**Supplementary Materials for**

*Spectral composition of light pollution affects melatonin suppression and West Nile virus infection resistance and mortality in the House Sparrow (Passer domesticus).*

Meredith E Kernbach, Vincent M Cassone, Thomas R Unnasch, Lynn B Martin

Includes:

Supplementary Methods (*p.2-3*)

Supplementary Tables S1-S26 (*p.4-9*)

**Materials and Methods**

**Experimental Procedures**

House sparrows (*Passer domesticus*; N=71) were captured in the Tampa Bay area using mist nets during the months of October and November 2018. All birds were captured between the hours of 5:30 and 9:30 AM. Following capture, birds were transported to the USF campus vivarium and housed individually in 13”x15”x18” cages in visual and auditory proximity to one another under assigned conditions for 2 weeks. Food (mixed seeds) and water were provided *ad libitum* throughout the study. All birds were housed under a 12L:12D cycle; control birds were housed in complete darkness (0 lux; N=24) at night, wildlife-safe ALAN exposed birds were housed under 5 lux of 1800K LED light at night (N=12), broad-spectrum ALAN exposed birds were housed under 5 lux of 3000K LED light at night (N=11), and cool-white ALAN exposed birds were housed under 5 lux of 5000K LED light at night (N=24). After the first two weeks, birds were transported to the USF ABSL-3 facility and housed under identical lighting conditions inside bioBUBBLE containment systems (bioBUBBLE Inc, Fort Collins CO).

All birds were exposed to 101 PFUs of NY’99 WNV one day after transfer to BSL-3. Half of the birds in the control group (N=12) and the 5000K ALAN group (N=12) were administered 200ug/mL crystalline melatonin dissolved in 0.5% EtOH in drinking water at night, a method used in similar experiments to elevate melatonin levels in pinealectomized house sparrows, in attempt to restore circadian rhythms and alleviate melatonin suppression caused by ALAN exposure. Following WNV exposure, birds were sampled on days 2, 4, 6, and 10, and serum was stored at -20°C until viral RNA extractions were performed. Body mass (to 0.1g) was measured at exposure and all sampling timepoints to quantify individual and group health; mortality was closely monitored daily. All birds were euthanized on day 10 post-exposure at the conclusion of the study.

To determine viremia, WNV RNA was first extracted from 10 uL of stored serum using the Qiagen QIAmp Viral Extraction Mini Kit (Qiagen Cat. No. 52906). Standards were also extracted from known concentrations using the same methods. Following extractions, RNA was quantified using quantitative real-time polymerase chain reaction (qRT-PCR) using a one-step Taqman kit (iTaq Universal Probes One-Step Kit; Bio-Rad Cat. No. 1725141). All samples were run in duplicate with negative controls to detect potential contamination.

**Data Analysis**

All data analyses were performed in R studio (1). Our first goal was to determine whether melatonin administration in the control or 5000K ALAN exposed birds affected viremia, mortality, and tolerance. We first used a generalized linear mixed model in R studio package ‘lme4’ (non-normal distribution indicated by Shapiro test) with WNV titer as the dependent variable, day, treatment (only including control and 5000K), melatonin administered or sham, and their interactions (two and three-way) as fixed effects; bird id was a random effect. Next, we asked whether melatonin influenced mortality rates or tolerance during the infectious-to-vector period. Indeed, melatonin, treatment, and their interaction had no significant effect on mortality or tolerance. Detailed statistical outputs are depicted below.

