When Night Becomes Day: Artificial Light at Night Alters Insect Behavior under Semi-Natural Conditions

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ABSTRACT

Light is the most important *Zeitgeber* for temporal synchronization in most organisms. Artificial light at night (ALAN) disrupts the natural light-dark rhythmicity and thus negatively affects animal behavior. However, to date, ALAN research has been mostly conducted under laboratory conditions. Here, we used the field cricket, *Gryllus bimaculatus*, to investigate the effect of ALAN on insect behavior under semi-natural conditions. Male crickets were placed individually in outdoor enclosures and exposed to ALAN conditions ranging from 0 to 1,500 lux intensity. The crickets' stridulation behavior was recorded for 14 consecutive days and nights and their daily stridulation activity patterns were analysed. ALAN impaired the crickets' stridulation rhythm, evoking a light-intensity-dependent increase in individual free-run behavior, and in the population's median activity cycle period. The observed ALAN-intensity-dependent desynchronization occurred despite the crickets' exposure to almost natural conditions. Our findings reconfirm and further establish the severe impacts of ALAN on animal behavioral patterns.

SIGNIFICANCE STATEMENT:

In most animals, the rhythmic cycle of day and night serves for timekeeping and for synchronizing daily activity within the individual animal and among the population. Artificial light at night (ALAN) disrupts this natural diurnal cycle. However, our knowledge on the negative impacts of ALAN on insect behavior is still limited. To this end, we exposed adult male crickets to different ALAN intensities under shaded natural lighting and temperature conditions and monitored their stridulation behavior for two consecutive weeks. Despite the semi-natural experimental conditions, our findings reveal ALAN-induced light-intensity-dependent free-run behavior, and thus loss of timekeeping and an ALAN-intensity-dependent desynchronization of the population. This demonstration of anthropogenic effects on animal behavior in natural settings underscores the ecological threats of ALAN.

INTRODUCTION

The daily rhythmicity of light and darkness serves most animals for the perception of time (1, 2) and has been reported to be the most important *Zeitgeber*, synchronizing internal and external events through entrainment of the animal's circadian clock mechanism (2, 3). Circadian rhythms are manifested in many behavioral patterns, such as locomotion, sleep, foraging, and singing, as well as in physiological processes, including hormonal regulation and gene expression (3, 4). The behavioral responses to the phases of daylight and darkness depend on the animal's visual system, its way of life (diurnal, nocturnal, or crepuscular), and the specific nature of the stimulus (5–8). The individuals' synchronization with external environmental rhythms (circadian, circalunar and circannual) is manifested in synchronization of the overall population.

Artificial light at night (ALAN), the use of anthropogenic outdoor illumination during the night-time, has become prevalent (9, 10) and is increasing worldwide by about 3-6% annually (11). ALAN disrupts the natural light-dark cycle, impacting animals' daily activity patterns and altering their temporal synchronization (1, 5, 12). It has been shown to have severe effects on human health (13), on wildlife, and on the environment (4, 14–17). In insects, nocturnal illumination has been reported to induce spatial disorientation (14, 18), resulting in higher predation risk (19, 20), increased mortality (19–22), reduced pollination (16, 23), and collectively impeding biodiversity (24).

Laboratory experiments offer a useful tool for ALAN research, enabling controlling and isolating the light stimuli from all other variables. Such experiments, however, lack

the complexity of the natural environment including related behavioral aspects such as social interactions. The natural environment incorporates, alongside light-dark cycles, a multitude of rhythmic processes, including thermoperiods, rhythmic soundscape due to the temporal changes in species' activities, as well as rhythmic intra- and interspecific (e.g., predator-prey) interactions. Ideally, ecological ALAN research would be conducted in the untouched, naturally dark environment, while introducing long-term ALAN manipulations. Such field experiments, however, present significant technical challenges. Semi-natural conditions therefore offer an excellent alternative, enabling monitoring the experimental animals under almost natural conditions, while limiting the biotic and abiotic variables by isolating the experimental individuals in dedicated enclosures.

Our experimental model, the field cricket, *Gryllus bimaculatus*, is a ground-dwelling species exhibiting distinct diel stridulation and locomotion cycles. Crickets demonstrate temporal shifts in both these activity patterns in response to changes in illumination regimes (12, 25–28). Additionally, temporal, circadian variations in gene expression patterns have been observed in the cricket (28, 29). We investigated the impact of ALAN on these nocturnal insects in a semi-natural experimental set-up. Individual crickets were subjected to various ALAN intensities in shaded outdoor conditions that incorporated natural temperature and soundscape rhythms. We monitored the individual insects' stridulation behavior for 14 consecutive days and nights. To the best of our knowledge, this is a first attempt to monitor the behavior of individual insects over such an extended period within a semi-natural setting. Our findings have uncovered the effects of ecologically-relevant ALAN intensities on behavioral patterns of these insects, under such natural conditions.

RESULTS

ALAN affected the type of stridulation activity patterns and their relative proportions

Individual crickets inside their outdoor enclosures were subjected to ALAN conditions at intensities ranging from 0 to 1,500 lux. The effects of these different ALAN treatments on stridulation rhythms were manifested in different relative proportions of two types of activity patterns: nocturnal synchronized rhythms with periods of 24 h (Figure 1A1); and free-run rhythms with periods deviating from 24 h (Figure 1A2). The ALAN conditions elicited a significant light-intensity-dependent increase in the

proportion of free-run behavior, from 10% in LD to 92% in $LL_{1,500}$ (Figure 1B, $\chi^2(6, N=98)=39.91$, *p<0.0001*), affecting overall 60% of the ALAN-exposed crickets (41 out of a total of 68 crickets).

ALAN altered stridulation activity in a light-intensity-dependent manner

Analysis of the medians of the period of the recorded behavioral stridulation rhythms revealed light-intensity-dependent differences (Figure 2A, Table S1). While the median period of stridulation activity cycles of the LA_2, LA_5, and LA_{15} groups did not differ significantly from those of LD (Figure 2A; Kruskal-Wallis test with Dunn's multiple comparisons test, $p > 0.9$ for all; for sample sizes, see Table S1), a significant increase in the period of stridulation was evident in all treatments of ALAN ≥ 100 lux (LA_{100}, LA_{400}, and $LL_{1,500}$; $p < 0.02$, $p < 0.04$, $p < 0.001$, respectively). Furthermore, ALAN also affected the variance of the calculated period: in the ALAN ≥ 100 lux treatments the stridulation displayed a significantly higher variance compared to in the LD (Figure 2A; one-tailed Brown-Forsythe test with Welch's correction, LA_{100}, LA_{400}, and $LL_{1,500}$; $p < 0.03$, $p < 0.04$, $p < 0.01$, respectively). The ALAN effects were also manifested in light-intensity-dependent changes in the activity onset and offset relative to the sunrise and sunset, respectively, i.e., the experimental crickets exposed to ALAN ≥ 100 lux delayed both their onset and offset of activity (Figure 2B; Kruskal-Wallis test, for onsets: LA_{100}, LA_{400}, and $LL_{1,500}$; $p < 0.01$, $p < 0.04$, $p < 0.003$, respectively; for offsets: LA_{100}, LA_{400}, and $LL_{1,500}$; $p < 0.03$, $p < 0.002$, $p < 0.02$, respectively; see also Table S3). Most conspicuously, the ALAN disruptive effects were accompanied by a discernible trend of an increasing percentage of diurnal stridulation (Figure 2C).

