into the hematocrit capillary tube, and centrifuged at 13,000 rpm for 15 min. The capillary tubes were photographed (Canon EOS 450D), and the length in pixels was quantified for the upper fat layer (FL) and solid bottom layer (SL) from the photographs using ImageJ software. Steatocrit was expressed as a fraction of fat in the non-aqueous matter of the sample; in other words, the length of a fat layer divided by the sum of the lengths of fat layer and solid layer. Repeatability (Lessells and Boag 1987) of steatocrit measurement (based on 10 samples analyzed in duplicate) was high ($r = 0.90$, $F_{1,9} = 15.2$, $p < 0.0001$).

Statistical analyses were performed with STATISTICA software (v. 10, StatSoft, Inc. 2011; www.statsoft.com). All dependent variables used in the analyses (steatocrit, tarsus length, body mass) were approximately normally distributed (based on visual inspection of the histograms, Kolmogorov-Smirnov test), fulfilling the models' assumptions. We analyzed the effect of light treatment on digestive efficiency using repeated measures analysis of variance (rmANOVA), using time, sex, treatment, and their interactions as predictors. We subsequently assessed treatment effects at different time points using parametric *t* tests. To improve our statistical power and because the influence of ALAN on steatocrit did not differ between the sexes (Table 1), we combined the data for males and females at each time point for our *t* tests. We used general linear models to analyze associations between digestive efficiency and growth parameters at separate time points, using treatment, sex, and their interaction as predictors. This study was carried out with the approval of Arizona State University's Institutional Animal Care and Use Committee and complies with the National Institutes of Health guidelines for the care and use of laboratory animals.

Results

ALAN exposure reduced digestive efficiency in growing king quail (Table 1, Fig. 1). When looking at each week

Table 1 Results of repeated measures ANOVA showing the effect of artificial light at night (ALAN) exposure on digestive efficiency of developing king quail (*Excalfactoria chinensis*)

Effect	df	*F*	*p*	η^2
ALAN	1,21	5.92	**0.024**	0.22
Sex	1,21	2.21	0.152	0.10
ALAN * sex	1,21	0.01	0.926	< 0.001
Time	5,105	4.63	**0.0007**	0.18
Time * sex	5,105	1.13	0.347	0.05
Time * ALAN	5,105	2.66	**0.026**	0.11
Time * ALAN * sex	5,105	0.83	0.529	0.05

Sample size 41 (20–21 per group). "Time" indicates weekly repeated measures from weeks 4–9 of the chicks' life. *df* degrees of freedom; η^2 effect size; * indicates interaction between variables. Boldfaced *p* values denote statistically significant results (i.e., $p < 0.05$)

