iScience, Volume *27*

Supplemental information

What happens when the lights are left on? Transcriptomic and phenotypic habituation to light pollution

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Supplementary materials for “*What happens when the lights are left on? Transcriptomic and phenotypic habituation to light pollution”*

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Table S1: Generalized linear mixed-effect model to test whether behavioral responses of naïve birds to ALAN (count of active minutes per night) depend on duration of activity / time of year animals were exposed (stage). There was no significant effect of sex, so it was dropped from the final model. Fixed effects include day (integer) and the interaction of ALAN (factor) and stage (factor), with individual as a random effect to account for repeated measures and a Poisson distribution.

Predictor	**IRR**[1]	**SE**	**CI(95%)**	***p***
Day	1.00	0.002	1.00, 1.00	0.433
ALAN	2.31	0.919	1.06, 5.04	**0.035**
ALAN * stage				
0 * 3	0.87	0.145	0.63, 1.21	0.407
1 * 3	1.27	0.539	0.55, 2.92	0.579

[1] IRR = Incidence Rate Ratio

Table S2: Linear mixed-effect models to test whether oxidative responses of naïve birds to ALAN depend on duration of captivity or time of year animals were exposed. Fixed effects include sex (factor), ALAN treatment group (factor) and day (integer), with individual bird as a random effect to account for repeated measures. There was no significant interaction of ALAN and day, so the interactive term was dropped from the final model. Results of models using a combined stress index as well as individual biomarkers (oxy absorbance and dROMs).

	Stress Index				Oxy Absorbance				dROMs			
Predictor	β	SE	CI (95%)	*p*	β	SE	CI (95%)	*p*	β	SE	CI (95%)	*p*
Sex	0.79	0.342	0.07, 1.5	**0.033**	-33	19.6	-73, 8.1	0.110	0.51	0.815	-1.2, 2.2	0.539
Day	-0.02	0.017	-0.06, 0.01	0.166	0.19	0.635	-1.1, 1.5	0.768	-0.06	0.039	-0.14, 0.02	0.134
ALAN	-0.09	0.288	-0.67, 0.48	0.748	-2.0	10.7	-24, 19	0.850	-0.25	0.657	-1.6, 1.1	0.704

Table S3. Gene annotation analysis of down- and upregulated genes for 1) naïve ALAN exposure compared to acclimation period (pre-ALAN); 2) Recovery 11 weeks post-exposure compared to during naïve exposures, and 3) Secondary re-exposure compared to naïve exposure. Gene annotation analysis conducted in Metascape [4]. Only top 20 results are shown, and all *q*-values of less than 0.05 are bolded. Full output available on GitHub (https://github.com/valaasam)

