

Artificial light at night influences clock-gene expression, activity, and fecundity in the mosquito *Culex pipiens f. molestus*

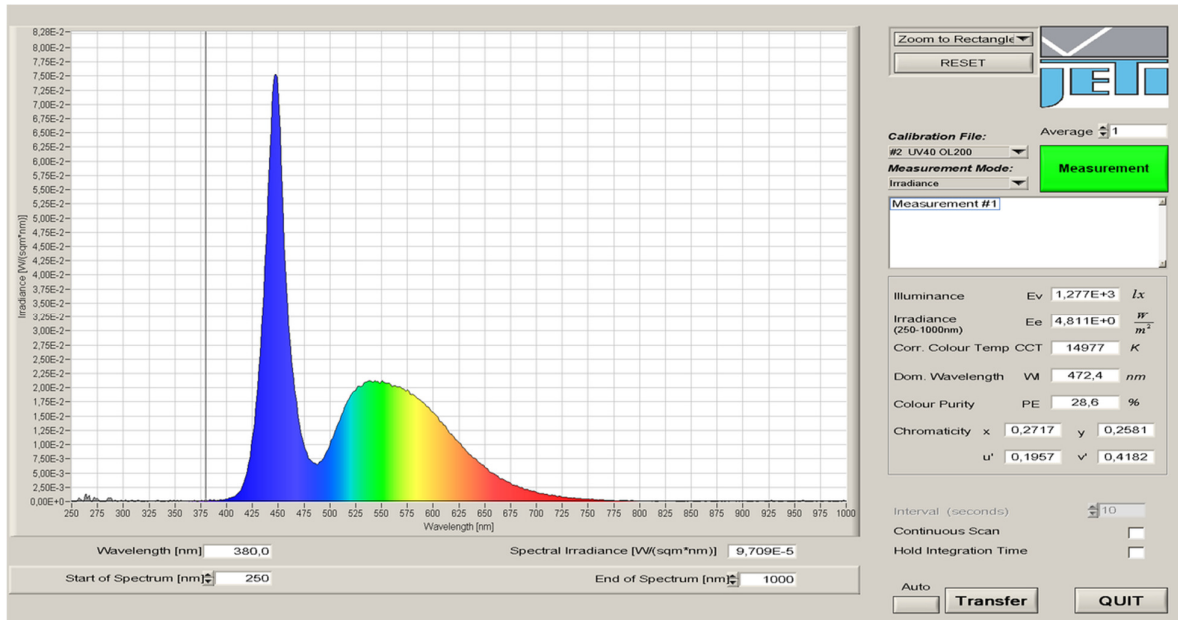
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Table S1. Light regulation in the two treatments in % of voltage and light intensity (lux) for each experiment.

| time of day | % voltage | gene expression / fecundity experiments | | Activity experiment | |
|-------------|-------------|---|-------------------|---------------------|-------------------|
| | | control [lux] | extra-light [lux] | control [lux] | extra-light [lux] |
| 04:00:00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 05:00:00 | 22.50 | 192.33 | 234.33 | 142.44 | 82.75 |
| 06:00:00 | 45.00 | 384.66 | 426.66 | 284.89 | 165.50 |
| 07:30:00 | 69.00 | 589.81 | 631.81 | 436.83 | 253.77 |
| 09:15:00 | 88.00 | 752.22 | 794.22 | 557.11 | 323.64 |
| 10:00:00 | 94.00 | 803.51 | 845.51 | 595.10 | 345.71 |
| 11:00:00 | 98.50 | 841.98 | 883.98 | 623.59 | 362.26 |
| 12:00:00 | 100.00 | 854.80 | 896.80 | 633.08 | 367.78 |
| 13:00:00 | 98.50 | 841.98 | 883.98 | 623.59 | 362.26 |
| 14:00:00 | 94.00 | 803.51 | 845.51 | 595.10 | 345.71 |
| 14:45:00 | 88.00 | 752.22 | 794.22 | 557.11 | 323.64 |
| 16:30:00 | 69.00 | 589.81 | 631.81 | 436.83 | 253.77 |
| 18:00:00 | 45.00 | 384.66 | 426.66 | 284.89 | 165.50 |
| 19:00:00 | 22.50 | 192.33 | 234.33 | 142.44 | 82.75 |
| 19:14:00 | 16.31 | 139.43 | 181.43 | 71.22 | 47.81 |
| 19:30:00 | 1.00 | 72.12 | 50.55 | 6.33 | 3.68 |
| 19:30:10 | 0.00/30.00* | 0.00 | 298.44 | 0.00 | 110.33 |
| 20:00:00 | 0.00/30.00* | 0.00 | 298.44 | 0.00 | 110.33 |
| 23:00:00 | 0.00/30.00* | 0.00 | 298.44 | 0.00 | 110.33 |
| 23:00:10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Entries marked with * show the % voltage in control/treatment, respectively.

Figure S1. Screenshot of the spectrum emitted by the LEDs used in the experiment (LED flex SMD, 24VDC, 24W, 1A, 60 LEDs/m, 500cm, cool-white single chip, Barthelme GmbH & Co. KG, Nuremberg, Germany; arranged in seven strips of 48 LEDs on a 88 x34 cm wooden board.) as measured with a SpecBos 1211UV (JETI Technische Instrumente GmbH, Jena, Germany). The human visible spectrum starts at about 400nm and ends around 700nm (colored area under the curve). Wavelengths shorter than 400nm belong to the ultraviolet and longer than 720nm belong to the infrared irradiance.