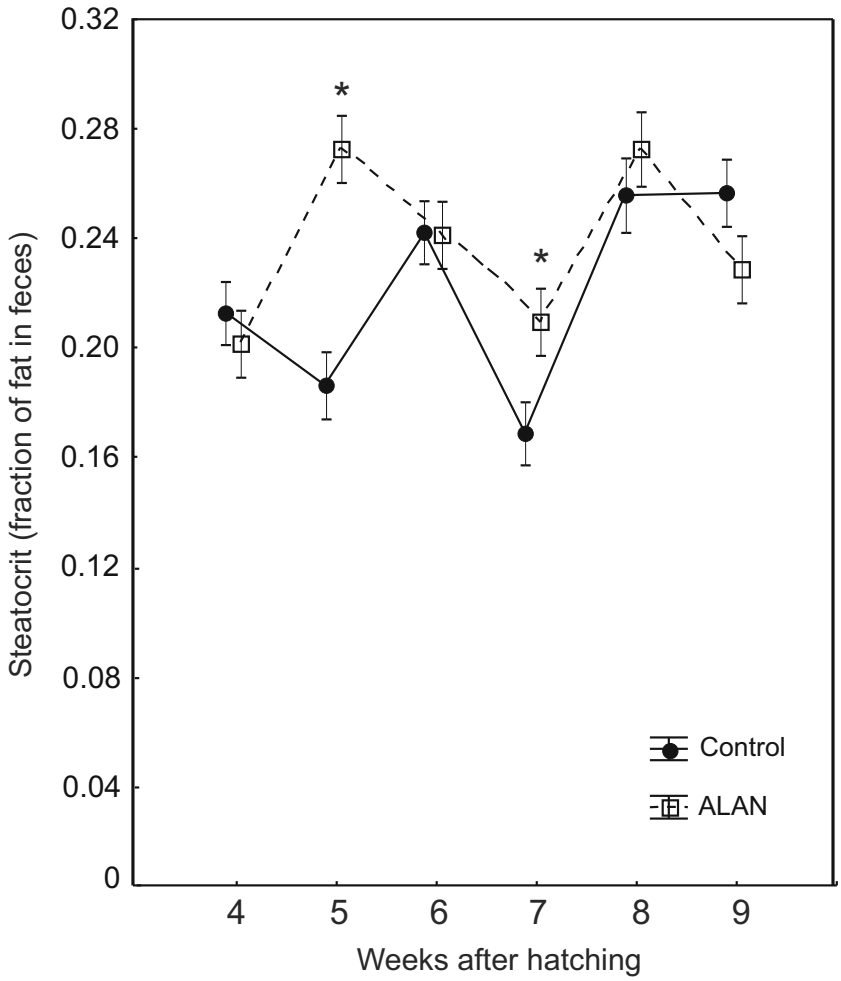

Fig. 1 Digestive efficiency (steatocrit; high values indicate low efficiency) differed between control ($n = 20$) and artificial light at night (ALAN)-exposed ($n = 21$) king quail (*Excalfactoria chinensis*) on weeks 4–9 after hatching. Mean ± standard errors for two groups are shown for each week. Asterisks indicate weeks where group differences were statistically significant

independently, digestive efficiency was significantly lower in ALAN-exposed birds than control birds in weeks 5 and 7 after hatching (Table 2, Fig. 1). This was also the period of fastest chick growth (Table 3), although exposure to ALAN did not affect growth parameters (body mass, tarsus length, body condition; Tables 4 and 5, Fig. 2). When examined week-by-week, steatocrit values did not predict body mass, tarsus length, or body condition on corresponding weeks, nor the growth rates between weeks (Table 6). However, on the week of fastest growth (i.e., week 5 after hatching), the association between steatocrit and tarsus length was nearly significant in the ALAN-exposed group (Fig. 3). All regressions between mass and tarsus except for week 9 were statistically significant ($p < 0.014$, $R^2 > 0.15$) and positive.

Table 2 Results of parametric *t* tests showing the effect of artificial light at night (ALAN) exposure on digestive efficiency (steatocrit) of growing king quail (*Excalfactoria chinensis*) from weeks 4–9 after hatching

Growth period	Control group mean ± SE (*N*, df)	ALAN group mean ± SE (*N*, df)	*t* value	*p*
Week 4	0.216 ± 0.011 (19, 35)	0.207 ± 0.015 (18, 35)	0.46	0.646
Week 5	0.192 ± 0.015 (19, 32)	0.268 ± 0.016 (15,32)	− 3.48	**0.001**
Week 6	0.243 ± 0.012 (20, 34)	0.237 ± 0.012 (16, 34)	0.37	0.715
Week 7	0.178 ± 0.010 (20, 36)	0.217 ± 0.016 (18,36)	− 2.13	**0.040**
Week 8	0.247 ± 0.018 (19, 36)	0.254 ± 0.017 (19,36)	− 0.30	0.763
Week 9	0.253 ± 0.015 (17, 33)	0.221 ± 0.015 (18, 33)	1.51	0.139

Sample sizes vary among weeks due to our inability to collect samples from all individuals during each sampling. N, sample size; *df* degrees of freedom, *SE* standard error. Boldfaced *p* values denote statistically significant results (i.e., $p < 0.05$)

Table 3 Growth rate (grams per week for body mass, mm per week for tarsus) of growing king quail (*Excalfactoria chinensis*) during the experimental period (when half of the birds were exposed to light at night)

