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To the scientific editors of *Plos Biology*,

Please consider our manuscript titled ‘Birds of a feather flock together: Social context exacerbates the effects of light pollution on circadian disruption’ for submission to *Plos Biology*. We present the first evidence of social regulation of circadian rhythms in vertebrates, using an integrative approach that links behavioral and molecular responses to light pollution in isolated and social settings. There is some evidence, across taxa, that social interactions could provide circadian rescue in highly altered light environments, we thought to test this amelioration to a pervasive environmental pollutant, light pollution. Opposite to our predictions, housing birds socially exacerbated responses to light pollution behaviorally and molecularly through advanced activity onset and disrupted expression of core circadian genes in both central and peripheral clock tissues. Moreover, hypothalamic gene expression predicted activity onset under light pollution, indicating synergist disruptions from biological organization to behavioral outputs.

Circadian rhythms are ubiquitous cross taxa, allowing organisms to synchronize their biological rhythms with the day-night cycle to align their behavioral, physiological, and molecular clocks (1). Artificial light at night disrupts circadian rhythms by affecting molecular, physiological, and behavioral processes, impacting health and biological functions across various organisms (2). It is known that social interactions play a pivotal role in shaping behavioral rhythms in invertebrate models (3). However, whether social context can modulate environmental effects on circadian rhythms remains largely unknown, especially in vertebrates.

Our findings demonstrate the profound influence of social interactions under light pollution. We analyzed birds exposed to light pollution compared to dark night controls in both isolated and socially housed conditions, measuring behavior, circulating concentrations of melatonin, and central and peripheral circadian gene expression in zebra finches. We found that artificial light at night advances activity onset and disrupts rhythmic circadian gene expression, all of which is exacerbated in social conditions. We also show light at night disrupts the peripheral clock in the liver of only socially housed birds. These data broaden our understanding of social species’ responses to light pollution and demonstrate for the first time to our knowledge of social interactions regulating the circadian clock on a molecular level in vertebrates.

We present a novel, integrative approach to understanding complex responses to one of the most widespread and rapidly increasing global pollutants, informing the fields of circadian biology, stress physiology, ecology, and social biology. Social context strongly affects gene expression and circadian misalignment, exacerbating responses to external stressors. Furthermore, results exemplify the value of accounting for social context to understand responses to environmental pollutants and stressors, which should interest the broad readership of *Plos Biology*.

We recommend the following reviewers:

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Works Cited

1. J. C. Dunlap, Molecular bases for circadian clocks. *Cell* **96**, 271-290 (1999).
2. K. M. Zielinska-Dabkowska, E. S. Schernhammer, J. P. Hanifin, G. C. Brainard, Reducing nighttime light exposure in the urban environment to benefit human health and society. *Science* **380**, 1130-1135 (2023).
3. O. Siehler, S. Wang, G. Bloch, Social synchronization of circadian rhythms with a focus on honeybees. *Philosophical Transactions of the Royal Society B-Biological Sciences* **376**, (2021).



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Birds of a feather flock together: Social context exacerbates the effects of light pollution on circadian disruption.

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Abstract

Artificial light at night (ALAN), a growing pervasive pollutant, disrupts physiological and behavioral rhythms across organisms. Social interactions play a significant role in shaping individual and group biological rhythms, but they are often overlooked in the context of environmental stressors, such as ALAN. We explore how dim ALAN affects zebra finches (*Taeniopygia guttata*) in social and isolated environments, examining behavioral, physiological, and molecular rhythms. We found that social birds under ALAN had an earlier activity onset and greater disruption in hypothalamic and liver circadian gene expression than control or isolated counterparts under ALAN. Additionally, we found that activity onset correlated negatively with hypothalamic *bmal1* and *cry1* expression and positively with *per2* expression in birds exposed to ALAN. Within ALAN-exposed birds, there was a larger disassociation between central and peripheral clock gene expression for social birds than in isolated birds. However, rhythmic melatonin concentrations did not differ among treatment groups. We show that social interactions may exacerbate the effects of ALAN, which highlights the impact of social interactions on circadian regulation at a molecular level and a critical need to consider social contexts in biological studies.