S2: Run settings for Stratageme MxPro3000/MxPro3005p

Thermal profile as reported in manuscript using ROX as reference dye.

Analysis term settings:

Baseline correction: adaptive baseline

Graphical temperature resolution Dissociation point separation: 0.5°

Threshold fluorescence: amplification-based threshold fluorescence lower range 5%, upper range 60%

Threshold has been set manually for each gene when exponential amplification was stable for all genes and used for all subsequent runs

Efficiency settings and thresholds:

Primer efficiency (E) is calculated as $E=10^{(-1/\text{slope})}$

cryptochrome2: R2: 0.981, slope: -3.323, Efficiency: 2.00, threshold: 0.090

period: R2: 0.994, slope: -3.557, Efficiency: 1.91, threshold: 0.080

timeless: R2: 0.993, slope: -3.323, Efficiency: 2.00, threshold: 0.080

Clock: R2: 0.992, slope: -3.834, Efficiency: 1.82, threshold: 0.190

cycle: R2: 0.998, slope: -3.276, Efficiency: 2.02, threshold: 0.190

ribosomal proteine 49: R2: 0.992, slope: -3.438, Efficiency: 1.95, threshold: 0.100

S3 GLMs of the target genes followed by pairwise comparison post-hoc tests.

Methods:

We then used generalized linear models (GLMs) to analyze changes in gene expression as a function of treatment, sex, length of exposure, and sampling time, as well as the interactions of treatment x sex, treatment x length of exposure, and treatment x sampling time. GLM analyses were performed for each gene separately in R 3.2.0 [59]. All models were run using a Gamma distribution with log link function with the significance level set to 0.05. The optimal link function, as well as the best-fit model, was determined using Akaike Information Criteria (AIC) and lowest residual deviance. To compare alternative models, we used the dropterm function within the mass package for R [60] by performing single deletion tests starting with the complete model. Within each model tested with the GLM extra-light was compared to control, males to females, 2- and 4-day lengths of exposure were compared to 1-day, and all timepoints were compared to timepoint 00:30. Significant contrasts in the interaction of treatment and time were examined with pairwise comparisons using the testInteractions function implemented in the phia package for R [61] with adjusted p-values using the Holm procedure. For this approach, every treatment is compared to every time point.

Table S3. GLM results for each gene (a=period, b=cryptochrome2, c=Sampling timeless, d=Clock, e=cycle) for the minimum adequate model. Significant terms are bold. Data is shown for extra-light compared to control, males to females, 2- and 4-day expositions to 1-day exposition and Sampling time in relation to timepoint 00:30. For genes with significant interaction terms (treatment x sampling time) in the minimum adequate GLM each table is followed by the results of the Holm post hoc interaction analysis as implemented in the phia package for R. Significant terms in bold.

a) GLM *period*

| Term | Estimate (<i>b</i>) | exp ^b | ± SE | t | P |
|-------------|-----------------------|------------------|------|-------|-----------------|
| Intercept | 0.37 | 1.45 | 0.07 | 5.02 | < 0.0001 |
| Extra-light | -0.66 | 0.52 | 0.10 | -6.38 | < 0.0001 |

Residual deviance 204.77 on 346 degrees of freedom

b) GLM timeless

| Term | Estimate (b) | exp^b | ± SE | t | P |
|-----------------------------------|---------------------|------------------------|-------------|----------|--------------------|
| Intercept | -1.49 | 0.23 | 0.27 | -5.43 | < 0.0001 |
| Extra-light | 0.37 | 1.45 | 0.36 | 1.03 | 0.30 |
| Sex | 0.43 | 1.54 | 0.11 | 3.80 | 0.0002 |
| Sampling time 17:30 | 0.56 | 1.74 | 0.36 | 1.53 | 0.13 |
| Sampling time 19:00 | 0.05 | 1.05 | 0.37 | 0.13 | 0.90 |
| Sampling time 19:20 | 1.62 | 5.06 | 0.37 | 4.40 | < 0.0001 |
| Sampling time 19:40 | 0.92 | 2.50 | 0.36 | 2.52 | 0.01 |
| Sampling time 20:00 | 1.55 | 4.70 | 0.38 | 4.07 | 0.0001 |
| Sampling time 20:30 | 1.16 | 3.19 | 0.36 | 3.19 | 0.002 |
| Sampling time 21:30 | 0.71 | 2.02 | 0.37 | 1.91 | 0.06 |
| Sampling time 22:30 | 0.12 | 1.13 | 0.37 | 0.34 | 0.74 |
| Sampling time 23:30 | 0.23 | 1.25 | 0.37 | 0.60 | 0.55 |
| Extra-light x Sampling time 17:30 | -0.79 | 0.45 | 0.51 | -1.53 | 0.13 |
| Extra-light x Sampling time 19:00 | 0.51 | 1.67 | 0.53 | 0.96 | 0.34 |
| Extra-light x Sampling time 19:20 | -1.40 | 0.25 | 0.50 | -2.80 | 0.01 |
| Extra-light x Sampling time 19:40 | 0.23 | 1.25 | 0.49 | 0.46 | 0.65 |
| Extra-light x Sampling time 20:00 | -0.98 | 0.38 | 0.52 | -1.87 | 0.06 |
| Extra-light x Sampling time 20:30 | -0.62 | 0.54 | 0.50 | -1.23 | 0.22 |
| Extra-light x Sampling time 21:30 | 0.03 | 1.03 | 0.52 | 0.06 | 0.95 |
| Extra-light x Sampling time 22:30 | -0.07 | 0.93 | 0.51 | -0.14 | 0.89 |
| Extra-light x Sampling time 23:30 | 0.49 | 1.63 | 0.51 | 0.95 | 0.34 |