ALAN did not alter the phase distribution of the stridulation activity

Acrophase refers to the phase of the peak of the fitted Cosinor curve and serves as a convenient phase marker of the behavioral rhythm. Acrophase analysis of the crickets' stridulation rhythmic cycles, conducted with increasing levels of ALAN intensity (Figure 3, Table S2), revealed no significant changes in the mean timing of acrophase fitted to each individual's stridulation period (Watson-Williams F-tests, $p>0.1$); nor did the phase distribution differ among treatments (Mardia–Watson–Wheeler test, $p = 0.37$). Pooling all the data together revealed an overall synchronized rhythmicity, expressed in the significant directionality of the mean phase of the population, as well as in a non-uniform distribution (Hotelling's Test, $p=0.003$, and Moore's Rayleigh Test,

p<0.001, respectively). This indicates an overall resilience of the acrophases of stridulation activity under semi-natural conditions, despite the exposure to ALAN.

Stridulation frequency is impacted by temperature but not ALAN

The search for possible differences in the stridulation frequencies among the various ALAN treatments yielded no discernible effect (Spearman's rank-order correlation, r_{113} = -0.11, p = 0.26). In contrast, during the experiments which took place over the course of two consecutive years, all the experimental crickets and treatments were subjected to daily and seasonal temperature cycles (Figure S2), which in total were found to significantly affect the stridulation frequency (r_{111} = 0.204, p < 0.001).

DISCUSSION

Crickets serve as prominent model insects for investigating diverse biological and life-history traits. Although studies on communication behavior or population genetics in crickets have occasionally been conducted in the field (30), chronobiology research has predominantly been limited to the controlled laboratory environment (3, 31). Similarly, investigations into the impact of ALAN on crickets have been primarily carried out under laboratory conditions, with the findings raising significant concerns regarding the impacts of ALAN on the crickets' overall wellbeing (12, 29, 32).

The findings of the current study, conducted under semi-natural conditions and incorporating behavioral monitoring over relatively long time periods, are in general agreement with those of our previous laboratory study (12): a light-intensity-dependent effect of ALAN was evident, expressed in a decrease in synchronized individual stridulation behavior. This suggests an ALAN-induced disruption in the population's behavioral synchronization. Notably, impairment of both the individuals' stridulation behavioral cycle and their free-run behavior was observed, even under relatively dim ALAN conditions of 2 lux, in both the laboratory and under semi-natural conditions. This sensitivity of crickets to light is not surprising, given that the field cricket *G. bimaculatus* is a nocturnal, ground-dwelling species, with a visual system well-adapted to near-dark conditions (33, 34).

While both our previous laboratory study and our current study exhibit similarities in their findings, as described above, they also present several key differences. It is important to acknowledge that under the semi-natural conditions, it was only from light intensities of 400 lux and above that over 80% of the experimental crickets demonstrated a transition towards desynchronized stridulation behavior (Figure 1B). This is in contrast to our earlier laboratory conditions, under which such a transition already occurred at light intensities of 2 lux and above (12). Moreover, another apparent difference between the semi-natural and the laboratory conditions is that, overall, the medians of the activity periods under semi-natural conditions were notably lower (24.0 h and 24.5 h in LD and LL, respectively) compared to those induced by lifelong ALAN exposure in the laboratory (ranging from 24.0 h in LD to 25.67 h in LL (12)). Interestingly, differences were also observed in the results of the acrophase analysis. While in the laboratory setting a significant impact on the mean phase vector and phase distribution under LL conditions (12) was evident, these same parameters remained stable under the semi-natural conditions (see Figure 3, Table S2).

These differences suggest that the behavior under semi-natural conditions is influenced by additional environmental factors, beyond that of the nocturnal light intensities *per se*. Such environmental factors may include the diurnal light intensity to which the experimental crickets were exposed, and thus also the absolute difference between diurnal and nocturnal light intensities. In addition, sunrise and sunset patterns, and the related changes in illumination spectra, may also have an effect. The potential impact of diurnal light intensities on the susceptibility to ALAN has remained unexplored, to date. However, it should be noted that the diurnal light intensities in the above-mentioned laboratory study were 40 lux (12), compared to 1,500 lux in the current semi-natural experiments. It is therefore highly plausible that these differences in light intensity could account for the observed differences in the stability of the activity period medians between the outdoor conditions and the laboratory conditions.

Taken together, our findings from both the earlier laboratory experiments (12) and the current semi-natural conditions underscore the crucial impact of ALAN on the cricket as a model insect. Our current study, conducted under semi-natural conditions, emphasizes the importance of field studies that enable multi-sensory modalities to be taken into account in order to comprehensively understand and accurately depict the effects of environmental pollutants on animal populations.

As noted, the experiments presented in this study took place outdoors under near-natural conditions, spanning from spring to autumn, over two consecutive years. Consequently, the experimental crickets were exposed to the natural daily temperature cycle (Figure S2), and to seasonal changes in temperature. Despite this temperature variability, it is striking that 90% of the control crickets maintained a consistent daily activity period of 24 h (Figure 1B, 2A). This finding reconfirms the remarkable phenomenon of temperature compensation (35, 36), which signifies the stability of the insects' daily activity period across a wide range of temperatures.

Temperature cycles have been reported to act as an exogenous pacemaker and effective *Zeitgeber* (3, 35, 37) in many animals, including insects. In fruit flies, different clock neurons have been identified as responsible for mediating entrainment by light and by temperature (38). Similarly, crickets that had been made arrhythmic by removing their optic lobes, regained their rhythmic locomotor or stridulatory activity upon exposure to 24-hour temperature cycles (36, 39, 40). The peak of the crickets' stridulation and locomotion behaviors occurred following the transition from high to low temperatures, accompanied by a slightly advanced locomotion activity onset (39, 40). Surprisingly, our findings indicate that over 85% of the crickets subjected to ALAN intensities exceeding 400 lux exhibited free-run stridulation behavior (Figure 1B), despite being exposed to natural temperature cycles (Figure S2). This clear ALAN intensity-dependent effect suggests that the daily temperature rhythms were insufficient to act as a *Zeitgeber* and entrain the crickets' activity patterns. The fact that, in our study, stridulation behavior was solely synchronized by light conditions and not affected by temperature changes reconfirms light as the most dominant environmental *Zeitgeber*, effectively entraining the circadian clock of adult insects (3, 35).