	Direction	**Category**	**Name**	**# of Genes**	***p***	***q***
Naïve Exposure	Down-regulated	Reactome Gene Sets	Cellular response to stress	15	< 0.001	**< 0.001**
			Neddylation	8	< 0.001	**0.004**
			Golgi Associated Vesicle Biogenesis	4	< 0.001	**0.044**
			Oxygen-dependent proline hydroxylation of Hypoxia-inducible Factor Alpha	4	< 0.001	0.074
		GO Biological Processes	Hemopoiesis	11	< 0.001	**0.014**
			Response to peptide	8	< 0.001	0.089
			Cellular response to decreased oxygen levels	5	< 0.001	0.089
Recovery	Down-regulated	KEGG Pathway	Platelet activation	19	< 0.001	**< 0.001**
			Focal adhesion	19	< 0.001	**< 0.001**
			Leukocyte transendothelial migration	11	< 0.001	**< 0.001**
			Thyroid hormone signaling	11	< 0.001	**< 0.001**
		WikiPathways	VEGFA-VEGFR2 signaling	22	< 0.001	**< 0.001**
			Myometrial relaxation and contraction	11	< 0.001	**< 0.001**
		Canonical Pathways	PID FAK	9	< 0.001	**< 0.001**
		Reactome Gene Sets	Muscle contraction	14	< 0.001	**< 0.001**
		GO Biological Processes	Response to wounding	29	< 0.001	**< 0.001**
			Actin cytoskeleton organization	28	< 0.001	**< 0.001**
			Positive regulation of cell migration	24	< 0.001	**< 0.001**
			Regulation of vesicle-mediated transport	23	< 0.001	**< 0.001**
			Cellular response to nitrogen compound	23	< 0.001	**< 0.001**
			Regulation of kinase activity	23	< 0.001	**< 0.001**
			Circulatory system process	21	< 0.001	**< 0.001**
			Cellular response to organic cyclic compound	19	< 0.001	**< 0.001**
			Regulation of cell-substrate junction organization	10	< 0.001	**< 0.001**
			Actomyosin structure organization	10	< 0.001	**< 0.001**
			Release of sequestered calcium ion into cytosol	8	< 0.001	**< 0.001**
			Cell-substrate junction assembly	7	< 0.001	**< 0.001**
	Up-regulated	WikiPathways	Translation factors	6	< 0.001	**< 0.001**
		Reactome Gene Sets	Asparagine N-linked glycosylation	9	< 0.001	**0.023**
			Hypusine synthesis from eIF5A-lysine	3	< 0.001	**< 0.001**
		GO Biological Processes	Translation	33	< 0.001	**< 0.001**
			Endomembrane system organization	17	< 0.001	**< 0.001**
			Protein catabolic process	16	< 0.001	**0.004**
			Ribosome biogenesis	13	< 0.001	**< 0.001**
			Regulation of cellular amide metabolic process	12	< 0.001	**0.033**
			Positive regulation of organelle organization	11	< 0.001	0.058
			Aerobic electron transport chain	10	< 0.001	**< 0.001**
			Cellular component disassembly	10	< 0.001	**0.006**

			Response to oxidative stress	9	< 0.001	0.074
			Mitochondrial gene expression	8	< 0.001	**0.001**
			Regulation of proteolysis involved in protein catabolic process	8	< 0.001	**0.017**
			Protein localization to organelle	7	< 0.001	**0.001**
			Vacuole organization	7	< 0.001	**0.022**
			Protein folding	7	< 0.001	0.068
			Regulation of ubiquitin protein ligase activity	4	< 0.001	**0.003**
			tRNA threonylcarbamoyladenosine metabolic process	3	< 0.001	**0.033**
		CORUM	Parvulin-associated pre-rRNP complex	3	< 0.001	**0.009**
Re-exposure	Down-regulated	KEGG Pathway	Regulation of actin cytoskeleton	21	< 0.001	**< 0.001**
			Platelet activation	17	< 0.001	**< 0.001**
			Leukocyte transendothelial migration	14	< 0.001	**< 0.001**
			Oxytocin signaling pathway	14	< 0.001	**< 0.001**
		WikiPathways	VEGFA-VEGFR2 signaling pathway	22	< 0.001	**< 0.001**
			Myometrial relaxation and contraction pathways	16	< 0.001	**< 0.001**
		Reactome Gene Sets	Hemostasis	35	< 0.001	**< 0.001**
			GPCR downstream signaling	25	< 0.001	**< 0.001**
			Cell-Cell communication	13	< 0.001	**< 0.001**
			Smooth Muscle Contraction	8	< 0.001	**< 0.001**
		GO Biological Processes	Actin filament-based process	35	< 0.001	**< 0.001**
			Regulation of body fluid levels	24	< 0.001	**< 0.001**
			Regulation of vesicle-mediated transport	24	< 0.001	**< 0.001**
			Positive regulation of cell migration	24	< 0.001	**< 0.001**
			Cell morphogenesis	23	< 0.001	**< 0.001**
			Regulation of anatomical structure size	21	< 0.001	**< 0.001**
			Regulation of GTPase activity	21	< 0.001	**< 0.001**
			Regulation of small GTPase mediated signal transduction	19	< 0.001	**< 0.001**
			Regulation of cell morphogenesis	17	< 0.001	**< 0.001**
			Small GTPase mediated signal transduction	15	< 0.001	**< 0.001**
	Up-regulated	Canonical Pathways	PID Telomerase Pathway	5	< 0.001	**0.012**
		GO Biological Processes	Immune system development	15	< 0.001	**0.003**
			Histone modification	11	< 0.001	**0.003**
			Neural tube closure	6	< 0.001	**0.006**
			Chaperone cofactor-dependent protein Refolding	4	< 0.001	**0.012**
			Negative regulation of macroautophagy	4	< 0.001	**0.016**
			Nucleocytoplasmic transport	7	< 0.001	**0.046**
			Heterochromatin formation	4	< 0.001	0.052
			Protein autophosphorylation	6	< 0.001	0.071
			Protein hydroxylation	3	< 0.001	0.091
			Nucleus localization	3	< 0.001	0.091
			Regulation of cell cycle process	11	< 0.001	0.100
			Regulation of dephosphorylation	5	< 0.001	0.100