To confirm that the 3000K and 5000K treatments did not differ, we constructed a linear mixed model in R studio package ‘nlme’ (normal distribution without heteroskedasticity indicated by Shapiro and Bartlett tests) with WNV titer as the dependent variable, day, treatment (only including 3000K and 5000K), and their interaction as fixed effects, and bird id as a random effect. Day was the only significant term in the model; the statistics are portrayed in the tables below. To further confirm that there was no statistical difference between the 3000K and 5000K treatment groups, we built a Cox proportional hazards analyses to determine effects of treatment, viremia, and vigor on mortality. We found that average viremia was the only significant predictor of mortality. Below are the specific statistics for this analysis. Finally, we confirmed that there was no difference between 3000K and 5000K groups regarding tolerance. To calculate tolerance, we first calculated the average change in body mass since exposure and the average viremia from days 2, 4, 6, and 10 post-exposure to summarize an individual’s health across the course of infection. The population average was then calculated and each individual’s residual from their predicted value served as their tolerance parameter (how much more body mass or less body mass did the individual maintain per their average viremia compared to all other individuals). We used a generalized linear model (non-normal distribution indicated by Shapiro test) with treatment, viremia, treatment\*viremia, and vigor as fixed effects. Vigor was the only significant predictor of tolerance in this model, so we concluded 3000K and 5000K bird tolerance did not differ. Model statistics are detailed below.

When assessing residual variation in health, we first calculated average percent change body mass since exposure across the course of infection and total average viremia in each individual. Using a linear regression, we could then calculate each residual value from their predicted average percent change body mass as a function of their average WNV viremia based on the population average. This residual value thus served as an individual’s measure of health; basically, how much more or less an individual maintained their body mass than was predicted based on their average viral burden. We used a generalized linear model (GLM) in R studio’s ‘lme4’ package where residual variance was the dependent variable and treatment, viremia, their interaction, and vigor were fixed effects.

Finally, the survival analyses were performed in R studio using ‘survival’, ‘survminer’, and ‘dplyr’ packages for the cox proportional hazards method. Time to death and censorship (died or survived until the conclusion of experiment) were incorporated as the dependent variables. The initial analysis was performed using just treatment as a predictor of mortality. Subsequently, once initial differences were detected, two different analyses were performed to avoid over-complicating models. The first iteration incorporated treatment, residual variation of health, their interaction, and vigor as fixed effects and the second iteration incorporated treatment, average viremia, their interaction, and vigor as fixed effects. This was done to determine if differences in mortality among treatment groups were driven by interactions between viremia and health status.

**Tables of Supplementary Statistics**

Below are the tables of supplementary statistics in order of appearance in the manuscript. We first show statistics to demonstrate the null results from melatonin administration. Then, we show the null results from comparing 3000K and 5000K treatments which justifies the combination of these wavelengths into a “broad-spectrum” wavelength group.

|  |  |  |
| --- | --- | --- |
| *Shapiro-Wilk Normality Test* | W value | P value |
| Viremia | 0.95821 | 0.0006078 |

**Table S1. Melatonin Administration Viremia Distribution.** Shapiro-Wilk test for normal distribution of data. Significant p-value indicated that WNV titer between MEL admin and sham individuals was not normally distributed.

|  |  |  |  |
| --- | --- | --- | --- |
| *Type III ANOVA* | Chi Sq value | Degrees of Freedom | P value |
| MEL | 0.0529 | 1 | 0.8181707 |
| Day | 17.5512 | 3 | 0.0005443 |
| MEL:day | 0.6182 | 3 | 0.8922593 |

**Table S2. Melatonin Administration Viremia Type III ANOVA.** Summary of main effects of type III ANOVA of the GLMM model below where WNV viremia (titer) is the dependent variable, melatonin (MEL), day, and their interaction are fixed effects, and bird id is a random effect. Indeed, melatonin and its interaction with day do not affect viremia.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *GLMM fit by ‘ML’, Gamma Distribution* | Estimate | Std error | T value | P value |
| MEL | -0.0024998 | 0.0108736 | -0.230 | 0.81817 |
| Day(L) | 0.1303988 | 0.0332830 | 3.918 | 8.93e-05\*\*\* |
| Day(Q) | 0.0860951 | 0.0283174 | 3.040 | 0.00236\*\* |
| Day(C) | 0.0148198 | 0.0232082 | 0.639 | 0.52311 |
| MEL:day(L) | -0.0157864 | 0.0208790 | -0.756 | 0.44960 |
| MEL:day(Q) | -0.0059293 | 0.0176832 | -0.335 | 0.73740 |
| MEL(C) | 0.0001207 | 0.0140931 | 0.009 | 0.99317 |