Temperature, however, did affect other aspects of our experimental crickets. Despite experiencing constant temperature conditions in their growth chamber (prior to the experiments), the stridulation frequencies of the individual crickets during the experiments were notably impacted by the outdoor temperature, while remaining unaffected by ALAN. This finding is in accord with previous findings (41, 42), suggesting the existence of some degree of plasticity in the stridulation behavior in response to changing environmental cues.

Male field crickets, *Gryllus bimaculatus*, were reported to respond aggressively (acoustically or physically) to male calls within a range of 2 m from their burrows, possibly identifying these as intruders (43). Furthermore, it was suggested that aggregations of calling crickets serve for intraspecific communication for both females and males, with 2 m being the most frequent distance between two calling males (Simmons, 1988, Figure 2 therein). This pattern of males responding with aggressive or territorial calls to the advertisement calls of other males has also been observed in the cricket *Teleogryllus commodus* (although these did not exhibit positive phonotaxis towards conspecific calls (44)). Given that some insects, such as honeybees and fruit flies, were reported to utilize social cues as *Zeitgebers* for entraining behavioral rhythms (45–47), and since stridulation serves for intraspecific communication in crickets, we hypothesized that our experimental crickets might use conspecific stridulation as a form of social *Zeitgeber*. Although the distance between each of the experimental cricket enclosures in our study was 285 cm (exceeding the 2 m distance noted above), the crickets were still able to audibly detect the stridulation of conspecifics (as evident in the sound recordings). Nevertheless, we found no evidence suggesting synchronization of the experimental individuals according to the cycles of conspecific stridulation. Rather, over 60% of the ALAN-exposed individuals demonstrated free-run stridulation behavior.

Furthermore, the natural environment surrounding the experimental enclosures featured a diverse and daily-cycling soundscape. This included the vocalizations of many bird species exhibiting circadian patterns, traffic noise, and other diurnal human activity. As noted, however, a majority of the crickets exposed to ALAN exhibited free-run behavior, essentially ignoring all these rhythmic environmental acoustic cues. The findings from our study thus demonstrate that the behavior of *G. bimaculatus* crickets is neither synchronized by conspecific stridulation nor by the broader acoustic soundscape. Rather, their behavioral patterns are, as previously suggested, primarily synchronized by light stimuli.

The semi-natural experimental system exhibited several unavoidable yet significant deviations from a fully natural setup. The crickets were isolated from interactions with females and prevented from engaging in reproductive activities, while also not being exposed to any risk of predation. Within their enclosures, these male crickets lacked the possibility to expand or change their habitat or microclimate. Importantly, unlike their

free-ranging counterparts in natural habitats that can select less illuminated areas, the experimental crickets were unavoidably exposed to ALAN due to the controlled conditions. These semi-natural conditions, however, effectively mirrored the considerably disrupted environment often encountered in the crickets' native habitats.

Our findings present evidence of free-run patterns of behavior also within the context of nearly natural conditions (in an illuminated habitat), as well as changes in the timing (onset and offset) of stridulation activity. This desynchronization may impair the crickets' courtship behavior and potentially put them at higher predation risk. The increased predation risk arises from two primary factors: first, individuals could become more visually conspicuous against an illuminated backdrop; and second, their acoustic signals could reveal their presence to diurnal predators and parasitoids. Indeed, certain possible predator species, including geckos and birds, have been documented to have adjusted their foraging patterns in response to ALAN, expanding or shifting from diurnal to nocturnal activities (48, 49).

The exposure of large areas to ALAN, as commonly observed in industrial zones or due to closely-positioned streetlights, can significantly disrupt the natural behaviors of a large variety of insects (14, 19). This disruption often results from the loss of dark niches, a phenomenon demonstrated in the present study, which may lead to lifelong exposure to ALAN. It is noteworthy, that in addition to acting as a source of attraction, and thus as a sink for flying insects (19, 21), ALAN has been reported to fragment the once-dark habitat by creating illuminated corridors, altering patterns of movement, such as positive and negative phototaxis, in various species (21, 50). As a consequence, ALAN, particularly that emanating from streetlights, may spatially and temporally reshape the distribution, behavior, and synchronization of insects across the landscape, ultimately resulting in significant declines in their overall fitness. Our growing understanding of the detrimental effects of the exposure to ALAN intensities, both on the natural behaviors of individual insects and on the broader dynamics of insect populations, offers a key opportunity for reassessing and reducing the prevalence of outdoor ALAN, with a specific focus on streetlights. Taking such actions would offer a vital step towards the enhanced protection of the natural environment.

MATERIALS AND METHODS

Rearing conditions

Crickets were reared in the laboratory under a constant temperature of 26±1°C in a chamber illuminated with white compact fluorescent light (CFL, NeptOn, 6500 K, 380-780 nm, peaks: 547 & 612 nm, Figure S1). The cricket colony was exposed to a 12 h light period of 320 lux (7:00 – 19:00) and a 12 h period of total darkness. Crickets were fed three times a week with dog chow, oats, and vegetables.

Experimental setup

The experiments took place outdoors, in the grounds of the I. Meier Segals Garden for Zoological Research at Tel Aviv University, from spring until autumn for two consecutive years (2020 & 2021). Male adult crickets from the indoor breeding colony were housed individually in transparent enclosures (20x12x13.5 cm each) attached to metal poles at a height of 1.3 m above the ground, 285 cm apart from each other. Each enclosure was lined with a layer of cocopeat, contained an egg carton for shelter, and was sealed with a metal mesh, allowing the cricket to experience natural lighting, temperature rhythms, and soundscape, while preventing its escape or predation. Each enclosure was equipped with a temperature logger and a Swift autonomous recording unit (Koch, 2016, Cornell Lab of Ornithology) placed 15 cm above the mesh (Figure 4A&B). Artificial lighting was supplied by CFL light bulbs of the same spectra described above, but of varying intensities, suspended above each enclosure (Figure 4A&B). Each light bulb was wrapped in aluminum foil to provide the required specific illumination intensity above each enclosure and prevent light-pollution from the surrounding area. The enclosures were shaded from direct sunlight by means of black cardboard affixed to the back of the poles (Figure 4A&B), preventing overheating and maintaining similar temperatures in all the enclosures (Figure S2). The different treatments, as detailed below, were conducted simultaneously, maintaining the variation in natural conditions higher between replicas of the same treatment than between different treatments. Crickets were supplied with dog chow and vegetables.