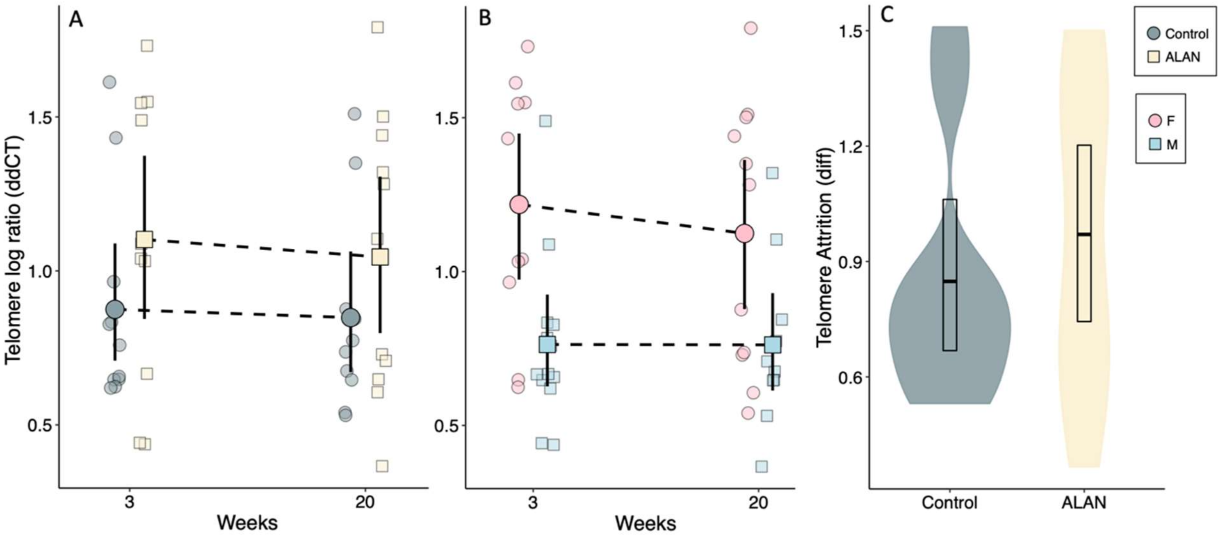

Figure S1. Telomere lengths did not differ between ALAN and control groups at either timepoint (A). Males consistently had shorter telomeres that females across timepoints (B). Individual changes in telomere length over time were all positive, indicating a shortening of telomeres over time, but there was no difference in degree of attrition between groups (C).