Residual deviance 219.12 on 315 degrees of freedom

Post hoc tests for timeless

| Term | Estimate (b) | χ^2 | df | P |
|---|---------------------|----------------------------|-----------|-------------|
| Control vs. Extra-light x 00:30 vs. 17:30 | 0.45 | 2.35 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 19:00 | 1.67 | 0.93 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 19:20 | 0.25 | 7.83 | 1 | 0.21 |
| Control vs. Extra-light x 00:30 vs. 19:40 | 1.25 | 0.21 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 20:00 | 0.38 | 3.51 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 20:30 | 0.54 | 1.51 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 21:30 | 1.03 | 0.00 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 22:30 | 0.93 | 0.02 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 23:30 | 1.63 | 0.91 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:00 | 3.68 | 5.94 | 1 | 0.53 |
| Control vs. Extra-light x 17:30 vs. 19:20 | 0.54 | 1.49 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:40 | 2.76 | 4.21 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 20:00 | 0.83 | 0.13 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 20:30 | 1.19 | 0.12 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 21:30 | 2.27 | 2.46 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 22:30 | 2.04 | 1.93 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 23:30 | 3.58 | 6.25 | 1 | 0.46 |
| Control vs. Extra-light x 19:00 vs. 19:20 | 0.15 | 13.49 | 1 | 0.01 |
| Control vs. Extra-light x 19:00 vs. 19:40 | 0.75 | 0.31 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 20:00 | 0.23 | 7.59 | 1 | 0.23 |
| Control vs. Extra-light x 19:00 vs. 20:30 | 0.32 | 4.68 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 21:30 | 0.62 | 0.80 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 22:30 | 0.55 | 1.22 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 23:30 | 0.97 | 0.00 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 19:40 | 5.08 | 11.51 | 1 | 0.03 |
| Control vs. Extra-light x 19:20 vs. 20:00 | 1.52 | 0.69 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 20:30 | 2.18 | 2.57 | 1 | 1 |

| | | | | |
|---|------|-------|---|-------------|
| Control vs. Extra-light x 19:20 vs. 21:30 | 4.18 | 7.91 | 1 | 0.20 |
| Control vs. Extra-light x 19:20 vs. 22:30 | 3.76 | 7.05 | 1 | 0.30 |
| Control vs. Extra-light x 19:20 vs. 23:30 | 6.59 | 14.48 | 1 | 0.01 |
| Control vs. Extra-light x 19:40 vs. 20:00 | 0.30 | 5.75 | 1 | 0.58 |
| Control vs. Extra-light x 19:40 vs. 20:30 | 0.43 | 3.07 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 21:30 | 0.82 | 0.15 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 22:30 | 0.74 | 0.37 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 23:30 | 1.30 | 0.28 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 20:30 | 1.43 | 0.50 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 21:30 | 2.74 | 3.62 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 22:30 | 2.47 | 3.01 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 23:30 | 4.32 | 8.01 | 1 | 0.20 |
| Control vs. Extra-light x 20:30 vs. 21:30 | 1.91 | 1.61 | 1 | 1 |
| Control vs. Extra-light x 20:30 vs. 22:30 | 1.72 | 1.17 | 1 | 1 |
| Control vs. Extra-light x 20:30 vs. 23:30 | 3.01 | 4.91 | 1 | 0.91 |
| Control vs. Extra-light x 21:30 vs. 22:30 | 0.90 | 0.04 | 1 | 1 |
| Control vs. Extra-light x 21:30 vs. 23:30 | 1.58 | 0.77 | 1 | 1 |
| Control vs. Extra-light x 22:30 vs. 23:30 | 1.75 | 1.21 | 1 | 1 |
| <hr/> | | | | |
| Residuals | 315 | | | |