Experimental illumination conditions

All the experimental crickets were exposed diurnally to natural, shaded lighting conditions of a maximum of 1,500 lux, measured at noon across several days using a digital light meter (TES-1337, TES, Taiwan). Each cricket was subjected to one of the following seven treatments: (i) LD (control): dark nights (light < 0.01 lux), (ii) LA_2: 2 lux, (iii): LA_5: 5 lux, (iv): LA_{15}: 15 lux, (v): LA_{100}: 100 lux, (vi): LA_{400}: 400 lux, (vii):

$LL_{1,500}$: 1,400-1,500 lux (constant light; LL). All light sources were constantly lit. However, as the light intensity in treatments (i) to (vi) was much lower than that of natural daylight, these experimental conditions represented ALAN conditions, while treatment (vii): $LL_{1,500}$, represented constant light, as its naturally shaded diurnal intensities were similar to the artificially lit nocturnal intensities. Light intensities were measured at all four corners at the top of the enclosures using the same light meter as above, and then averaged.

Behavioral monitoring

Stridulation behavior and behavioral rhythms under ALAN exposure were monitored using Swift autonomous recording units (Figure S2B), at 32,000 Hz, 16 bit, and 30 dB microphone gain. Each individual cricket was recorded for up to 14 consecutive days and nights.

Data processing and statistical analysis

Stridulation data extraction and processing followed Levy et al. (2021), using ”R”, version v.3.4.1. (52), the “Rraven” open source package (53), and RavenPro1.5 (54). All bioacoustic data, aka stridulation events, were manually validated to avoid false positives/negatives and to eliminate other acoustic sources in the semi-natural conditions, such as bird vocalizations. Only stridulation activity patterns containing at least five consecutive days and nights of behavioral data were used for further analysis. Data processing and statistical analyses were conducted in Python version 3.7 (PyCharm, JetBrains), SPSS version 21 (IBM Corp. Armonk, NY, USA), and Prism 8 (GraphPad Software, San Diego, California USA). The number of detected stridulation events was assessed per animal in 10-minute bouts. For rhythmicity and periodogram analyses, values were normalized for each individual by dividing that individual’s combined values by its own maximum value, resulting in an activity index ranging from 0 (no activity) to 1 (maximal activity). Periodogram analyses of the activity rhythm periods were determined using the ImageJ plugin ActogramJ (55), while ClockLab (Actimetrics) was used for onsets and offsets of activity evaluations.

A $\chi 2$ test was used to evaluate a possible connection between ALAN intensity and stridulation rhythm types. The medians of the periods were compared using a Kruskal-Wallis test with Dunn’s multiple comparisons post-hoc test. Variance comparison was

conducted using a one-tailed Brown-Forsythe test for equality of variance, followed by Welch's correction. Mean onsets and offsets of activity were calculated by subtracting the time of sunset from that of the onset of activity and subtracting the offsets from sunrise, further converting the results into minutes. A Kruskal-Wallis test was used for means comparisons. Spearman's rank-order correlation was used to assess a possible connection between ALAN and the frequency of stridulation behavior.

The mean acrophase, presenting the peak of the rhythms evaluated for a minimum of 5 days, was calculated for each animal and period using the CosinorPy package (56). Mean phases as well as circular statistical analyses were conducted using the Oriana software, v. 4 (Kovach Computing Wales, UK) (57). The Watson-Williams F-Test was used for comparisons among the treatments' mean arrow angles and the Mardia–Watson–Wheeler test was used for distribution comparisons among treatments.

REFERENCES

1. R. M. Borges, Impacts of Artificial Light at Night on Biological Timings. *Indian J. Entomol.* **84**, 483–492 (2022).

2. J. Aschoff, Zeitgeber des tierischen Tagesperiodik. *Naturwissenschaften* **41**, 49–56 (1954).

3. K. Beer, C. Helfrich-Förster, Model and Non-model Insects in Chronobiology. *Front. Behav. Neurosci.* **14**, 601676 (2020).

4. C. Rich, T. Longcore, *Ecological Consequences of Artificial Night Lighting* (Island Press, 2006).

5. B. Helm, *et al.*, Two sides of a coin: Ecological and chronobiological perspectives of timing in the wild. *Philos. Trans. R. Soc. B Biol. Sci.* **372**, 20160246 (2017).

6. E. J. Warrant, The remarkable visual capacities of nocturnal insects: Vision at the limits with small eyes and tiny brains. *Philos. Trans. R. Soc. B Biol. Sci.* **372** (2017).

7. N. Mrosovsky, Masking: History, definitions, and measurement. *Chronobiol.*

Int. **16**, 415–429 (1999).

8. N. Kronfeld-Schor, T. Dayan, Partitioning of Time as an Ecological Resource. *Annu. Rev. Ecol. Evol. Syst.* **34**, 153–181 (2003).

9. F. Falchi, *et al.*, Light pollution in USA and Europe: The good, the bad and the ugly. *J. Environ. Manage.* **248**, 109227 (2019).

10. C. C. M. Kyba, M. Newhouse, Citizen scientists report global rapid reductions in the visibility of stars from 2011 to 2022. *Science (80-.).* **379**, 265–268 (2023).

11. F. Hölker, *et al.*, The dark side of light: a transdisciplinary research agenda for light pollution policy. *Ecol. Soc.* **15**, 215–224 (2010).

12. K. Levy, Y. Wegrzyn, R. Efronny, A. Barnea, A. Ayali, Lifelong exposure to artificial light at night impacts stridulation and locomotion activity patterns in the cricket *Gryllus bimaculatus*. *Proc. R. Soc. B Biol. Sci.* **288**, 20211626 (2021).

13. 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 (80-.).* **380**, 1130–1135 (2023).

14. A. C. S. Owens, *et al.*, Light pollution is a driver of insect declines. *Biol. Conserv.* **241**, 108259 (2020).

15. A. J. A. Stewart, Impacts of artificial lighting at night on insect conservation. *Insect Conserv. Divers.* **14**, 163–166 (2021).

16. S. Giavi, S. Blösch, G. Schuster, E. Knop, Artificial light at night can modify ecosystem functioning beyond the lit area. *Sci. Rep.* **10**, 1–12 (2020).

17. A. K. Jägerbrand, K. Spoelstra, Effects of anthropogenic light on species and ecosystems. *Science (80-.).* **380**, 1125–1130 (2023).

18. J. J. Foster, *et al.*, Light pollution forces a change in dung beetle orientation behavior. *Curr. Biol.* **31**, 3935-3942.e3 (2021).

19. G. Eisenbeis, "Artificial night lighting and insects: attraction of insects to streetlamps in a rural seting in Germany" in *Ecological Consequences of Artificial Night Lighting*, (2006).

