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The intensity and spectrum of artificial light at night alters crayfish interactions

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**ABSTRACT**

Ecological light pollution (ELP) is quickly becoming a worldwide concern and can negatively affect aquatic ecosystems. The given intensity and spectrum of a light source can influence how organisms function within their environment. These properties of artificial lighting at night (ALAN) and their impacts on the physiology and behaviour of crayfish were examined in this work. Hemolymph was obtained from crayfish to quantify a physiological response. Behavioural data were measured as the number, duration, and maximum intensity of agonistic fights. Exposure to higher intensities of light and the presence of ultraviolet light induced a behavioural trend, resulting in significantly altered social interactions within both species of crayfish. The number and maximum intensity of lights significantly decreased, whereas the duration of time spent fighting significantly increased. Due to the importance of freshwater environments and the role crayfish play as a keystone species, examining how crayfish are impacted from ALAN is imperative to maintaining the health of aquatic ecosystems.

**ARTICLE HISTORY**

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**KEYWORDS**

Artificial lighting at night; intensity; physiology; social behaviour; spectrum

**Introduction**

Ecological light pollution (ELP) is the addition of artificial light at night (ALAN) that changes natural light-dark cycles (Longcore and Rich 2004). Over 18% of the world has been polluted with ALAN of various intensities and spectral compositions, which is causing an approximate 6% increase in global night-time illumination annually (Cinzano et al. 2001; Hölscher et al. 2010a; Gaston et al. 2015; Falchi et al. 2016; Kyba et al. 2017; Kyba 2018). Given that ALAN is increasing worldwide and a large portion of ELP occurs near aquatic habitats, the impact of this apparent, but often missed, type of pollution on aquatic ecosystems needs to be investigated (Davies et al. 2014; Falchi et al. 2016; Zapata et al. 2019). ALAN detrimentally threatens aquatic ecosystems by transforming the functioning of waterways through alterations in community structure, behaviour, and physiology (Dudgeon et al. 2006; Swaddle et al. 2015; Zapata et al. 2019). Despite the growing threat and investigations into the effects on marine systems, there has been less examination of

Although there has been a shift towards investigating higher biological orders, such as predator-prey dynamics (Miller et al. 2017), previous research focuses on the consequences of ALAN on individual behaviour. Impacts such as shifted foraging rates and tactics (Yurk and Trites 2000; Dwyer et al. 2013; Manriquez et al. 2019), reproductive physiology (Davies et al. 2014), disorientation (Salmon 2003), movement patterns (Moore et al. 2000; Riley et al. 2013), and activity levels (Duarte et al. 2019) have been noted in a variety of organisms. Since light is an important sensory cue used by many organisms, changes to lighting intensity or spatial patterns can create behavioural alterations (Ragni and D’Alcalá 2004; Gaston et al. 2013). These modifications in behaviour, changes in circadian rhythms, timing of mating, and signal detection, due to ALAN can be initiated by explicit changes in the physiology of animals (Pulgar et al. 2019). Not only can the intensity of light directly influence functions necessary for survival (Illnerova et al. 1978; Dauchy et al. 1997; Cos et al. 2006; Navara and Nelson 2007; Bedrosian et al. 2011), but the wavelength emitted can also alter the physiology of animals (Thorington 1985). Natural and artificial lighting have spectral signatures that will illuminate light at differing intensities within wavelengths along the electromagnetic spectrum (Thorington 1985; Gaston et al. 2013). Exposure to wavelengths both in and outside the visible light portion of the spectrum, such as ultraviolet light (UV) (200–400 nm), facilitates specific biological processes within organisms, such as altering corticosterone levels (Maddocks et al. 2001, 2002; Migaud et al. 2007; Ouyang et al. 2015). Corticosterone and cortisol are the main glucocorticoids found within vertebrate species and is pertinent for regulating immune functioning and stress. Exposure to ALAN influences stress levels within organisms (Navara and Nelson 2007), that could impact the biological processes and behaviour of an individual when participating in social interactions (Creel et al. 2013).