**Table S3. Melatonin Administration Alone on Viremia GLMM Output**. Statistical output for a GLMM in R studio ‘lme4’ package. Treatment term as a fixed effect was dropped from the model as the model would not converge at this level of complexity and our main question was regarding the role of melatonin in viral resistance. This confirms that melatonin had no effect on WNV titer.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *CoxPH Survival Analysis* | Coef | Exp(coef) | Se(coef) | Z score | P value |
| Treatment | -1.42e+01 | 6.84e-07 | 1.47e+01 | -0.97 | 0.333 |
| MEL | -1.20e+01 | 5.95e-06 | 8.63e+00 | -1.39 | 0.163 |
| Viremia | -2.90e+00 | 5.51e-02 | 2.23e+00 | -1.30 | 0.193 |
| Vigor | 4.66e-01 | 1.59e+00 | 2.48e-01 | 1.88 | 0.061 |
| Treatment:MEL | 1.33e+01 | 6.20e+05 | 1.03e+01 | 1.29 | 0.196 |
| Treatment:viremia | 3.08e+00 | 2.17e+01 | 2.56e+00 | 1.21 | 0.228 |
| MEL:viremia | 2.09e+00 | 8.06e+00 | 1.49e+00 | 1.40 | 0.161 |
| Treatment:MEL:viremia | -2.64e+00 | 7.11e-02 | 1.81e+00 | -1.46 | 0.145 |

**Table S4. Melatonin Administration\*Treatment on Viremia GLMM Output.** Statistical output of cox proportional hazards analysis to determine whether melatonin administration influenced mortality rates. Indeed, the only marginally significant predictor of mortality was vigor (body condition in absence of infection).

|  |  |  |
| --- | --- | --- |
| *Shapiro-Wilk Normality Test* | W value | P value |
| Residual Variation in Body Mass | 0.84972 | 2.137e-05 |

**Table S5. Residual Variation in Body Mass Distribution.** Shapiro-Wilk test to determine normality. The p-value is significant, indicating that the data is not normally distributed.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *GLM Residual Var Body Mass* | Estimate | Std. Error | T value | P value |
| Treatment | -0.0002726 | -0.1652612 | -0.002 | 0.998692 |
| MEL | -0.0093786 | 0.1023457 | 0.092 | 0.927456 |
| Viremia | -0.0027277 | 0.0233191 | -0.117 | 0.907483 |
| Vigor | 0.0112710 | 0.0031364 | 3.594 | 0.000903\*\*\* |
| Treatment:MEL | -0.0353297 | 0.1171529 | -0.302 | 0.764583 |
| Treatment:Viremia | -0.0011187 | 0.0268656 | -0.042 | 0.966997 |
| MEL:Viremia | -0.0028335 | 0.0175894 | -0.161 | 0.872851 |
| Treatment:MEL:Viremia | 0.0063113 | 0.0198555 | 0.318 | 0.752285 |

**Table S6. Melatonin Administration\*Treatment\*Viremia on Residual Variation in Body Mass GLM.** Statistical output of a generalized linear model where we asked whether treatment, melatonin, viremia, or vigor predicted tolerance measurements. Indeed, no differences in tolerance existed between melatonin administered and sham individuals and the only significant predictor of tolerance was vigor.

|  |  |  |
| --- | --- | --- |
| *Shapiro-Wilk Normality Test* | W value | P value |
| Viremia | 0.97697 | 0.1145 |

**Table S7. Viremia Distribution between 3000K and 5000K Treatments.** Shapiro-Wilk test to determine normal distribution of data. Non-significant P-values indicate normal distribution.