c) GLM *cryptochrome-2*

| Term | Estimate (b) | exp ^b | ± SE | t | P |
|----------------------------------|--------------|------------------|------|-------|-----------------|
| Intercept | -0.96 | 0.38 | 0.22 | -4.35 | < 0.0001 |
| Extra-light | 0.30 | 1.35 | 0.29 | 1.03 | 0.30 |
| Sex | 0.23 | 1.26 | 0.09 | 2.55 | 0.01 |
| Sampling time17:30 | 0.38 | 1.46 | 0.29 | 1.30 | 0.20 |
| Sampling time19:00 | 0.39 | 1.48 | 0.30 | 1.33 | 0.18 |
| Sampling time19:20 | 0.05 | 1.05 | 0.30 | 0.16 | 0.87 |
| Sampling time19:40 | 0.12 | 1.13 | 0.30 | 0.40 | 0.69 |
| Sampling time20:00 | 0.29 | 1.34 | 0.31 | 0.96 | 0.34 |
| Sampling time20:30 | 0.28 | 1.32 | 0.29 | 0.94 | 0.35 |
| Sampling time21:30 | 1.12 | 3.06 | 0.30 | 3.77 | 0.0002 |
| Sampling time22:30 | 0.54 | 1.71 | 0.30 | 1.82 | 0.07 |
| Sampling time23:30 | 0.32 | 1.38 | 0.30 | 1.07 | 0.28 |
| Extra-light x Sampling time17:30 | -0.76 | 0.47 | 0.41 | -1.84 | 0.07 |
| Extra-light x Sampling time19:00 | -0.83 | 0.43 | 0.43 | -1.94 | 0.05 |
| Extra-light x Sampling time19:20 | -0.28 | 0.76 | 0.40 | -0.69 | 0.49 |
| Extra-light x Sampling time19:40 | -0.08 | 0.92 | 0.40 | -0.20 | 0.84 |
| Extra-light x Sampling time20:00 | -0.69 | 0.50 | 0.42 | -1.66 | 0.10 |
| Extra-light x Sampling time20:30 | -0.50 | 0.60 | 0.40 | -1.25 | 0.21 |
| Extra-light x Sampling time21:30 | -1.09 | 0.34 | 0.42 | -2.62 | 0.01 |
| Extra-light x Sampling time22:30 | -0.62 | 0.54 | 0.41 | -1.51 | 0.13 |
| Extra-light x Sampling time23:30 | -0.14 | 0.87 | 0.41 | -0.35 | 0.73 |

Residual deviance 139.58 on 315 degrees of freedom

Post hoc test for cryptochrome-2

| Term | Estimate (b) | χ^2 | df | P |
|---|---------------------|----------------------------|-----------|----------|
| Control vs. Extra-light x 00:30 vs. 17:30 | 0.47 | 3.40 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 19:00 | 0.43 | 3.77 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 19:20 | 0.76 | 0.48 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 19:40 | 0.92 | 0.04 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 20:00 | 0.50 | 2.75 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 20:30 | 0.60 | 1.56 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 21:30 | 0.34 | 6.87 | 1 | 0.40 |
| Control vs. Extra-light x 00:30 vs. 22:30 | 0.54 | 2.28 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 23:30 | 0.87 | 0.12 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:00 | 0.93 | 0.03 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:20 | 1.62 | 1.44 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:40 | 1.98 | 2.85 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 20:00 | 1.07 | 0.03 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 20:30 | 1.29 | 0.41 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 21:30 | 0.72 | 0.62 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 22:30 | 1.15 | 0.11 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 23:30 | 1.86 | 2.29 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 19:20 | 1.74 | 1.76 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 19:40 | 2.13 | 3.21 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 20:00 | 1.16 | 0.11 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 20:30 | 1.39 | 0.62 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 21:30 | 0.77 | 0.35 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 22:30 | 1.23 | 0.24 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 23:30 | 2.00 | 2.64 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 19:40 | 1.22 | 0.26 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 20:00 | 0.66 | 1.03 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 20:30 | 0.80 | 0.33 | 1 | 1 |

| | | | | |
|---|------|------|---|------|
| Control vs. Extra-light x 19:20 vs. 21:30 | 0.44 | 4.02 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 22:30 | 0.71 | 0.74 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 23:30 | 1.15 | 0.12 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 20:00 | 0.54 | 2.25 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 20:30 | 0.65 | 1.16 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 21:30 | 0.36 | 6.17 | 1 | 0.57 |
| Control vs. Extra-light x 19:40 vs. 22:30 | 0.58 | 1.82 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 23:30 | 0.94 | 0.02 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 20:30 | 1.20 | 0.21 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 21:30 | 0.67 | 0.91 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 22:30 | 1.07 | 0.03 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 23:30 | 1.73 | 1.77 | 1 | 1 |
| Control vs. Extra-light x 20:30 vs. 21:30 | 0.56 | 2.08 | 1 | 1 |
| Control vs. Extra-light x 20:30 vs. 22:30 | 0.89 | 0.09 | 1 | 1 |
| Control vs. Extra-light x 20:30 vs. 23:30 | 1.44 | 0.82 | 1 | 1 |
| Control vs. Extra-light x 21:30 vs. 22:30 | 1.60 | 1.26 | 1 | 1 |
| Control vs. Extra-light x 21:30 vs. 23:30 | 2.58 | 5.29 | 1 | 0.92 |
| Control vs. Extra-light x 22:30 vs. 23:30 | 1.62 | 1.39 | 1 | 1 |
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| Residuals | 315 | | | |