A suite of behaviours that are intimately tied to the physiology of organisms is the aggression associated with social behaviour (Alexander 1974; Wilson 1975). Both the endocrine and neuroendocrine system are directly linked to the expression of social behaviours (Creel 2001; Adkins-Regan 2005; Carter et al. 2008). For example, non-territorial African cichlids (Haplochromis burtoni) had higher amounts of cortisol than territorial individuals in stable environments, indicating the hormone is related to the social status of the fish (Fox et al. 1997). The social standing of the individual, as well as the behaviours they engage in, are fundamental variables contributing to access of mates, territory, and food (Alexander 1974; Wilson 1975; Pellegrini 2008; Bourke 2011; Hofmann et al. 2014). The alteration of time budgets to obtain resources, such as increased time spent behaving more aggressively in social interactions, can influence the movement of nutrients through ecosystems (Peckarsky et al. 2008). Various anthropogenic chemicals can alter the social behaviour of aquatic organisms that compete with one another to gain resources, including crayfish (Burba 1999; Bergman and Moore 2003; Zala and Penn 2004; Fero et al. 2007; Cook and Moore 2008; Olsén 2011).

Crayfish are nocturnal organisms that are commonly found within freshwater environments around the world (Crandall and Buhay 2008; Reynolds et al. 2013; Souty-Grosset and Fetzner Jr 2016). Having developed compound eyes that are sensitive to variations in light, crayfish will respond to visual stimuli in their environment and readily engage in agonistic interactions over the acquisition of resources (Bergman and Moore 2003; Fero et al. 2007; Moore 2007). Serotonin, a biogenic amine found in decapod crustaceans, not only regulates
the stress and anxiety-like behaviours within crayfish (Fossat et al. 2014, 2015) but is also involved in the expression of social behaviours (Kravitz 1988; Edwards and Kravitz 1997). Specifically, serotonin modifies the aggression levels and, consequently, the agonistic behaviour of crustaceans based on the dominance standing of an individual (Huber, Orzesyna, et al. 1997). Because crayfish rely on small amounts of light for detecting predators, objects, and securing resources (Glantz 2001; Tuthill and Johnsen 2006; Clark and Moore 2018), the behavioural and physiological impacts from exposure to ALAN could both affect the survival of crayfish.

Previous work has identified ALAN effects on different crayfish species. Noble crayfish (Astacus astacus) housed under higher light intensities of ALAN displayed less exploratory behaviour (Abeel et al. 2016). Additionally, increased time spent in shelters, and decreased duration of interactions were measured when signal crayfish (Pacifastacus leniusculus) were exposed to differing light intensities of high-pressure sodium lights (Thomas et al. 2016). While light intensity is known to impact the behaviours of individual crayfish (Bruski and Dunham 1987), how ALAN affects the social behaviour and physiology has been minimally investigated. Furthermore, few studies have examined the impacts of light wavelength on crayfish (Fanjul-Moles et al. 1998; Fischer 2016). New river crayfish (Cambarus chasmodactylus) and spiny stream crayfish (Faxonius cristavarius) displayed more sheltering behaviour and less activity when exposed to warm (2600 K) and cool (5500 K) light-emitting diode (LED) lights (Fischer 2016). The aspects of warm and cool lighting pertain to the wavelengths of the visual light spectrum, with warm evoking longer red wavelengths (620–750 nm) and cool focusing on shorter blue wavelengths (400–495 nm). However, there has yet to be a study exploring the effects UV has on the physiology and behaviour of crayfish. Crayfish have corneas that specifically absorb ultraviolet wavelengths, which could impact how they respond to variations in spectral composition of ALAN (Goldsmith and Fernandez 1968). It has been noted that changes in physiology and behaviour in crayfish, such as serotonin levels or learning, can lead to the reformation of dominance hierarchies most likely through changes in neurotransmitter function (Yeh et al. 1996, 1997; Hardy and Briffa 2013). Physiological and behavioural changes within crayfish, foraging patterns and social dynamics, can cause significant impacts to aquatic environments, as they serve as keystone species and ecosystem engineers (Belanger et al. 2017). Therefore, exploring how the agonistic behaviour and physiology of crayfish is altered from exposure to ALAN is imperative to the understanding of the organism itself and its role in the ecosystems they inhabit.