|  |  |  |  |
| --- | --- | --- | --- |
| *Bartlett Test of Homogeneity* | K squared | Degrees of Freedom | P value |
| Viremia~treatment\*day | 11.175 | 7 | 0.1312 |

**Table S8. Bartlett Test of Homogeneity.** Bartlett test to determine homogeneity of variances. Non-significant P-values indicate there is no heteroskedasticity or unequal variance among groups.

|  |  |  |  |
| --- | --- | --- | --- |
| *Type III ANOVA* | Chi Sq | Degrees of Freedom | P value |
| Treatment | 4.8539 | 3 | 0.1828 |

**Table S9. 3000K and 5000K Type III ANOVA.** Type III ANOVA test of main effects of the linear mixed model built in ‘nlme’. Day is the only significant fixed effect in this model, so we concluded there are no difference between 3000K and 5000K treatments on viremia.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Cox PH Model* | Coef | Exp(coef) | SE(coef) | Z score | P value |
| Treatment | 0.19382 | 1.21388 | 3.19598 | 0.061 | 0.95164 |
| Viremia | 1.09994 | 3.00397 | 0.35933 | 3.061 | 0.00221\*\* |
| Vigor | -0.03809 | 0.96263 | 0.25329 | -0.150 | 0.88047 |
| Treatment:Viremia | 0.03031 | 1.03077 | 0.43621 | 0.069 | 0.94460 |

**Table S10. 3000K and 5000K Effects on Mortality in a Cox Proportional Hazards Model.** Statistical output for the cox proportional hazards model to determine effects of survival where treatment, viremia, their interaction, and vigor were integrated as fixed effects. The only significant predictor of mortality was viremia, so we concluded that treatments 3000K and 5000K again did not statistically differ in mortality rates.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *GLM, Gamma Distribution* | Estimate | Std. Error | T value | P value |
| Treatment | 0.009165 | 0.041454 | 0.221 | 0.8266 |
| Viremia | -0.001570 | 0.005246 | -0.299 | 0.7668 |
| Vigor | 0.010455 | 0.004215 | 2.480 | 0.0192\* |
| Treatment:Viremia | -0.001771 | 0.006367 | -0.278 | 0.7829 |

**Table S11. 3000K and 5000K Effects on Body Mass in a GLM.** Statistical output of the generalized linear model used to determine whether 3000K or 5000K treatments differed in change in body mass. Vigor was the only significant predictor of tolerance, therefore, we concluded that the treatments did not differ regarding tolerance statistically.

**Tables of Main Statistics**

Below are the tables containing full statistical output for all analyses discussed in the manuscript in order of appearance.

|  |  |  |  |
| --- | --- | --- | --- |
| *Type III ANOVA* | ChiSq | Degrees of Freedom | P value |
| Treatment | 0.02255 | 1 | 0.8806 |
| Time | 0.69236 | 1 | 0.4054 |
| Treatment:Time | 0.17504 | 1 | 0.6757 |

**Table S12. Melatonin Pre-Exposure**. Type III ANOVA output to determine melatonin pre-exposure concentrations between the control and treatment groups. No significant differences existed between groups.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *GLM Gamma Distribution* | Estimate | Std Error | T value | P value |
| Treatment | 4.131e-04 | 2.749e-03 | 0.150 | 0.8809 |
| Time | -9.931e-05 | 1.193e-04 | -0.832 | 0.4075 |
| Treatment:Time | -6.109e-05 | 1.461e-04 | -0.418 | 0.6768 |

**Table S13. Melatonin Pre-Exposure.** Output of the generalized linear model where melatonin concentration was the dependent variable, and treatment, time, and their interaction were fixed effects pre-exposure. We confirmed that there were no significant pre-existing differences.

|  |  |  |  |
| --- | --- | --- | --- |
| *Type III ANOVA* | ChiSq | Degrees of Freedom | P value |
| Pre-/Post-Exposure | 7.7698 | 1 | 0.005313\*\* |
| Time | 2.8776 | 1 | 0.089817 |
| Pre-/Post-Exposure:Time | 1.9442 | 1 | 0.163214 |