Growth period	Sample size	Mean change in mass (g/week) ± SE	Mass growth rate compared to prior week		Mean change in tarsus length (mm/week) ± SE	Tarsus growth rate compared to prior week	
			t value	*p*		*t* value	*p*
Week 4–5	41	5.87 ± 0.25	–	–	0.52 ± 0.06	–	–
Week 5–6	41	3.04 ± 0.19	10.15	**< 0.0001**	0.11 ± 0.03	6.55	**< 0.0001**
Week 6–7	40	2.77 ± 0.29	0.78	0.442	0.003 ± 0.04	2.00	0.052
Week 7–8	39	2.56 ± 0.49	0.54	0.595	− 0.05 ± 0.03	0.80	0.429
Week 8–9	39	3.90 ± 0.83	−1.40	0.170	− 0.007 ± 0.04	−0.68	0.502

t tests were used to compare changes in growth rates between weeks. *SE* standard error. Boldfaced *p* values denote statistically significant results (i.e., $p < 0.05$)

Discussion

We found that growing king quail experimentally exposed to ALAN showed an impaired digestive efficiency compared to control birds. We found that this effect was only apparent at specific and potentially key life stages, coinciding with a period of rapid skeletal growth in the quail. At the same time, body mass, growth, and body condition were not affected by ALAN exposure, and birds with lower digestive efficiency did not gain less weight. This suggests that, although the physiology of developing birds was affected by ALAN, these effects might be either compensated for or concealed by growth achieved by longer overnight time available for feeding.

While large fluctuations in steatocrit values were observed between weeks, independent of the treatment, steatocrit values were lower in ALAN-exposed birds at two time points out of six during the developmental period. These included weeks 5 and 7 of life (note that treatment started at week 4). For the first week of treatment (i.e., week 4 of life), it is possible that the effect of ALAN on digestion had not yet manifested, potentially because, in the short-term, melatonin can buffer the adverse effects of bright light at night on captive birds (Malek et al. 2020). While it is difficult to explain why the effect of ALAN on digestive efficiency varied among the weeks during development, some general patterns can be suggested. First, quail skeletal growth was fastest during one of these time points: the second week of the treatment (week 5 of life). This possibly led to higher demands on energy consumption and metabolic efficiency. After week 6 of life, the birds continued to gain some weight, though skeletal growth seemed to be completed. In addition, it has been shown that younger quail with smaller body size have higher resting heart rates, which are correlated with the higher metabolic demands of thermoregulation at small body masses (Pearson et al. 1998), leading to additional pressure on metabolic efficiency during growth. During

Table 4 Repeated measures ANOVA table showing the effect of exposure to artificial light at night (ALAN) on body mass and tarsus length of growing king quail (*Excalfactoria chinensis*)

	Body mass				Tarsus length			
Effect	df	*F*	*p*	η^2	df	*F*	*p*	η^2
ALAN	1,35	0.015	0.904	0.0004	1,35	0.06	0.815	0.002
Sex	1,35	11.04	**0.002**	0.24	1,35	0.05	0.821	0.001
ALAN * sex	1,35	0.06	0.804	0.002	1,35	0.05	0.818	0.002
Time	5,175	195.56	**< 0.0001**	0.85	5,175	41.06	**< 0.0001**	0.54
Time * ALAN	5,175	0.76	0.582	0.02	5,175	0.36	0.88	0.01
Time * sex	5,175	18.56	**< 0.0001**	0.35	5,175	1.83	0.11	0.05
Time * ALAN * sex	5,175	0.22	0.954	0.006	5,175	1.19	0.32	0.03

"Time" indicates weekly repeated measures from weeks 4–9 of the chicks' life. *df* degrees of freedom; η^2 effect size; * indicates interaction between variables. Boldfaced *p* values denote statistically significant results (i.e., $p < 0.05$).

Table 5 Repeated measures ANOVA table showing the effect of artificial light at night (ALAN) exposure on body condition of growing female and male king quail (*Excalfactoria chinensis*)

	Females				Males			
Effect	df	*F*	*p*	η^2	df	*F*	*p*	η^2
ALAN	1,20	0.24	0.628	0.01	1,15	0.003	0.958	0.0002
Time	5,100	4.41	**0.001**	0.18	5,75	56.95	**< 0.0001**	0.79
Time * ALAN	5,100	0.51	0.765	0.03	5,75	0.16	0.98	0.01

df degrees of freedom; η^2 effect size; * indicates significant interaction between variables. Boldfaced *p* values denote statistically significant results (i.e., $p < 0.05$)

this demanding period, the addition of stress caused by ALAN could have led to lower digestive efficiency. For example, it has been shown in broiler chickens that, compared to continuous lighting, intermittent lighting schedules improve feed conversion and metabolism (Apeldoorn et al. 1999).