The objective of this study was to examine how both the intensity and spectra of ALAN affect the way crayfish function in their environment. For this study, virile (Faxonious virilis) and rusty (Faxonius rusticus) crayfish were chosen as the model organisms, based on their availability near the study site. Both species coexist within the same bodies of water and are frequently fighting over resources and shelters (Moore per obs). It was hypothesized that: (1) exposure to ALAN would decrease the number of social interactions due to an overall reduction in activity, (2) cause longer fights and heightened agonistic behaviours as a result to the resource acquisition of the shelters and food, (3) higher light intensities would increase serotonin levels within both species of crayfish, and (4) the addition of UV spectrum would initiate lower levels of serotonin.
Materials and methods

Animal collection and housing

A total of 200 *F. rusticus* and 200 *F. virilis* crayfish were collected using hand nets from Burt Lake, MI (45°28’N, 84°40’W). Both *F. virilis* and *F. rusticus* are sympatric in northern Michigan. Crayfish were form II (non-reproductive) females equipped with all sensory appendages. We chose non-reproductive females due to availability and to reduce confounding variables by including males (both reproductive and non-reproductive) and reproductive females. After acquisition, crayfish were communally housed in large metal horse troughs (239 cm x 0.76 cm x 60 cm; L x W x H). These flow through troughs were supplied with unfiltered river water from the East Branch of the Maple River (Pellston, MI), producing a continuous flow of oxygen and detritus for the crayfish to feed on. Troughs were also equipped with pieces of broken pottery for areas of shelter. Each species resided in a separate trough and were maintained on a natural light-dark cycle (~15h:9h; L:D). The actual light and dark cycle changed throughout the 9-week experimental period.

In preparation for use, crayfish were removed from communal housing and individually isolated in plastic containers (ranging from 6.3 to 9.5 cm deep) for at least 1 week to eliminate previous social familiarity (Bergman et al. 2003). Isolation occurred within an artificial stream that was constructed from cinder blocks and plastic sheeting (300 cm x 79 cm x 29 cm; L x W x H). The flow through stream was likewise supplied with unfiltered water from the East Branch of the Maple River and kept on a natural light-dark cycle (~15h:9h; L:D). Crayfish were size matched within a 10% difference of each other using carapace and chelae length to remove dominance bias (Pavey and Fielder 1996).

Experimental design

All trials took place at the University of Michigan Biological Station (UMBS) Stream Research Facility in Pellston, MI. A 5 × 2 fully factorial design was implemented to examine the impacts of ALAN on crayfish social behaviours and physiology. The first factor was the ALAN condition with five treatments: (1) no ALAN (physiological control), (2) high light intensity (32 ± 2 lux), (3) low light intensity (4 ± 2 lux), (4) high light intensity with UV (32 ± 2 lux, 0.4 ± 0.2 µ-mol), (5) low light intensity with UV (4 ± 2 lux, 0.4 ± 0.2 µ-mol). A LI-COR light meter (LI-250A) was utilized to measure the light intensity being emitted from the LED bulb, and an Apogee Instruments UV meter (MU-200) was used to quantify the light spectrum of the UV bulbs. All experimental light measurements were taken at the bottom of the experimental arenas (approximately 30 cm of water). Light measurements were also taken at the site where the animals were collected throughout the moon cycle, to assess the amount of natural light in the given environment. The highest light intensity identified at the collection site was 0.5 lux (2-days post full moon), whereas no UV was detected. All measurements of UV light occurred at the surface of the water, as the UV meter was not submersible. The second factor was crayfish species, which entailed two treatments: (1) four *F. virilis* individuals per trial, and (2) four *F. rusticus* individuals per trial. Ten trials were conducted for each species and treatment, except for the physiological control that had five trials because behavioural analysis was not possible without light. For the entire experiment, a total of 90 trials were executed.
This design lacks what can be considered a true control (no light). Even the addition of infrared radiation (IR) light that might allow analysis of the social behaviour would be the presence of additional light at night. Given that it was impossible to analyse the social behaviour under no light conditions, this part of the experiment did not include that type of control. It was included with the physiological data (below).