20. T. W. Davies, J. Bennie, K. J. Gaston, Street lighting changes the composition of invertebrate communities. *Biol. Lett.* **8**, 764–767 (2012).

21. J. Bolliger, *et al.*, Effects of traffic-regulated street lighting on nocturnal insect abundance and bat activity. *Basic Appl. Ecol.* **47**, 44–56 (2020).

22. D. Sanders, R. Kehoe, D. Cruse, F. J. F. van Veen, K. J. Gaston, Low Levels of Artificial Light at Night Strengthen Top-Down Control in Insect Food Web. *Curr. Biol.* **28**, 2474-2478.e3 (2018).

23. E. Knop, *et al.*, Artificial light at night as a new threat to pollination. *Nature* **548**, 206–209 (2017).

24. J. Falcón, *et al.*, Exposure to Artificial Light at Night and the Consequences for Flora, Fauna, and Ecosystems. *Front. Neurosci.* **14**, 1–39 (2020).

25. K. Levy, A. Barnea, A. Ayali, Exposure to a nocturnal light pulse simultaneously and differentially affects stridulation and locomotion behaviors in crickets. *Front. Physiol.* **14**, 1151570 (2023).

26. K. Tomioka, Y. Chiba, Post-embryonic development of circadian rhythm in the cricket, *Gryllus bimaculatus*: A rhythm reversal. *J. Comp. Physiol. A* **147**, 299–304 (1982).

27. W. Loher, Circadian rhythmicity of locomotor behavior and oviposition in female *Teleogryllus commodus*. *Behav. Ecol. Sociobiol.* **5**, 253–262 (1979).

28. Y. Moriyama, *et al.*, Timeless Plays an Important Role in Compound Eye-Dependent Photic Entrainment of the Circadian Rhythm in the Cricket *Gryllus bimaculatus*. *Zoolog. Sci.* **39**, 1–9 (2022).

29. K. Levy, B. Fishman, A. Barnea, A. Ayali, E. Tauber, Transcriptional Response of Circadian Clock Genes to an 'Artificial Light at Night' Pulse in the Cricket *Gryllus bimaculatus*. *Int. J. Mol. Sci.* **23**, 11358 (2022).

30. R. Rodríguez-Muñoz, A. Bretman, J. Slate, C. A. Walling, T. Tregenza, Natural and sexual selection in a wild insect population. *Science (80-.).* **328**, 1269–1272 (2010).

31. K. Tomioka, A. Matsumoto, "The circadian system in insects: Cellular, molecular, and functional organization" in *Advances in Insect Physiology*, 1st Ed., (Elsevier Ltd., 2019), pp. 73–115.

32. J. Durrant, *et al.*, Constant illumination reduces circulating melatonin and impairs immune function in the cricket *Teleogryllus commodus*. *PeerJ* **3**, e1075 (2015).

33. R. V. Frolov, E. V. Immonen, M. Weckström, Performance of blue- and green-sensitive photoreceptors of the cricket *Gryllus bimaculatus*. *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.* **200**, 209–219 (2014).

34. F. Zufall, M. Schmitt, R. Menzel, Spectral and polarized light sensitivity of photoreceptors in the compound eye of the cricket (*Gryllus bimaculatus*). *J. Comp. Physiol. A* **164**, 597–608 (1989).

35. J. Aschoff, "Biological Rhythms" in *Handbook of Behavioral Neurobiology*, first, J. Aschoff, Ed. (Plenum Press, 1981), p. 563.

36. D. S. Saunders, C. G. H. Steel, X. Vafopoulou, R. D. Lewis, "Circadian Rhythms of Activity in Individual Insects" in *Insect Clocks*, First, (Elsevier B.V, 2002), pp. 7–42.

37. R. George, R. Stanewsky, Peripheral Sensory Organs Contribute to Temperature Synchronization of the Circadian Clock in *Drosophila melanogaster*. *Front. Physiol.* **12**, 622545 (2021).

38. S. Yadlapalli, *et al.*, Circadian clock neurons constantly monitor environmental temperature to set sleep timing. *Nature* **555**, 98–102 (2018).

39. B. Rence, W. Loher, Arrhythmically singing crickets: Thermoperiodic reentrainment after bilobectomy. *Science (80-.).* **190**, 385–387 (1975).

40. N. N. Kannan, Y. Tomiyama, M. Nose, A. Tokuoka, K. Tomioka, Temperature Entrainment of Circadian Locomotor and Transcriptional Rhythms in the Cricket, *Gryllus bimaculatus*. *Zoolog. Sci.* **36**, 95 (2019).

41. J. A. Doherty, Temperature Coupling and 'Trade-Off' Phenomena in the Acoustic Communication System of the Cricket, *Gryllus bimaculatus* De Geer

(Gryllidae). *J. Exp. Biol.* **114**, 17–35 (1985).

42. L. B. Symes, R. L. Rodríguez, G. Höbel, Beyond temperature coupling: Effects of temperature on ectotherm signaling and mate choice and the implications for communication in multispecies assemblages. *Ecol. Evol.* **7**, 5992–6002 (2017).

43. L. W. Simmons, The calling song of the field cricket, *Gryllus bimaculatus* (de geer): constraints on transmission and its role in intermale competition and female choice. *Anim. Behav.* **36**, 380–394 (1988).

44. E. K. Thompson, N. L. Cullinan, T. M. Jones, G. R. Hopkins, Effects of artificial light at night and male calling on movement patterns and mate location in field crickets. *Anim. Behav.* **158**, 183–191 (2019).

45. T. Fuchikawa, A. Eban-Rothschild, M. Nagari, Y. Shemesh, G. Bloch, Potent social synchronization can override photic entrainment of circadian rhythms. *Nat. Commun.* **7**, 11662 (2016).

46. G. Bloch, E. D. Herzog, J. D. Levine, W. J. Schwartz, Socially synchronized circadian oscillators. *Proc. R. Soc. B Biol. Sci.* **280**, 20130035 (2013).

47. S. R. Lone, V. K. Sharma, Social synchronization of circadian locomotor activity rhythm in the fruit fly *Drosophila melanogaster*. *J. Exp. Biol.* **214**, 3742–3750 (2011).

48. J. Baxter-Gilbert, *et al.*, Nocturnal foraging and activity by diurnal lizards: Six species of day geckos (Phelsuma spp.) using the night-light niche. *Austral Ecol.* **46**, 501–506 (2021).

49. D. M. Dominoni, B. Helm, M. Lehmann, H. B. Dowse, J. Partecke, Clocks for the city: Circadian differences between forest and city songbirds. *Proc. R. Soc. B Biol. Sci.* **280** (2013).

50. A. B. Allema, *et al.*, Effect of light quality on movement of *Pterostichus melanarius* (Coleoptera: Carabidae). *J. Appl. Entomol.* **136**, 793–800 (2012).