We also found that the negative effects of ALAN on digestive efficiency did not affect growth or weight gain. This may be because food was provided *ad libitum*. However, since our study design lacked a measure of food intake, we cannot confirm if the lowered digestive efficiency was indeed compensated by increased feeding in ALAN-exposed birds. Nevertheless, studies from the broiler chicken industry have shown that, although average daily feed intake of broilers in intermittent lighting was significantly less than for broilers in continuous lighting conditions, feed conversion of broilers raised under a 4L:4D photoperiod was significantly more efficient than broilers in continuous lighting (Yang et al. 2015). It is also possible that differences in digestive efficiency were too small to affect growth or condition or that quail exposed to ALAN slept more during the day and thus spent less energy, which compensated for their lower digestion efficiency in terms of growth. In wild great tits, daily energy expenditure is indeed lower in birds exposed to ALAN (Welbers et al. 2017). It is known that ALAN may cause other physiological changes, mainly by suppression of melatonin rhythmicity and metabolic function (Rybnikova et al. 2016). Receptors for melatonin have been identified in the digestive system, indicating that melatonin may have a role in digestive physiopathology (Motilva et al. 2001). Though we did not measure melatonin levels in our study, we still expect this hormone to be a mediator of ALAN's effects, because ALAN has been shown to suppress melatonin levels (Dominoni et al. 2013). Future studies experimentally manipulating melatonin levels and measuring digestive efficiency should clarify the role of this mechanism in birds.

Another possible mediator between ALAN and feeding control mechanisms is the disruption of the neuroendocrine system in the developing brain. A recent study in domestic chicks showed that circadian disruption by ALAN affected brain development through changes in pineal steroid hormone activity during early life (Haraguchi et al. 2019). Accordingly, impacts of ALAN on animal health should not be assessed solely based on traditional morphological markers like body mass, growth rate, or body condition but include direct physiological metrics like hormone levels, nutrient deficiencies, or immune status. As an example, we have previously shown that exposure to ALAN increased the bactericidal capacity of plasma in growing king quail (Saini et al. 2019), which could be linked to increased inflammation in the possibly sleep-deprived animals, but this effect could have also been a reflection of increased immune investment early in life. In addition, recent studies have indicated that immunity is suppressed in ALAN-exposed zebra finches (*Taeniopygia guttata*, Mishra et al. 2019) and Australian black field crickets (*Teleogryllus commodus*, Durrant et al. 2019).

Given the global extent of artificial light pollution (affecting nearly 40% of the terrestrial area; Falchi et al. 2016), large numbers of land animals are exposed to ALAN. If, as we found in this study, wild animals also experience ALAN-induced reductions in fat digestive efficiency, and if these effects of ALAN on digestive efficiency are substantial enough to have consequences on bird fitness, ALAN may affect the foraging ecology of wild animal communities, especially when food is not freely and continuously available. ALAN-exposed animals may have to extend their foraging efforts to meet energetic demands, especially during development and growth. Clearly, extended foraging has been observed in ALAN-affected animals, though it is often attributed to the direct and circadian effects of light on activity levels (Dominoni et al. 2016). More work is required to disentangle the contribution of environmental and physiological mechanisms to increased activity in ALAN-affected organisms. More broadly, ALAN-induced reductions in fat digestive efficiency could affect community trophic interactions, further disrupting populations and communities globally (Sanders and Gaston 2018).