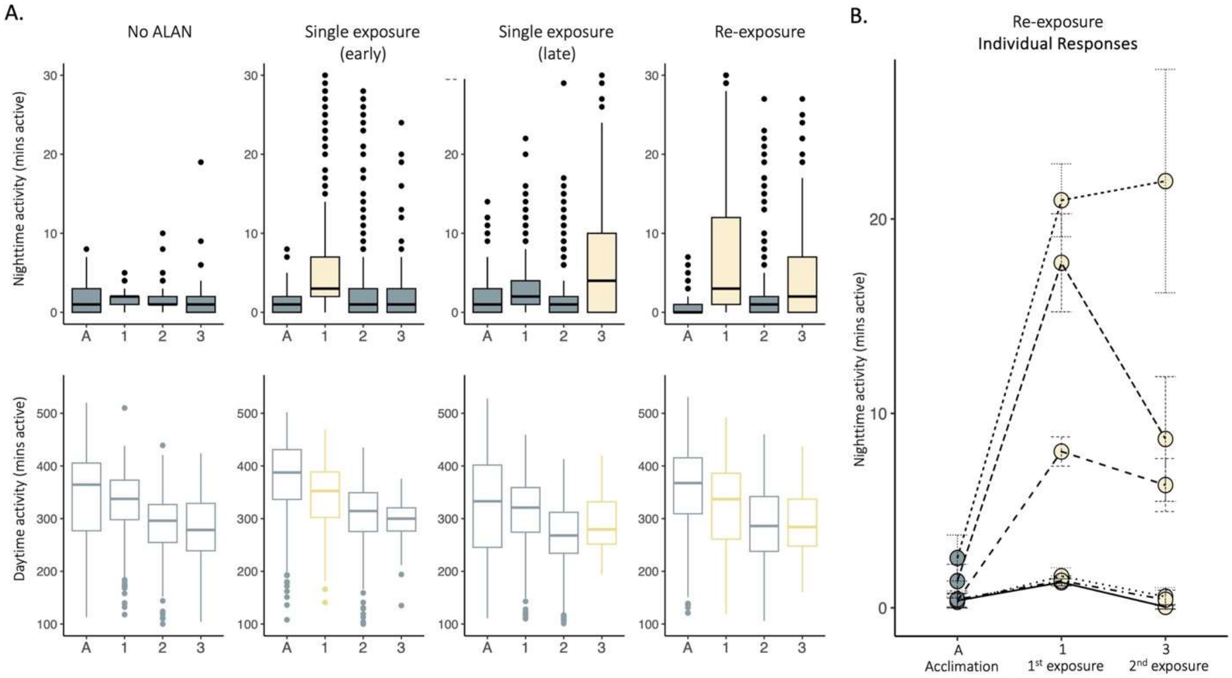

Figure S2. Effect of ALAN on activity, shown for treatment groups. (A). Boxplot version of Figure 2 in main text, showing raw data. Nighttime (top) and daytime (bottom) activity, measured as total minutes active in the night and/or day, across stages of the experiment. Stage "A" represents acclimation period. Note differences in y axis between daytime and nighttime plots. (B). A closer look at individual responses to repeated ALAN exposure. Here we show individual birds (n=6) in our experimental group that were exposed to ALAN in both stages 1 and 3. Points represent individual averages (across days of exposure), and error bars show standard errors. Stage 2 here was omitted, to illustrate the change in magnitude of response between first and second exposure (*i.e.*, habituation).