**Experimental arena**

Treatments arenas were constructed out of cinder blocks lined with 0.01 cm plastic sheets (81 cm x 81 cm x 40 cm; L x W x H) ([Figure 1](#)). Natural river water from the East Branch of the Maple River was pumped into a constant head tank (208 L) equipped with nylon mesh stockings to filter excess detritus. Each head tank fed water directly into two streams through four garden hoses (1 cm interior diameter, ranging 69–100 cm in length). Two identical head tanks were used to distribute water to a total of four treatment arenas. A cinder block lined with window screening mesh (13 cm x 14 cm; L x W) was placed inside each stream. Outside of each stream, LED lights (a) and ultraviolet bulbs (b) were set up. Security cameras were placed directly above each test arena. A total of four test arenas were created for this project.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trials conducted</th>
</tr>
</thead>
<tbody>
<tr>
<td>No ALAN</td>
<td>N = 10 (5 <em>F. virilis</em>, 5 <em>F. rusticus</em>)</td>
</tr>
<tr>
<td>High, UV-</td>
<td>N = 20 (10 <em>F. virilis</em>, 10 <em>F. rusticus</em>)</td>
</tr>
<tr>
<td>Low, UV-</td>
<td>N = 20 (10 <em>F. virilis</em>, 10 <em>F. rusticus</em>)</td>
</tr>
<tr>
<td>High, UV+</td>
<td>N = 20 (10 <em>F. virilis</em>, 10 <em>F. rusticus</em>)</td>
</tr>
<tr>
<td>Low, UV+</td>
<td>N = 20 (10 <em>F. virilis</em>, 10 <em>F. rusticus</em>)</td>
</tr>
</tbody>
</table>

*Figure 1.* Diagram of experimental test arena setup. Unfiltered river water was pumped into two streams through garden hoses connected to a constant head tank. Four PVC shelters were placed on the sides and food pellets were centered in the middle of each stream. Outside of each stream, LED lights (a) and ultraviolet bulbs (b) were set up. Security cameras were placed directly above each test arena. A total of four test arenas were created for this project.
downstream all treatment arenas, creating a flow through system to prevent any chemical social cues from building up in the system (Wofford et al. 2017). Four 81 x 15 cm (L x W) strips of black plastic weed barrier were cut and placed along the top of the sides of the arenas, to reduce the possibility of crayfish escaping during trials. Each arena was lined with black plant potter mesh (81 cm x 81 cm; L x W) to serve as substrate and held in place by six ceramic tiles (20 cm x 20 cm; L x W) that were spray painted black to match the mesh substrate.

Two types of resources were placed in the arena to create competition and agonism between crayfish. Four PVC half-pipe shelters (10 cm x 8.9 cm; L x W) and four pre-made fish gelatin capsules were included in every arena. Fish gelatin capsules were made by homogenizing a ¼ can of sardines (Ocean Prince – 3.75 oz) in a blender. Two packets of unflavored gelatin (Knox – 0.25 oz) and 300 mL of boiling water were combined with the sardine mixture in the blender. The solution was then administered into 3.5 mL scintillation vial capsules with a turkey baster and allowed to set for 24 h. All food capsules had a strip of Velcro® (1 cm x 1 cm; L x W) attached to the bottom. A piece of plexiglass (11 cm x 16 cm; L x W) had the opposing piece of Velcro® to affix the capsules in place. The plexiglass was placed into the center of each arena as a food source for the crayfish.