d) GLM cycle

| Term | Estimate (b) | exp^b | ± SE | t | P |
|-----------------------------------|---------------------|------------------------|-------------|----------|--------------------|
| Intercept | -0.74 | 0.48 | 0.27 | -2.75 | 0.01 |
| Extra-light | 1.45 | 4.25 | 0.34 | 4.20 | < 0.0001 |
| Sex | 0.30 | 1.35 | 0.11 | 2.78 | 0.01 |
| 2-day exposition | -0.27 | 0.77 | 0.13 | -2.02 | 0.04 |
| 4-day exposition | -0.59 | 0.56 | 0.13 | -4.40 | < 0.0001 |
| Sampling time 17:30 | 0.94 | 2.56 | 0.34 | 2.73 | 0.01 |
| Sampling time 19:00 | 0.89 | 2.43 | 0.35 | 2.54 | 0.01 |
| Sampling time 19:20 | 0.75 | 2.12 | 0.35 | 2.15 | 0.03 |
| Sampling time 19:40 | 0.67 | 1.95 | 0.35 | 1.91 | 0.06 |
| Sampling time 20:00 | 0.88 | 2.41 | 0.36 | 2.45 | 0.02 |
| Sampling time 20:30 | 0.62 | 1.87 | 0.35 | 1.79 | 0.07 |
| Sampling time 21:30 | 1.72 | 5.56 | 0.35 | 4.92 | < 0.0001 |
| Sampling time 22:30 | 1.10 | 3.02 | 0.35 | 3.17 | 0.002 |
| Sampling time 23:30 | 0.58 | 1.79 | 0.35 | 1.65 | 0.10 |
| Extra-light x Sampling time 17:30 | -1.89 | 0.15 | 0.49 | -3.89 | 0.0001 |
| Extra-light x Sampling time 19:00 | -1.83 | 0.16 | 0.51 | -3.62 | 0.0003 |
| Extra-light x Sampling time 19:20 | -1.63 | 0.20 | 0.48 | -3.39 | 0.001 |
| Extra-light x Sampling time 19:40 | -1.13 | 0.32 | 0.47 | -2.39 | 0.02 |
| Extra-light x Sampling time 20:00 | -1.74 | 0.17 | 0.49 | -3.56 | 0.0004 |
| Extra-light x Sampling time 20:30 | -1.40 | 0.25 | 0.48 | -2.91 | 0.004 |
| Extra-light x Sampling time 21:30 | -2.09 | 0.12 | 0.49 | -4.26 | < 0.0001 |
| Extra-light x Sampling time 22:30 | -1.11 | 0.33 | 0.49 | -2.29 | 0.02 |
| Extra-light x Sampling time 23:30 | -0.45 | 0.64 | 0.48 | -0.93 | 0.36 |

Residual deviance 205.37 on 310 degrees of freedom

Post hoc test for cycle

| Term | Estimate (b) | χ^2 | df | P |
|---|--------------|----------|----|--------------|
| Control vs. Extra-light x 00:30 vs. 17:30 | 0.15 | 15.10 | 1 | 0.004 |
| Control vs. Extra-light x 00:30 vs. 19:00 | 0.16 | 13.10 | 1 | 0.01 |
| Control vs. Extra-light x 00:30 vs. 19:20 | 0.20 | 11.50 | 1 | 0.03 |
| Control vs. Extra-light x 00:30 vs. 19:40 | 0.32 | 5.70 | 1 | 0.58 |
| Control vs. Extra-light x 00:30 vs. 20:00 | 0.17 | 12.67 | 1 | 0.02 |
| Control vs. Extra-light x 00:30 vs. 20:30 | 0.25 | 8.44 | 1 | 0.14 |
| Control vs. Extra-light x 00:30 vs. 21:30 | 0.12 | 18.14 | 1 | 0.001 |
| Control vs. Extra-light x 00:30 vs. 22:30 | 0.33 | 5.24 | 1 | 0.73 |
| Control vs. Extra-light x 00:30 vs. 23:30 | 0.64 | 0.86 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:00 | 1.06 | 0.01 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:20 | 1.30 | 0.31 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:40 | 2.16 | 2.65 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 20:00 | 1.16 | 0.09 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 20:30 | 1.63 | 1.03 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 21:30 | 0.82 | 0.16 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 22:30 | 2.18 | 2.58 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 23:30 | 4.25 | 8.98 | 1 | 0.11 |
| Control vs. Extra-light x 19:00 vs. 19:20 | 1.23 | 0.17 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 19:40 | 2.03 | 2.08 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 20:00 | 1.09 | 0.03 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 20:30 | 1.54 | 0.73 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 21:30 | 0.77 | 0.25 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 22:30 | 2.05 | 2.02 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 23:30 | 4.00 | 7.60 | 1 | 0.22 |
| Control vs. Extra-light x 19:20 vs. 19:40 | 1.65 | 1.18 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 20:00 | 0.89 | 0.06 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 20:30 | 1.25 | 0.22 | 1 | 1 |

| | | | | |
|---|------|-------|---|-------------|
| Control vs. Extra-light x 19:20 vs. 21:30 | 0.63 | 0.91 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 22:30 | 1.67 | 1.16 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 23:30 | 3.26 | 6.17 | 1 | 0.45 |
| Control vs. Extra-light x 19:40 vs. 20:00 | 0.54 | 1.70 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 20:30 | 0.76 | 0.36 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 21:30 | 0.38 | 4.12 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 22:30 | 1.01 | 0.00 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 23:30 | 1.97 | 2.11 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 20:30 | 1.41 | 0.49 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 21:30 | 0.71 | 0.48 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 22:30 | 1.88 | 1.66 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 23:30 | 3.66 | 7.12 | 1 | 0.27 |
| Control vs. Extra-light x 20:30 vs. 21:30 | 0.50 | 1.98 | 1 | 1 |
| Control vs. Extra-light x 20:30 vs. 22:30 | 1.34 | 0.36 | 1 | 1 |
| Control vs. Extra-light x 20:30 vs. 23:30 | 2.60 | 3.99 | 1 | 1 |
| Control vs. Extra-light x 21:30 vs. 22:30 | 2.65 | 3.97 | 1 | 1 |
| Control vs. Extra-light x 21:30 vs. 23:30 | 5.16 | 11.40 | 1 | 0.03 |
| Control vs. Extra-light x 22:30 vs. 23:30 | 1.95 | 1.91 | 1 | 1 |
| <hr/> | | | | |
| Residuals | 310 | | | |