51. R. Koch, Swift recorder users guide. Revision 2.1. 1–13 (2016).

52. R Core Team, R: A language and environment for statistical computing. 2020

(2020).

53. M. Araya-Salas, Rraven: connecting R and Raven Sound Analysis Software (2017).

54. Bioacoustics Research Program, Raven Pro: Interactive Sound Analysis Software (Version 1.5). Ithaca, NY: The Cornell Lab of Ornithology. Available from http://www.birds.cornell.edu/raven. 2014 (2013).

55. B. Schmid, C. Helfrich-Förster, T. Yoshii, A new ImageJ plug-in "actogramJ" for chronobiological analyses. *J. Biol. Rhythms* **26**, 464–467 (2011).

56. M. Moškon, CosinorPy: a python package for cosinor-based rhythmometry. *BMC Bioinformatics* **21**, 1–12 (2020).

57. W. L. Kovach, Oriana – Circular Statistics for Windows (2011).

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AUTHOR CONTRIBUTION

AA, AB, and KL conceived the study and wrote the manuscript; KL and SM carried out the experiments; KL and YW conducted the data analysis.

The authors have no competing interests related to this study.

DATA AND MATERIAL AVAILABILITY

The supplementary materials and data for this article can be found online at: https://figshare.com/s/b9c3ebc0b1c4c94ed5f2

doi:10.6084/m9.figshare.24058008

FIGURES AND CAPTIONS

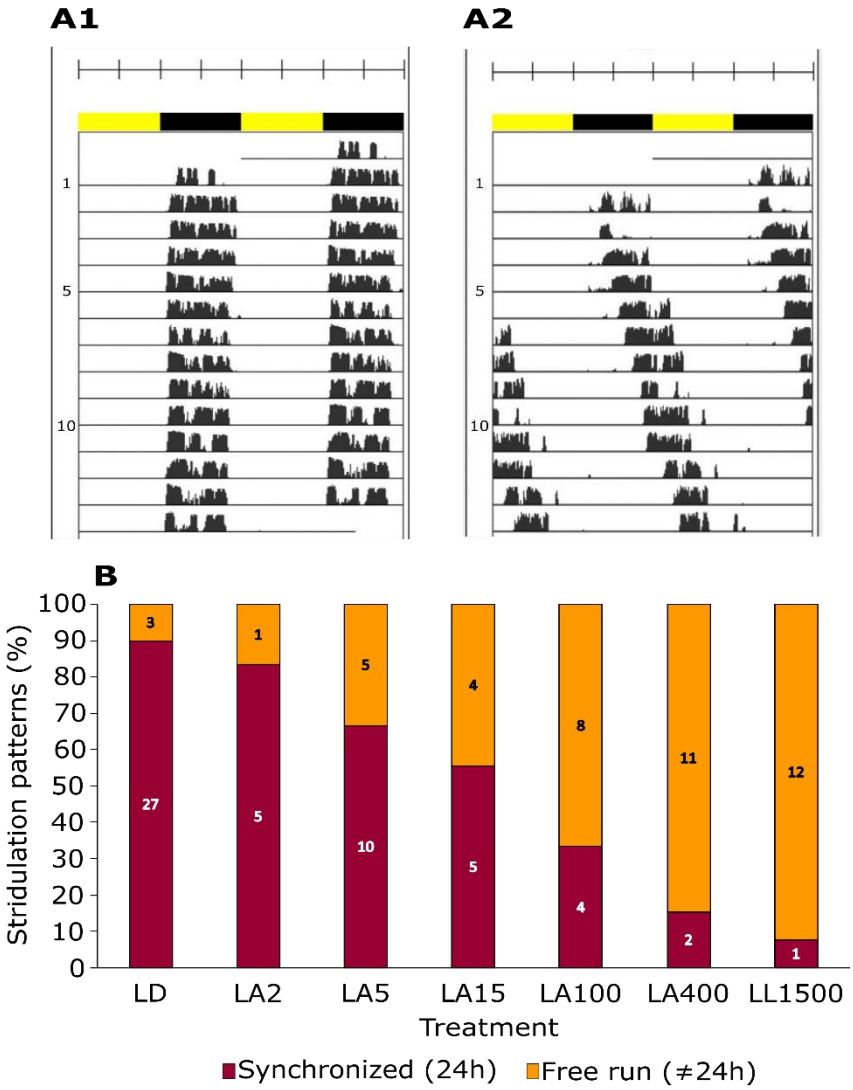

Figure 1. Daily stridulation activity patterns and their percentage in adult male crickets exposed to different ALAN intensities under semi-natural conditions: (**A**) Double-plotted actograms representing two rhythmic patterns: (**A1**) synchronized stridulation, with a 24 h period; (**A2**) free-running behavior over a period longer than 24 h (25.7 h in the example shown). (**B**) Percentage of synchronized (dark red), and free-run (orange) stridulation activity patterns observed in the experimental crickets. The number of synchronized and free-running individual crickets is indicated in the bars (in white and black, respectively; $n_{tot.}$=98). Yellow and black bars indicate diurnal and nocturnal phases, respectively.