**Table S14. Pre-/Post-ALAN Exposure Effects on Melatonin [Within-Group].** Main effects determined by Type III ANOVA for Pre-/Post-Exposure comparison of ALAN exposed birds GLM model with a gamma distribution. There is a significant main effect of exposure on melatonin concentration.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *GLM Gamma Distribution* | Estimate | Std Error | T value | P value |
| Pre-/Post-Exposure | 4.155e-02 | 1.556e-02 | 2.670 | 0.00897\*\* |
| Time | -1.604e-04 | 9.504e-05 | -1.688 | 0.09485 |
| Pre-/Post-Exposure | -1.135e-03 | 8.176e-04 | -1.389 | 0.16827 |

**Table S15. Pre-/Post-ALAN Exposure Effects on Melatonin [Within-Group].** Output of the generalized linear model where melatonin concentration was the dependent variable, and pre-/post-exposure, time, and their interaction were fixed effects. ALAN exposure had a significant effect on melatonin concentration.

|  |  |  |  |
| --- | --- | --- | --- |
| *Type III ANOVA* | ChiSq | Degrees of Freedom | P value |
| Treatment | 2.952 | 1 | 0.08575 |
| Pre-/Post-Exposure | 0.829 | 1 | 0.36262 |
| Time | 6.369 | 1 | 0.01161\* |
| Treatment:Exposure | 186.419 | 1 | < 2e-16\*\*\* |

**Table S16. Effects of ALAN Exposure on Melatonin Concentrations.** Main effects determined by Type III ANOVA for ALAN-exposed vs control melatonin concentration GLM model with a gamma distribution. There is a significant main effect of the interaction between treatment and exposure on melatonin concentration, indicating that melatonin concentrations were only suppressed when individuals are exposed to ALAN.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *GLM Gamma Distribution* | Estimate | Std. Error | T value | P value |
| Treatment | -7.254e-04 | 4.294e-04 | -1.689 | 0.0929 |
| Pre-/Post-Exposure | -4.104e-04 | 4.531e-04 | -0.906 | 0.3663 |
| Time | -1.555e-04 | 6.173e-05 | -2.518 | 0.0127\* |
| Treatment:Exposure | 2.099e-02 | 2.245e-03 | 9.349 | < 2e-16\*\*\* |

**Table S17. Effects of ALAN Exposure on Melatonin Concentrations.**  Output of the generalized linear model where melatonin concentration was the dependent variable, and treatment, time, and their interaction were fixed effects post-exposure. The interaction between treatment and exposure significantly affected melatonin concentrations, further confirming that only exposure to ALAN affected melatonin.

|  |  |  |  |
| --- | --- | --- | --- |
| *Type III ANOVA* | ChiSq | Degrees of Freedom | P value |
| Day | 76.3587 | 3 | 2e-16\*\*\* |
| Treatment | 6.9942 | 2 | 0.03029\* |
| Day:Treatment | 8.7008 | 6 | 0.19111 |

**Table S18. Main Effects of Treatment, Time, and Their Interaction on WNV Viremia.** Main effects on viremia determined by Type III ANOVA for GLMM fit by ‘ML’ and gamma distribution. Treatment and day are both significant main effects on viremia (log10 WNV PFU).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *GLMM fit by ‘ML’, Gamma Distribution* | Estimate | Std Error | T value | P Value |
| Day (linear) | 0.101127 | 0.012972 | 7.796 | 6.41e-15\*\*\* |
| Day (quadratic) | 0.073140 | 0.010849 | 6.742 | 1.57e-11\*\*\* |
| Day (cubic) | 0.014650 | 0.008698 | 1.684 | 0.09212 |
| 1800K Treatment | 0.031555 | 0.012474 | 2.530 | 0.01142\* |
| 3000+5000K Treatment | 0.002687 | 0.009257 | 0.290 | 0.77165 |
| Day(L):1800K | 0.067238 | 0.024680 | 2.724 | 0.00644\*\* |
| Day(Q):1800K | 0.041429 | 0.020729 | 1.999 | 0.04565\* |
| Day(C):1800K | 0.015780 | 0.016147 | 0.977 | 0.32842 |
| Day(L):3000+5000K | 0.006164 | 0.018069 | 0.341 | 0.73300 |
| Day(Q):3000+5000K | 0.006026 | 0.015218 | 0.396 | 0.69214 |
| Day(C):3000+5000K | -0.003912 | 0.011954 | -0.327 | 0.74351 |