e) GLM Clock

| Term | Estimate (b) | exp ^b | ± SE | t | P |
|----------------------------------|--------------|------------------|------|-------|---------------|
| Intercept | -0.57 | 0.56 | 0.19 | -2.96 | 0.003 |
| Extra-light | 0.07 | 1.08 | 0.26 | 0.29 | 0.77 |
| Sex | 0.30 | 1.35 | 0.08 | 3.72 | 0.0002 |
| Sampling time 17:30 | 0.18 | 1.19 | 0.26 | 0.69 | 0.49 |
| Sampling time 19:00 | 0.11 | 1.12 | 0.26 | 0.44 | 0.66 |
| Sampling time 19:20 | -0.38 | 0.68 | 0.26 | -1.48 | 0.14 |
| Sampling time 19:40 | -0.35 | 0.71 | 0.26 | -1.35 | 0.18 |
| Sampling time 20:00 | -0.04 | 0.96 | 0.27 | -0.14 | 0.89 |
| Sampling time 20:30 | 0.03 | 1.03 | 0.26 | 0.11 | 0.92 |
| Sampling time 21:30 | 0.53 | 1.70 | 0.26 | 2.04 | 0.04 |
| Sampling time 22:30 | 0.28 | 1.33 | 0.26 | 1.08 | 0.28 |
| Sampling time 23:30 | -0.03 | 0.97 | 0.26 | -0.12 | 0.91 |
| Extra-light x Sampling time17:30 | -0.65 | 0.52 | 0.36 | -1.79 | 0.07 |
| Extra-light x Sampling time19:00 | -0.62 | 0.54 | 0.38 | -1.62 | 0.11 |
| Extra-light x Sampling time19:20 | -0.35 | 0.70 | 0.35 | -0.99 | 0.32 |
| Extra-light x Sampling time19:40 | 0.10 | 1.10 | 0.35 | 0.28 | 0.78 |
| Extra-light x Sampling time20:00 | -0.66 | 0.52 | 0.37 | -1.81 | 0.07 |
| Extra-light x Sampling time20:30 | -0.32 | 0.72 | 0.35 | -0.91 | 0.36 |
| Extra-light x Sampling time21:30 | -0.73 | 0.48 | 0.37 | -1.99 | 0.05 |
| Extra-light x Sampling time22:30 | -0.50 | 0.61 | 0.36 | -1.37 | 0.17 |
| Extra-light x Sampling time23:30 | 0.01 | 1.01 | 0.36 | 0.04 | 0.97 |

Residual deviance: 137.08 on 315 degrees of freedom

Post hoc tests for Clock

| Term | Estimate (b) | χ^2 | df | P |
|---|---------------------|----------------------------|-----------|----------|
| Control vs. Extra-light x 00:30 vs. 17:30 | 0.52 | 3.22 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 19:00 | 0.54 | 2.62 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 19:20 | 0.70 | 0.99 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 19:40 | 1.10 | 0.08 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 20:00 | 0.52 | 3.28 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 20:30 | 0.72 | 0.83 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 21:30 | 0.48 | 3.94 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 22:30 | 0.61 | 1.88 | 1 | 1 |
| Control vs. Extra-light x 00:30 vs. 23:30 | 1.01 | 0.00 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:00 | 1.03 | 0.01 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:20 | 1.35 | 0.72 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 19:40 | 2.11 | 4.55 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 20:00 | 0.99 | 0.00 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 20:30 | 1.39 | 0.85 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 21:30 | 0.93 | 0.04 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 22:30 | 1.17 | 0.18 | 1 | 1 |
| Control vs. Extra-light x 17:30 vs. 23:30 | 1.94 | 3.40 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 19:20 | 1.31 | 0.52 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 19:40 | 2.05 | 3.72 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 20:00 | 0.96 | 0.01 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 20:30 | 1.34 | 0.62 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 21:30 | 0.90 | 0.08 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 22:30 | 1.13 | 0.10 | 1 | 1 |
| Control vs. Extra-light x 19:00 vs. 23:30 | 1.88 | 2.77 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 19:40 | 1.56 | 1.73 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 20:00 | 0.73 | 0.77 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 20:30 | 1.03 | 0.01 | 1 | 1 |

| | | | | |
|---|------|------|---|------|
| Control vs. Extra-light x 19:20 vs. 21:30 | 0.69 | 1.11 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 22:30 | 0.86 | 0.17 | 1 | 1 |
| Control vs. Extra-light x 19:20 vs. 23:30 | 1.44 | 1.08 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 20:00 | 0.47 | 4.62 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 20:30 | 0.66 | 1.51 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 21:30 | 0.44 | 5.42 | 1 | 0.89 |
| Control vs. Extra-light x 19:40 vs. 22:30 | 0.55 | 2.88 | 1 | 1 |
| Control vs. Extra-light x 19:40 vs. 23:30 | 0.92 | 0.06 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 20:30 | 1.40 | 0.90 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 21:30 | 0.94 | 0.03 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 22:30 | 1.18 | 0.21 | 1 | 1 |
| Control vs. Extra-light x 20:00 vs. 23:30 | 1.96 | 3.47 | 1 | 1 |
| Control vs. Extra-light x 20:30 vs. 21:30 | 0.67 | 1.27 | 1 | 1 |
| Control vs. Extra-light x 20:30 vs. 22:30 | 0.84 | 0.24 | 1 | 1 |
| Control vs. Extra-light x 20:30 vs. 23:30 | 1.40 | 0.91 | 1 | 1 |
| Control vs. Extra-light x 21:30 vs. 22:30 | 1.26 | 0.39 | 1 | 1 |
| Control vs. Extra-light x 21:30 vs. 23:30 | 2.09 | 4.15 | 1 | 1 |
| Control vs. Extra-light x 22:30 vs. 23:30 | 1.66 | 2.01 | 1 | 1 |