Wooden frames (112 cm x 112 cm x 122 cm; L x W x H) were placed on top of streams and covered in black tarps (601 cm x 601 cm x 0.03 cm; L x W x H) to prevent any natural or artificial outside night lighting from entering the arenas that could influence the study. DVR security cameras (Swann SWDVK-430004) were mounted to the wooden frames 120 cm directly above the waterline in the center of each test arena. Since crayfish are nocturnal, these cameras recorded behaviour from 2200 to 0700 every night throughout the duration of the study.

**Lighting**

One LED bulb (A19 LED 100 W, model number: 6A19ED14301DB, Great Eagle) was placed inside an aluminium reflector and positioned to illuminate the system without interfering with the video recording. The high light intensity treatment was standardized at 32 ± 2 lux at the center of each stream, whereas the low light intensity treatment was 4 ± 2 lux. An inline light dimmer was connected to alter the light intensity to desired range. An UV bulb (E27 UV ultraviolet 38LED, model number: HandPC-53867, Fashion Outlet) was placed next to the LED bulb. UV intensity was set at 0.4 ± 0.2 µ-mol at the center of each stream. Lights were hooked up to a power strip and connected to a 24-h light timer (model: HT1025, Intertek).

**Experimental protocol**

Once the week of acclimation in isolation was complete, all size matched individuals were distinctively marked with a white-out pen for identification purposes before placement into treatments. Lighting treatments were randomized across the four different arenas to eliminate bias based on individual arena. To start a trial, the plexiglass with food capsules was placed into each test arena after the lights turned on at 2200. The cameras also began recording at 2200. A total of four crayfish were put into each treatment arena. Lights and cameras remained on until 0700 the following day. Therefore, tested crayfish were still maintained under a 15h:9h light-dark cycle, where the 9 h of darkness became 9 h of...
exposure to the designated treatment. After the 9 h of recording, crayfish were removed from the test area and used only once.

**Hemolymph collection**

When removed from the treatment, 50–100 µL of hemolymph was collected from each individual crayfish utilizing a 0.5 mL plastic insulin syringe and needle. One syringe was inserted into the fifth walking leg, where hemolymph was drawn up, transferred, and stored in labeled 1.5 mL polypropylene tubes. Hemolymph extracted from all four crayfish used in each trial was pooled to yield one sample. A total of five samples per treatment and species were obtained. Immediately after collection, tubes were flash frozen utilizing liquid nitrogen and transported on ice back to the UMBS Alfred H. Stockard Lakeside Laboratory where they were placed in a −20°C freezer. Following this, a freeze dryer (FreeZone® 1 L Benchtop Freeze Dry Systems, model number: 77400, Labconco) was utilized to remove excess water and samples were dried for at least 8 h. Samples were then removed from the freeze dryer and remained in the laboratory on desiccant until chemical analysis.

**Serotonin evaluation**

Serotonin extraction and analysis followed the methods outlined in Watson et al. (1996). Hemolymph samples were extracted three times in 0.75 mL 10% sodium bicarbonate (NaHCO₃) in an ultrasonic bath, sonicated, and centrifuged to yield the supernatant. An additional 10% NaHCO₃ was added to the extracts to bring the total volume to 5 mL. In a fume hood, 0.5 mL acetic anhydride (C₄H₆O₃) was added at ambient temperature with constant stirring, to give rise to the n-acetyl serotonin derivative. The final pH of the solution was approximately 3.5 and optimal for the extraction of acidic compounds into organic solvents. Again, the solution was extracted three times with 325 µL of methylene chloride (CH₂Cl₂) and evaporated to dryness in a Thermo Savant Vacufuge set at 45°C. An internal standard of ibuprofen was then added to the solution, reacting with 15 µL MSTFA at 80°C for 30 min. The residue was then analysed by GC/MS in selection ion monitoring (SIM) mode. The detection limit was 0.01 ng serotonin on-column with a 1:50 split.