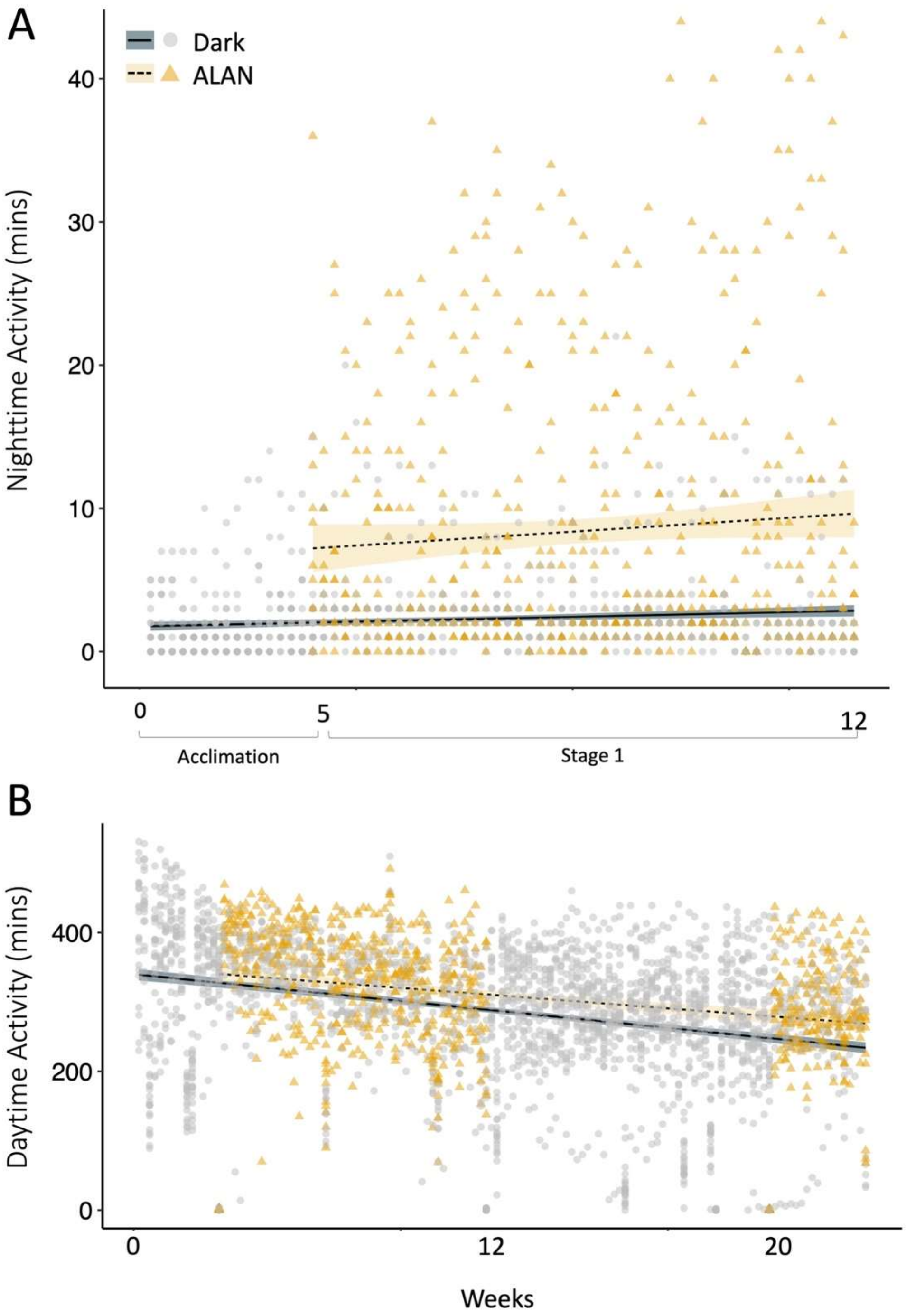

Figure S3. Raw activity data over time. (A). In stage 1 (8 weeks), we found that for birds under ALAN, nighttime activity increases slightly over time (day), while birds under dark nights showed consistently low nighttime activity. Points represent raw counts of minutes active per night for all birds (n=11 per ALAN and Dark groups). (B). Daytime activity decreased across the duration of experiment, regardless of ALAN exposure.