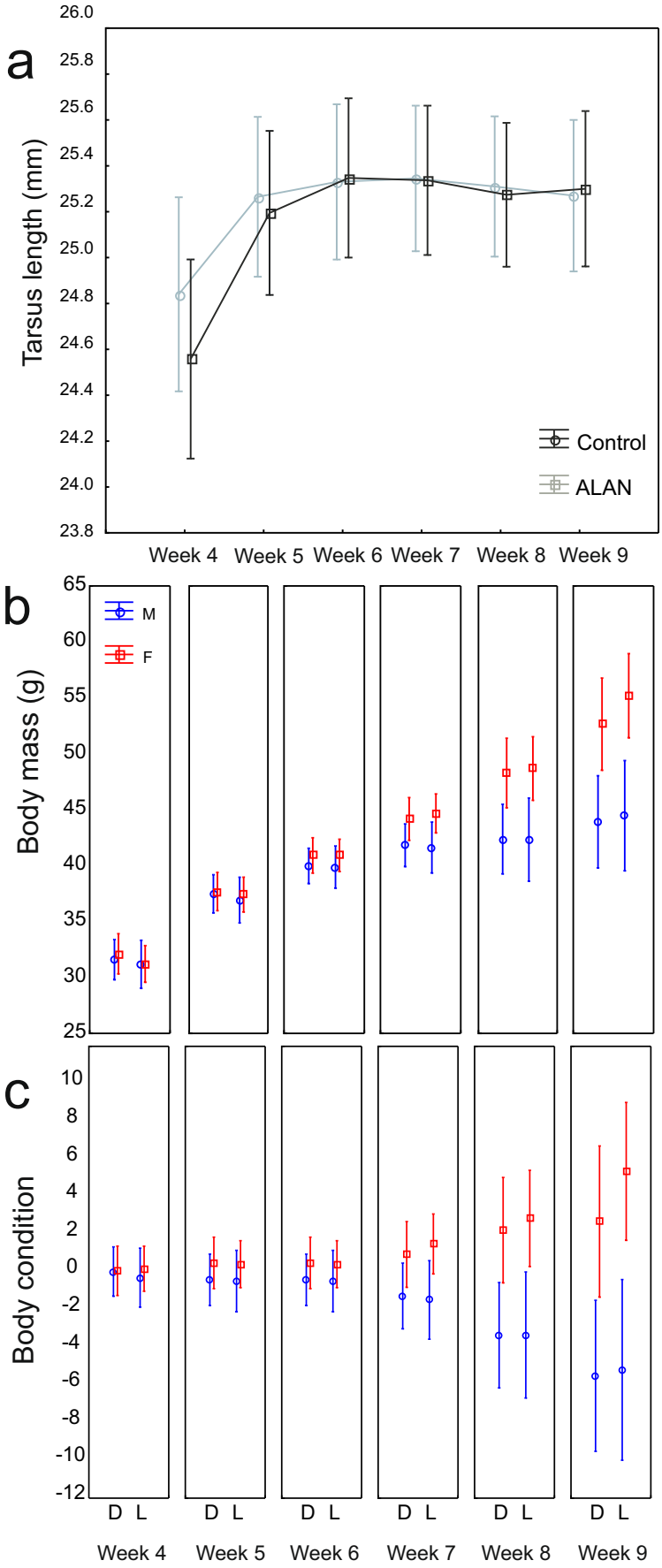

Fig. 2 Artificial light at night (ALAN) treatment did not affect tarsus length (**a**), body mass (**b**), or body condition (**c**) in king quail (*Excalfactoria chinensis*), but females gained more weight during development than males. No differences between sexes were seen in tarsus length. Due to faster weight gain, females had higher values for body condition than males. Means and standard errors for each group are shown. Control male $n = 10$, control female $n = 10$, experimental male $n = 9$, experimental female $n = 12$

Table 6 Association between the weekly values of steatocrit and other growth and condition parameters (general linear models) in king quail (*Excalfactoria chinensis*)

Week	Body mass (g)			Tarsus length (mm)			Body condition			Change of body mass			Change of tarsus length		
	F	df	*p*	*F*	df	*p*	*F*	df	*p*	*F*	df	*p*	*F*	df	*p*
4	0.01	1,35	0.914	0.10	1,35	0.749	0.21	1,35	0.651	–	–	–	–	–	–
5	0.27	1,32	0.607	0.19	1,32	0.676	0.28	1,32	0.597	1.57	1,32	0.220	0.41	1,32	0.524
6	1.86	1,34	0.181	0.71	1,34	0.405	1.08	1,34	0.305	0.005	1,43	0.943	2.44	1,34	0.127
7	0.13	1,32	0.724	0.75	1,36	0.391	0.05	1,32	0.83	0.0007	1,34	0.979	–	–	–
Sex:	6.69		**0.014**				8.60		**0.006**	9.29		**0.004**			
8	0.0002	1,35	0.990	1.96	1,36	0.170	0.46	1,35	0.502	0.36	1,35	0.554	–	–	–
Sex:	14.87		**0.0005**				17.15		**0.0002**	16.41		**0.0002**			
9	0.13	1,32	0.716	0.37	1,33	0.550	0.03	1,32	0.868	2.97	1,33	0.09	–	–	–
Sex:	11.67		**0.001**				12.39		**0.001**						