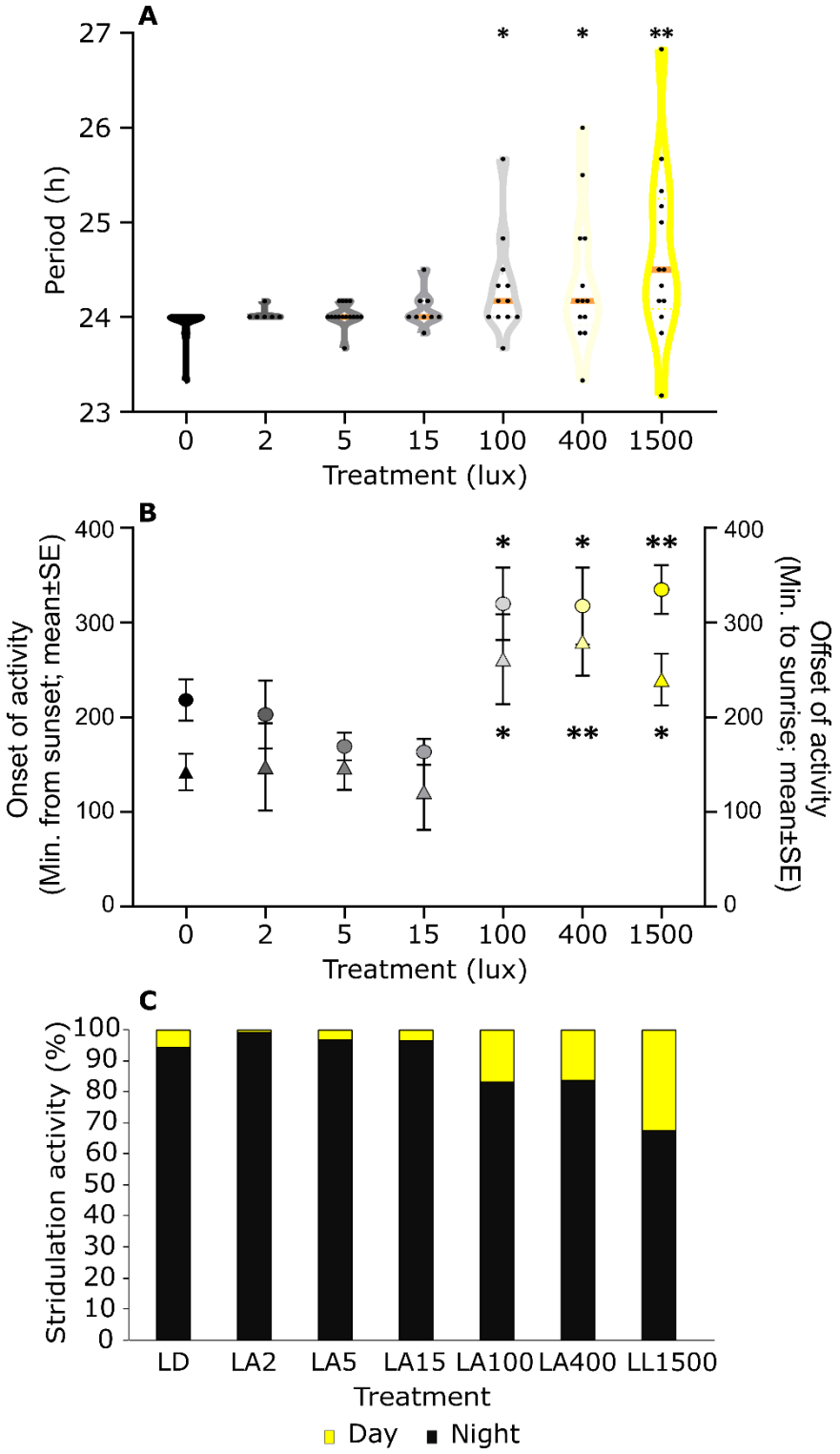

Figure 2. ALAN affects the periods, onset, offset, and timing of stridulation behavior. An increase: (**A**) in the medians of the cricket populations' stridulation periods; (**B**) in the mean timespan from sunset to the onset of activity (circles, upper asterisks), as well as from the offset of activity to sunrise (triangles, lower asterisks); and (**C**) in the percentage of diurnal and nocturnal stridulation. Medians are colored orange; each individual male is represented by a dot. When comparing only variances, 0 lux was found to differ from 100, 400, and 1,500 lux. $n_{tot.}$=98; * p<0.05, ** p<0.01.

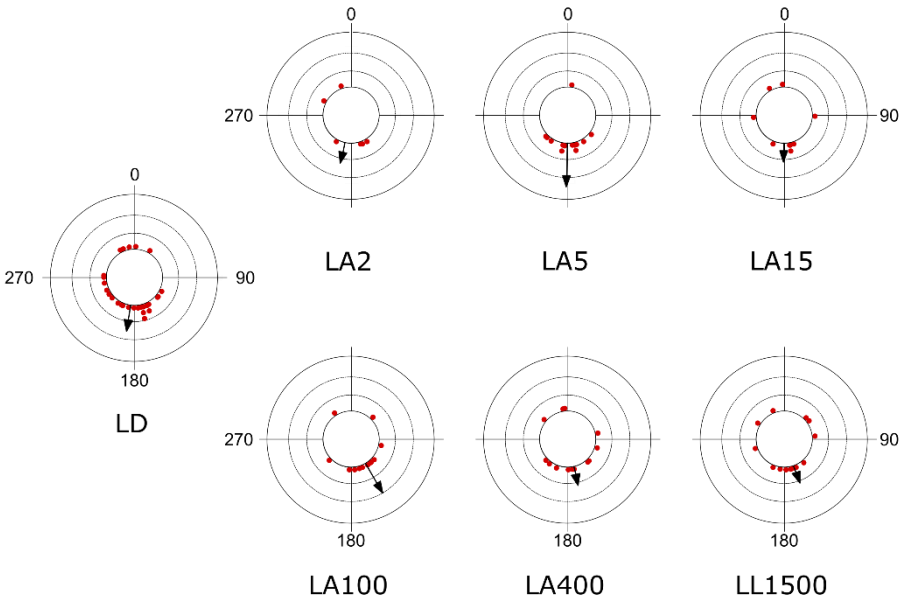

Figure 3. The stridulation acrophase calculations: Under semi-natural conditions, the individual male crickets' acrophase of stridulation behavior was not significantly affected by ALAN. Each red point in the circular plots represents the mean phase of a minimum of 5 days of stridulation behavior (n_{LD} =30; n_{LA2} =6; n_{LA5} =15; n_{LA15} =9; n_{LA100} =12; n_{LA400} =13; n_{LL1500} =13). Circle grid lines = 1.25. Each black arrow represents the mean vector of the phase, calculated for each experimental group.

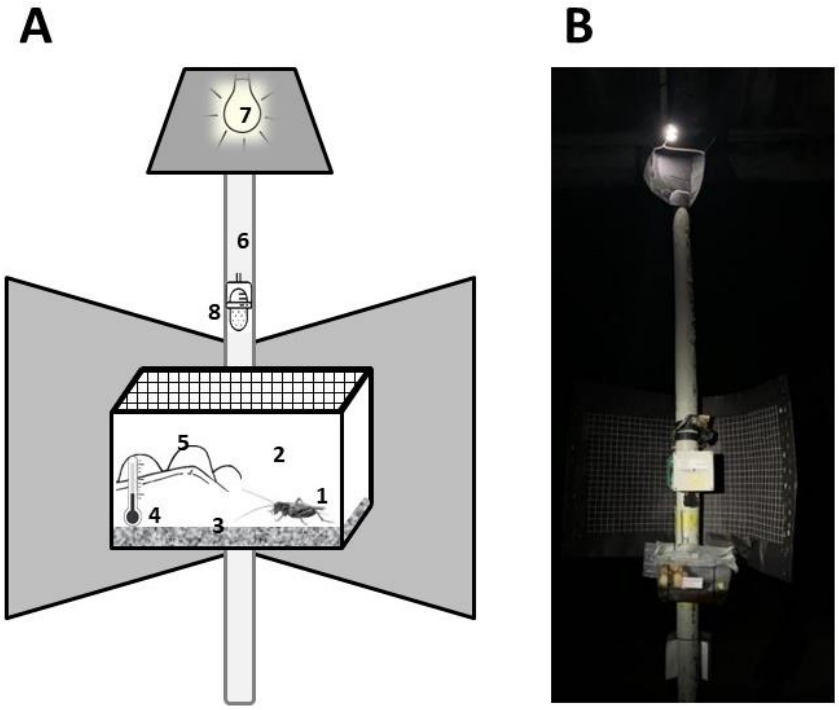

Figure 4: The set-up of the semi-natural ALAN experiment: (**A**) A cricket (1) was placed within a shaded, transparent enclosure (2), lined with cocopeat as substrate (3), a temperature logger (4), and an egg carton (5), attached to a metal pole (6), as well as an ALAN light source (7), and a Swift autonomous sound recording device 15 cm above the enclosure (8), which continuously recorded stridulation behavior. (**B**) The 5-lux ALAN treatment.