**Table S19. Detailed Statistics of Treatment, Time, and Their Interaction on WNV Viremia GLMM Output.** Main statistic output for the GLMM fit by ‘ML’ and gamma distribution to determine the effects of treatment, day, and their interaction on WNV viremia (log10 PFU). Day as a linear and quadratic function are both highly significant, which is unsurprising because it is just saying that viremia is different across time. The 1800K treatment and its interaction across time are both significant.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *GLMM fit by ‘ML’, Gamma Distribution* | Estimate | Std Error | T value | P Value |
| 1800K (2) | -0.0117398 | 0.0099515 | -1.180 | 0.2381 |
| 3000+5000K (3) | 0.0006638 | 0.0074137 | 0.090 | 0.9287 |
| Day (linear) | -0.0064685 | 0.0046877 | -1.380 | 0.1676 |
| Day (quadratic) | -0.0035572 | 0.0044511 | -0.799 | 0.4242 |
| Day (cubic) | 0.0017896 | 0.0043874 | 0.408 | 0.6834 |
| 1800K:Day (L) | 0.0026512 | 0.0073996 | 0.358 | 0.7201 |
| 3000+5000K:Day (L) | 0.0130326 | 0.0062909 | 2.072 | 0.0383\* |
| 1800K:Day (Q) | 0.0064073 | 0.0068622 | 0.934 | 0.3505 |
| 3000+5000K:Day (Q) | 0.0019145 | 0.0060900 | 0.314 | 0.7532 |
| 1800K:Day (C) | -0.0031822 | 0.0066844 | -0.476 | 0.6340 |
| 3000+5000K:Day (C) | -0.0044053 | 0.0060896 | -0.723 | 0.4694 |

**Table S20. Detailed Statistics of Treatment, Time, and Their Interaction on Percent Change Body Mass GLMM Output.** GLMM output for percent change body mass across the course of infection where treatment, day, and their interaction were fixed effects and bird ID was a random effect. There was a significant interaction between broad-spectrum (3000+5000K) and day as a quadratic function.

|  |  |  |  |
| --- | --- | --- | --- |
| *Cox Proportional Hazards Model* | X2 | DF | P Value |
| Treatment | 5.7400 | 2 | 0.0567 |
| Vigor | 0.8902 | 1 | 0.3454 |

**Table S21. Effects of Treatment and Vigor on Mortality.** Cox proportional hazards model in Rstudio using packages ‘survival’ and ‘survminer’. Vigor has no effect on mortality, as demonstrated here, so it is not included in further iterations of the model.

|  |  |  |  |
| --- | --- | --- | --- |
| *Cox Proportional Hazards Model* | X2 | DF | P Value |
| Treatment | 6.0217 | 2 | 0.0493\* |

**Table S22. Effects of Treatment Alone on Mortality.** Cox proportional hazards model after removing vigor from the model and only accounting for fixed effects of treatment on survival.

|  |  |  |  |
| --- | --- | --- | --- |
| *Type III ANOVA* | X2 | DF | P Value |
| Treatment | 7.3586 | 2 | 0.02524\* |
| Average % Δ Body Mass | 4.0220 | 1 | 0.04491\* |
| Average Viremia | 15.2721 | 1 | 9.31e-05\*\*\* |
| Treatment:Average % Δ Body Mass | 3.9696 | 2 | 0.13741 |
| Treatment:Average Viremia | 6.5208 | 2 | 0.03837\* |
| Average % Δ Body Mass:Average Viremia | 4.3120 | 1 | 0.03785\* |
| Treatment:Avg%ΔBodyMass:AvgViremia | 4.1748 | 2 | 0.12401 |