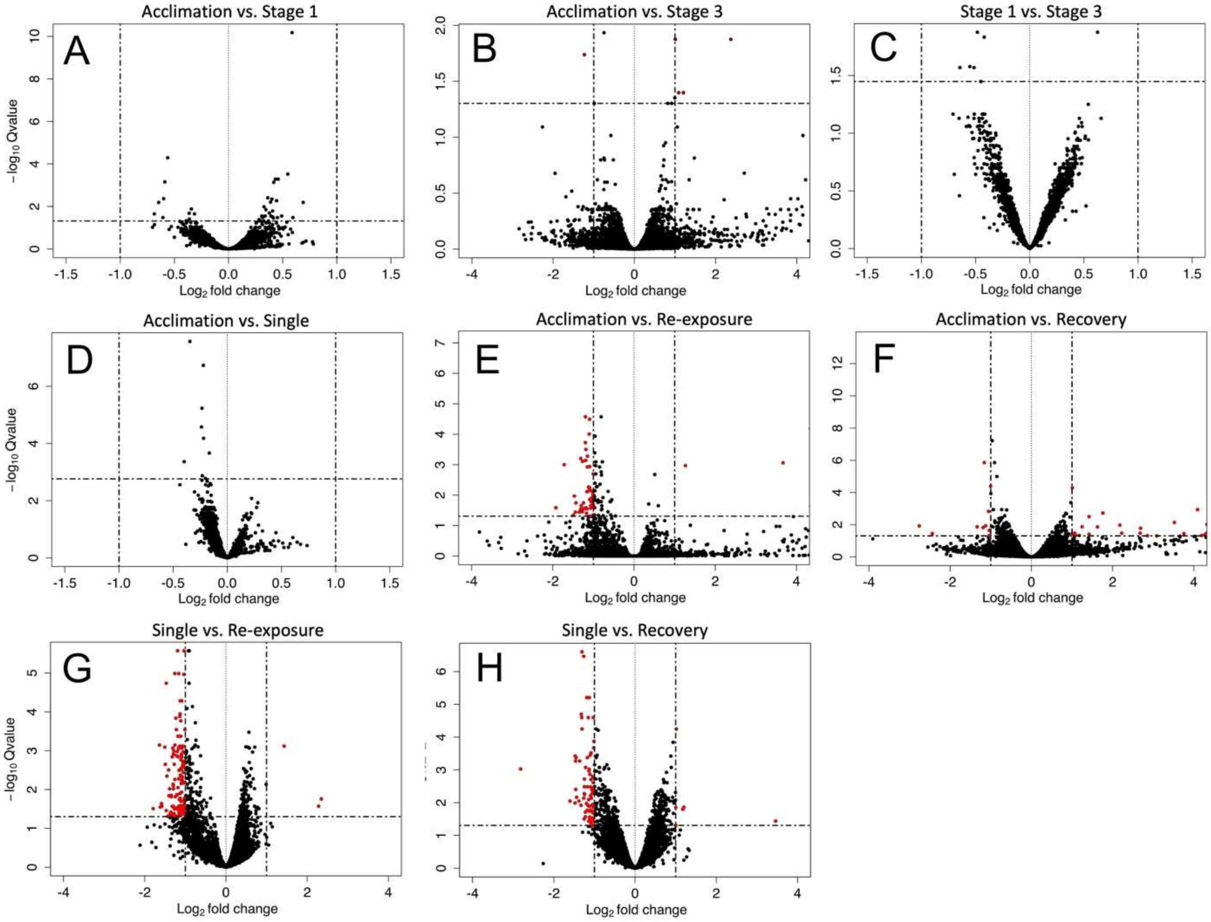

Figure S4. Volcano plots representing differentially expressed genes for comparisons between treatment groups. **(A-C)** Pairwise comparisons between timepoints of our control group, maintained in dark-night conditions for the duration of the experiment, performed to validate the impact of ALAN treatment. **(D-H)** Pairwise comparisons of various groups exposed to ALAN (see Figure 1). "Acclimation" represents birds under dark-night conditions, with no previous ALAN exposure. "Single" represents naïve birds during first ALAN exposures. "Recovery" represents birds returned to dark-night conditions, 8 weeks *after* first ALAN exposures. "Re-exposure" represents birds during secondary exposures to ALAN.

Supplementary References

1. Cawthon RM (2009) Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res 37:e21–e21. https://doi.org/10.1093/nar/gkn1027

2. Criscuolo F, Bize P, Nasir L, et al (2009) Real-time quantitative PCR assay for measurement of avian telomeres. J Avian Biol 40:342–347. https://doi.org/10.1111/j.1600-048X.2008.04623.x
3. Morinha F, Magalhães P, Blanco G (2020) Different qPCR master mixes influence telomere primer binding within and between bird species. J Avian Biol 51:
4. Zhou Y, Zhou B, Pache L, et al (2019) Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun 10:1523. https://doi.org/10.1038/s41467-019-09234-6