SUPPLEMENTARY FIGURES:

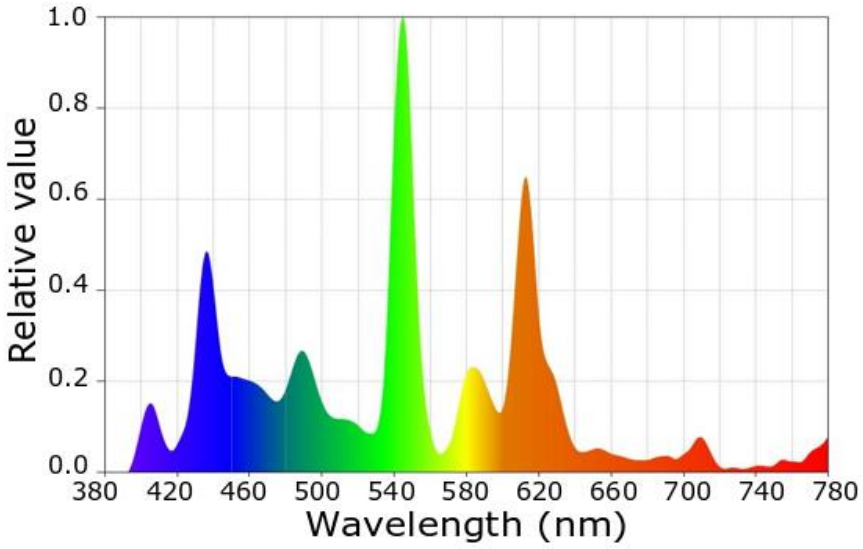

Figure S1. Spectrograms of the white, compact, fluorescent light bulb (CFL, NeptOn, 6500 K, 380-780 nm, peak: 547 & 612 nm). The light spectra were recorded using a Sekonic Sprectromaster C-700 (North White Plans, NY, USA).

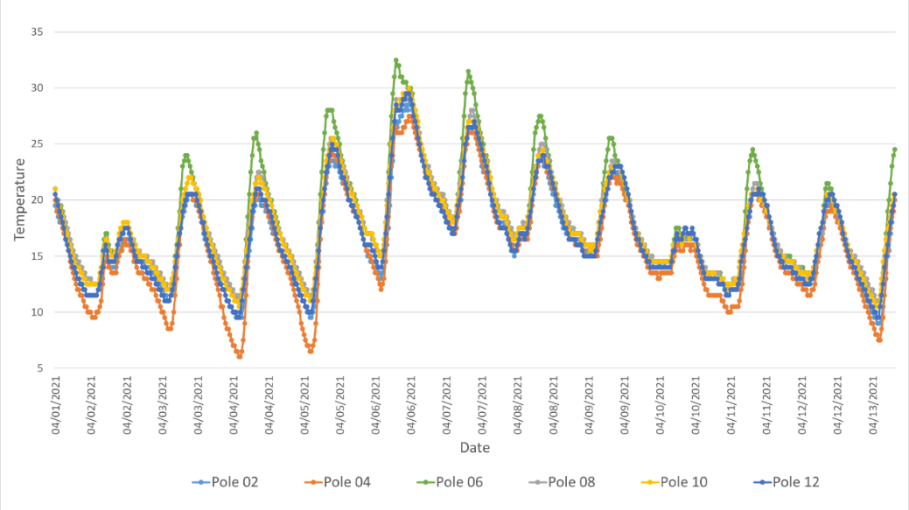

Figure S2. An example of a two-week-long period (01-13/04/2021), representing daily temperature rhythms of the six experimental poles in the semi-natural ALAN experiment.

Table S1: Sample sizes (N) and medians of stridulation activity rhythm periods of adult male crickets (*Gryllus bimaculatus*) exposed to seven ALAN treatments under semi-natural conditions. Only individuals with data presenting the activity of more than five days and nights, and with significant activity rhythm periods, are included.

Treatment	N	Median
LD	30	24.00
LA_2	6	24.00
LA_5	15	24.00
LA_{15}	9	24.00
LA_{100}	12	24.17
LA_{400}	13	24.17
$LL_{1,500}$	13	24.50

Table S2: Acrophase analyses of stridulation activity of individual crickets from the seven artificial light at night treatments under almost natural conditions.

Treatment	N	Mean (°)	Vector Length	Median (°)	Variance (°)	Circular SD (°)	SE (°)	95% CI (+/-)
LD	30	188.723	0.473	172.432°	0.527	70.109	14.693	159.919
LA_2	6	193.394	0.374	186.306°	0.626	80.319	60.877	74.05
LA_5	15	181.595	0.775	185.405°	0.225	40.947	11.621	158.814
LA_{15}	9	181.461	0.339	170.631°	0.661	84.288	51.113	81.259

LA_{100}	12	149.818	0.608	153.333°	0.392	57.154	17.712	115.095
LA_{400}	13	167.94	0.344	168.468°	0.656	83.722	37.5	94.425
LL_{1500}	13	160.914	0.329	168.468°	0.671	85.448	40.178	82.15

Table S3: Activity onset and offset analyses of stridulation activity of individual *Gryllus bimaculatus* adult male crickets exposed to seven artificial light at night treatments.

Treatment	N	Mean onset of activity (absolute minutes to sunset ± SE)	Mean offset of activity (absolute minutes from sunrise ± SE)
LD	30	221.0 ± 22.2	143.9 ± 19.5
LA_2	6	205.4 ± 36.4	149.3 ± 46.8
LA_5	15	171.2 ± 14.8	148.8 ± 24.1
LA_{15}	9	165.4 ± 13.9	122.6 ± 40.5
LA_{100}	12	323.9 ± 38.7	264.6 ± 48.1
LA_{400}	13	321.6 ± 41.1	283.4 ± 36.5
$LL_{1,500}$	13	339.2 ± 26.2	242.7 ± 27.7

Movie S1. A cricket from the LA_{400} treatment exhibiting diurnal stridulation. Please note the rooster in the background.