**Table S23. Main Effects of Treatment, Average Percent Change Body Mass, and Average Viremia on Mortality.** Type III ANOVA for main effects of the Cox proportional-hazards model in R studio with treatment, average % change body mass days 2 and 4 post-exposure), and average viremia (days 2 and 4 post-exposure) as fixed effects. We also asked about the interaction between these fixed effects.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Cox Proportional-Hazards Model* | Coef | Exp(coef) | SE(coef) | Z score | P value |
| 1800K (2) | 1.557e+01 | 5.802e+06 | 9.729e+00 | 1.601 | 0.10943 |
| 3000+5000K (3) | 1.950e+01 | 2.953e+08 | 8.843e+00 | 2.205 | 0.02743\* |
| Avg%ΔMass | -5.311e+00 | 4.939e-03 | 2.932e+00 | -1.811 | 0.07014 |
| AvgViremia | 3.577e+00 | 3.578e+01 | 1.162e+00 | 3.079 | 0.00208\* |
| (2):Avg%ΔMass | 2.685e+00 | 1.466e+01 | 5.821e+00 | 0.461 | 0.64465 |
| (3):Avg % Δ Mass | 5.158e+00 | 1.738e+02 | 2.946e+00 | 1.751 | 0.07993 |
| (2):Avg Viremia | -2.028e+00 | 1.316e-01 | 1.313e+00 | -1.544 | 0.12260 |
| (3):Avg Viremia | -2.561e+00 | 7.723e-02 | 1.170e+00 | -2.189 | 0.02863\* |
| %ΔMass:AvgViremia | 7.055e-01 | 2.025e+00 | 3.776e-01 | 1.868 | 0.06172 |
| (2):Avg%ΔMass:AvgViremia | -3.491e-01 | 7.053e-01 | 8.921e-01 | -0.391 | 0.69558 |
| (3):Avg%ΔMass:AvgViremia | -6.864e-01 | 5.034e-01 | 3.796e-01 | -1.809 | 0.07052 |

**Table S24. Detailed Output of Treatment, Average Percent Change Body Mass, and Average Viremia on Mortality.** Output from the Cox proportional-hazards analysis with treatment, average % change body mass days 2 and 4 post-exposure), and average viremia (days 2 and 4 post-exposure) as fixed effects. We also asked about the interaction between these fixed effects.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *One-way ANOVA* | DF | Sum Sq | Mean Sq | F value | P value |
| Treatment | 2 | 13.51 | 6.754 | 3.561 | 0.0341\* |
| Residuals | 65 | 123.30 | 1.897 |  |  |

**Table S25. Comparison of Days Until Death Between Treatments.** One-way ANOVA analysis to determine whether the residual of the mean of days until death (i.e. mortality occurs earlier or later than predicted based on viremia) in treatments differ.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *Tukey Comparison of Means* | Diff | Lower | Upper | P value adjusted |
| 1800K - control | -0.2397 | -1.4253 | 0.9458 | 0.8787 |
| 3000+5000K - control | -0.9612 | -1.8651 | -0.0573 | 0.0346\* |
| 3000+5000K - 1800K | -0.7215 | -1.8307 | 0.3878 | 0.2702 |

**Table S26. Pairwise Comparison of Time Until Death Between Treatments.** Tukey multiple comparison of means analysis used to determine between which treatment groups there were significant differences. Here, we see that the significant effect of treatment is driven by the difference between the control and broad-spectrum [3000+5000K] ALAN group. This indicates that individuals in the broad-spectrum ALAN group are dying earlier than anticipated based on the residual variation of the means of time until death based on average viremia days 2 and 4 post-exposure.