Residuals

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Table S4. Pairwise Mann-Whitney-U –tests for differences among activity phases within treatment groups for LD (A) and DD (B) separately. Above diagonal: Z-values for each comparison; below diagonal: 2tailed P-values. Bonferroni-corrected significance level= 0.005; significant values in bold.

A)

| LD | control | | | | |
|-------------|---------------|---------------|---------------|---------------|-------------|
| | night | dawn | day | dusk | extra-light |
| night | ~ | -24.466 | -35.434 | -23.816 | -17.484 |
| dawn | 0.0001 | ~ | -14.212 | -10.663 | -24.685 |
| day | 0.0001 | 0.0001 | ~ | -25.947 | -32.023 |
| dusk | 0.0001 | 0.0001 | 0.0001 | ~ | -26.559 |
| extra-light | 0.0001 | 0.0001 | 0.0001 | 0.0001 | ~ |

| LD | extra-light | | | | |
|-------------|---------------|---------------|---------------|---------------|-------------|
| | night | dawn | day | dusk | extra-light |
| night | ~ | -27.96 | -34.894 | -29.457 | -25.635 |
| dawn | 0.0001 | ~ | -13.262 | -3.006 | -10.499 |
| day | 0.0001 | 0.0001 | ~ | -16.548 | -22.644 |
| dusk | 0.0001 | 0.003 | 0.0001 | ~ | -8.453 |
| extra-light | 0.0001 | 0.0001 | 0.0001 | 0.0001 | ~ |

B)

| DD | control | | | | |
|-------------|-------------------|---------------|---------------|---------------|---------|
| | phase | night | dawn | day | dusk |
| night | ~ | -15.784 | -33.679 | -1.392 | -19.531 |
| dawn | <0.0001 | ~ | -15.739 | -14.058 | -0.268 |
| day | 0.0001 | 0.0001 | ~ | -30.454 | -17.172 |
| dusk | 0.164 | 0.0001 | 0.0001 | ~ | -16.085 |
| extra-light | 0.0001 | 0.789 | 0.0001 | 0.0001 | ~ |

| DD | extra-light | | | | |
|-------------|---------------|---------------|---------------|---------|---------|
| | phase | night | dawn | day | dusk |
| night | ~ | -17.193 | -20.097 | -20.761 | -20.91 |
| dawn | 0.0001 | ~ | -5.343 | -24.613 | -22.705 |
| day | 0.0001 | 0.0001 | ~ | -30.693 | -29.724 |
| dusk | 0.0001 | 0.0001 | 0.0001 | ~ | -2.82 |
| extra-light | 0.0001 | 0.0001 | 0.0001 | 0.005 | ~ |

Table S5. Detailed results for the statistical analyses of activity A) Effect of ALAN on combined female and male activity within phases (dawn, day, dusk, trial, dark) for light:dark cycles (LD) and in constant darkness (DD); B) Female activity and C) male activity using MWU tests. n = number of ranks for each group, U = Mann-Whitney U, W= Wicoxons W, Z = test statistic corrected for ties.

A) Activity over all individuals

| stage | phase | mean activity control | mean activity extra-light | $p_{2tailed}$ | n_{ranks} | W | U | Z |
|-------|-------|-----------------------|---------------------------|-------------------|-------------|-----------|-----------|--------|
| LD | dawn | 0.098 | 0.053 | 0.001 | 743/790 | 578042500 | 265597500 | -3469 |
| | day | 0.010 | 0.008 | 0.205 | 825/825 | 672159000 | 331431000 | -1267 |
| | dusk | 0.105 | 0.038 | <0.0001 | 757/715 | 408142000 | 152172000 | -15030 |
| | trial | 0.419 | 0.051 | <0.0001 | 478/520 | 136165000 | 705000 | -27369 |
| | night | 0.251 | 0.213 | <0.0001 | 797/750 | 492681500 | 211056500 | -10052 |
| DD | dawn | 0.209 | 0.074 | <0.0001 | 790/790 | 450141500 | 137696500 | -19549 |
| | day | 0.076 | 0.050 | <0.0001 | 825/825 | 586193000 | 245468000 | -10105 |
| | dusk | 0.314 | 0.234 | <0.0001 | 715/715 | 437380500 | 181410500 | -9537 |
| | trial | 0.172 | 0.200 | <0.0001 | 520/520 | 228871500 | 93411500 | -8695 |
| | night | 0.295 | 0.114 | <0.0001 | 750/750 | 306408000 | 24783000 | -30723 |