Sex and treatment (light at night vs. control) were used as co-factors in initial general linear models but only kept in final models if significant. Since tarsus length did not change after week 6, results are given only for the first 2 weeks of the experiment. *df* degrees of freedom. Boldfaced *p* values denote statistically significant results (i.e., $p < 0.05$)

To conclude, we have shown for the first time that ALAN may affect the ability of growing birds to absorb nutrients from their food. Although growth rate might not be affected when food is provided *ad libitum*, additional food might not always be available in ALAN-exposed wild populations due to resource limitations, and, when possible, additional intake of calories might lead to unhealthy weight gain. In addition to increased food consumption, alterations in metabolism or changes in overall activity could also account for the missing effect of ALAN-lowered digestion efficiency on chick growth. To better understand the biological importance of our findings and the observed variation in steatocrit levels, more studies are needed that investigate the links between steatocrit and changes in nutrient absorption in birds, but also between steatocrit and other physiological, morphological, and behavioral variables. Another avenue for future research would be to explore whether there are physiological consequences of reduced digestive efficiency under ALAN, if food is not provided *ad libitum*. This could be achieved with experimental studies that include monitoring food intake and/or removing food overnight. Since the steatocrit method for assessing digestion efficiency is cheap and easily applicable in natural conditions, we recommend the inclusion of this measurement in future studies assessing the impact of ALAN exposure on wild birds.

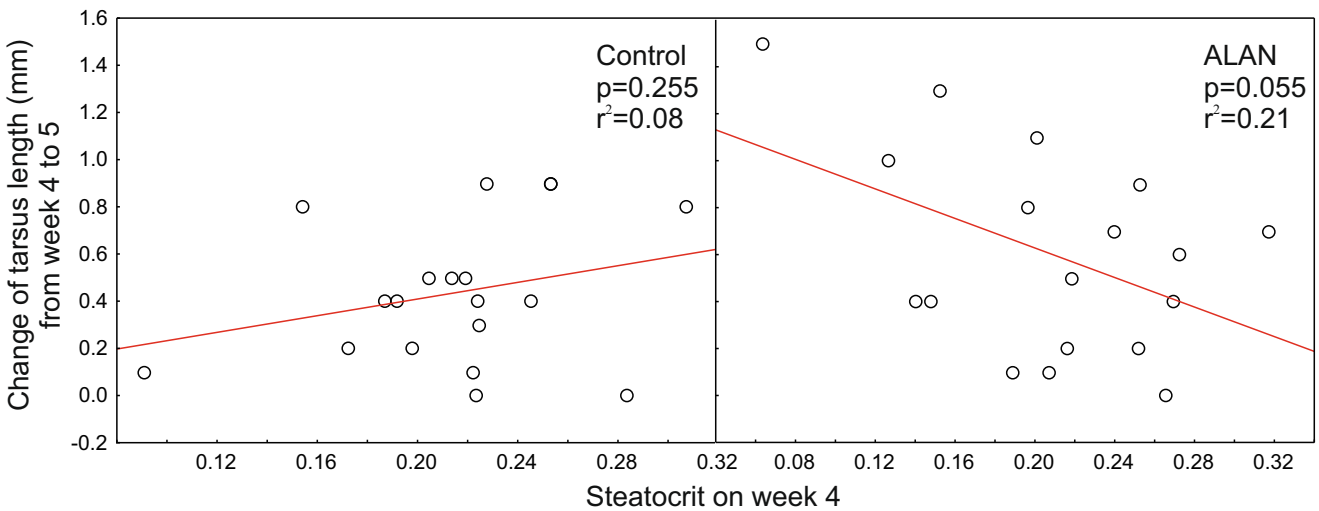

Fig. 3 Relationship between steatocrit and change of tarsus length on the period of fastest growth (weeks 4 to 5) in control and artificial light at night (ALAN)-exposed king quail (*Excalfactoria chinensis*)

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Data availability Data for this article is available in supplementary materials.

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

Ethics approval This study was carried out with the approval of Arizona State University's IACUC and complies with the National Institutes of Health guidelines for the care and use of laboratory animals.**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00114-020-01715-9.

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