B) Activity females

| stage | phase | mean activity control | mean activity extra-light | $p_{2tailed}$ | n_{ranks} | W | U | Z |
|-----------|-------|-----------------------|---------------------------|-------------------|-------------|----------|------------|--------|
| LD | dawn | 0.132 | 0.103 | 0.006 | 10402/5530 | 43612387 | 28319172 | -2.775 |
| | day | 0.017 | 0.029 | <0.0001 | 11550/5775 | 99644738 | 32937712.5 | -5.4 |
| | dusk | 0.148 | 0.102 | <0.0001 | 10598/5005 | 37822281 | 25294766 | -7.939 |
| | trial | 0.538 | 0.141 | <0.0001 | 6692/3640 | 14047810 | 7421190 | -38.72 |
| | night | 0.334 | 0.425 | <0.0001 | 11158/5250 | 86994110 | 24738049 | -19.09 |
| DD | dawn | 0.252 | 0.120 | <0.0001 | 10744/5530 | 40864567 | 25571352 | -20.83 |
| | day | 0.097 | 0.085 | 0.009 | 11220/5775 | 94935308 | 31985497.5 | -2.606 |
| | dusk | 0.329 | 0.352 | <0.0001 | 9655/5005 | 69546840 | 22932500 | -6.138 |
| | trial | 0.202 | 0.365 | <0.0001 | 6968/3640 | 35053720 | 10773724 | -16.92 |
| | night | 0.307 | 0.208 | <0.0001 | 10170/5250 | 37398090 | 23614215 | -15.35 |

C) Activity males

| stage | phase | mean activity control | mean activity extra-light | $p_{2tailed}$ | n_{ranks} | W | U | Z |
|-----------|-------|-----------------------|---------------------------|-------------------|-------------|---------------|------------|--------|
| LD | dawn | 0.063 | 0.051 | <0.0001 | 8186/10270 | 93925871 | 41184286 | -6.152 |
| | day | 0.003 | 0.001 | 0.015 | 9075/10725 | 10610000 0 | 48588787.5 | -2.435 |
| | dusk | 0.061 | 0.021 | <0.0001 | 8327/9295 | 80358493 | 37155332.5 | -13.53 |
| | trial | 0.307 | 0.026 | <0.0001 | 5258/6760 | 35573766 | 12721586 | -43.63 |
| | night | 0.166 | 0.198 | 0.664 | 8934/9750 | 90986655 | 43450530 | -0.435 |
| DD | dawn | 0.189 | 0.084 | <0.0001 | 8269/10270 | 90823054 | 38081468.5 | -20.5 |
| | day | 0.062 | 0.054 | <0.0001 | 8580/10725 | 10250000 0 | 45019260 | -6.82 |
| | dusk | 0.359 | 0.278 | <0.0001 | 7436/9295 | 74500355 | 31297194.5 | -13.17 |
| | trial | 0.171 | 0.225 | <0.0001 | 5408/6760 | 31774704 | 17148768 | -8.392 |
| | night | 0.333 | 0.116 | <0.0001 | 7920/9750 | 78162780 | 30626655 | -33.13 |

Table S6. Sex-specific activity levels (i.e. female activity compared to male activity) determined within treatment group and phase based on data points for each individual per 2-minute time interval recoded into an active/inactive matrix (see Methods). LD= light:dark cycles, DD=constant darkness. Calculations were done using Mann-Whitney-U tests. N= total number of ranks, Z= test statistic corrected for ties.

| LD | control | | | extra-light | | |
|-------------|---------|---------|---------------|-------------|---------|---------------|
| | N | Z | $P_{2tailed}$ | N | Z | $P_{2tailed}$ |
| night | 20092 | -27.091 | 0.0001 | 15000 | -42.574 | 0.0001 |
| dawn | 18588 | -15.456 | 0.0001 | 15800 | -17.504 | 0.0001 |
| day | 20625 | -9.552 | 0.0001 | 16500 | -16.241 | 0.0001 |
| dusk | 18925 | -19.028 | 0.0001 | 14300 | -21.28 | 0.0001 |
| extra-light | 11950 | -25.242 | 0.0001 | 10400 | -24.288 | 0.0001 |

| DD | control | | | extra-light | | |
|-------------|---------|--------|---------------|-------------|---------|---------------|
| | N | Z | $P_{2tailed}$ | N | Z | $P_{2tailed}$ |
| night | 18090 | -3.584 | 0.0001 | 15000 | -10.905 | 0.0001 |
| dawn | 19013 | -10.03 | 0.0001 | 15800 | -5.125 | 0.0001 |
| day | 19800 | -8.915 | 0.0001 | 16500 | -17.26 | 0.0001 |
| dusk | 17091 | -3.95 | 0.0001 | 14300 | -14.424 | 0.0001 |
| extra-light | 12376 | -4.247 | 0.0001 | 10400 | -12.939 | 0.0001 |

Table S7: Egg diameter variation over time tested using pairwise MWUs comparing sampling periods for each light treatment separately. Above diagonal are Z-scores, below diagonal are the p-values for the respective comparisons.

| | control | | | extra-light | | |
|---------|-----------------|-------------------|--------------------|-----------------|-------------------|--------------------|
| | March (n=93) | August (n=110) | October (n=271) | March (n=88) | August (n=125) | October (n=224) |
| March | | -8083 | -9584 | | -2813 | -1154 |
| August | <0.0001 | | -2844 | 0.005 | | -2700 |
| October | <0.0001 | 0.004 | | 0.248 | 0.007 | |