**Data analyses**

The fighting behaviour of the crayfish was examined across the 80 trials of exposure to lighting treatments, because interactions were unable to be observed in the no ALAN treatment. Behaviour was quantified from 2300 to 0700 for every trial by a blind observer utilizing a modified 11-point ethogram (Table 1) (Bruski and Dunham 1987; Bergman and Moore 2003). Number of fights, duration of fights (in seconds), and maximum intensity of the fight achieved during each interaction was recorded for every trial conducted.

Fight duration and maximum intensity have multiple measures within each trial, so a repeated measures statistical design is necessary. The Shapiro–Wilk test was used to assess normality of these two measures (Shapiro and Wilk 1965). In addition, the experiment involved four separate stream setups. To evaluate the effect of treatments on fight duration and maximum intensity, a separate repeated measures mixed model was run using the lmer function in R (Bates et al. 2015; RStudio Inc., Boston, MA; R Core Team 2017). Within the model, light intensity, light spectrum, and species were
Table 1. Ethogram of agonistic interactions utilized throughout the duration of the study (Bruski and Dunham 1987; Bergman and Moore 2003).

<table>
<thead>
<tr>
<th>Intensity level</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>−2</td>
<td>Rapid movement of the tail in reverse without chelae grab</td>
</tr>
<tr>
<td>−1</td>
<td>Lower intensity walking away from opponent</td>
</tr>
<tr>
<td>1</td>
<td>Lower intensity walking toward opponent</td>
</tr>
<tr>
<td>2</td>
<td>Quick high intensity approach with meral spread</td>
</tr>
<tr>
<td>3</td>
<td>No forceful movement, just chelae touches</td>
</tr>
<tr>
<td>4</td>
<td>Hitting the opponent with antennae</td>
</tr>
<tr>
<td>5</td>
<td>Closed chelae straight forward holding opponent away</td>
</tr>
<tr>
<td>6</td>
<td>Pushing with chelae that are closed</td>
</tr>
<tr>
<td>7</td>
<td>Open chelae touching, no pushing or closing</td>
</tr>
<tr>
<td>8</td>
<td>Open chelae pushing, no closing</td>
</tr>
<tr>
<td>9</td>
<td>Closed chelae around appendage, no pulling</td>
</tr>
<tr>
<td>10</td>
<td>High intensity and rapid movement attempting to rip claws or appendages</td>
</tr>
<tr>
<td>11</td>
<td>Flipping opponent over</td>
</tr>
</tbody>
</table>

Results

Number of fights

There was a significant interaction of species, light intensity, and light spectrum on the number of fights \( (p < 0.0001; \text{Table 2; Figure 2}) \). \textit{F. rusticus} engaged in significantly more fights in the low light intensity treatment (Low, UV−) compared to all other treatments (Tukey-HSD, \( p < 0.0001 \)). No other treatment was significantly different from any treatment.
The mean amount of fights that occurred in the Low, UV-treatment for *F. rusticus* was 25.6 ± 0.81, whereas all other treatments had means ranging from 2.9 ± 0.35 to 7.9 ± 1.49 fights.

**Duration of fights**

There was a significant interaction between species and light intensity on the duration of fights (p = 0.014; Table 3; Figure 3). *F. rusticus* spent significantly more time fighting under high light intensities (102.09 ± 10.32 s) compared to low light intensities (43.16 ± 3.1 s; Tukey-HSD, p < 0.0001). Similarly, *F. rusticus* under low light intensity conditions had significantly longer durations of social interactions than *F. virilis* under high light intensities (69.89 ± 6.49 s; Tukey-HSD, p < 0.005). In addition, there was a significant effect (with no interactions) of light spectrum on fight durations (p < 0.001; Table 3). Crayfish spent

### Table 2. Statistical results from the linear mixed models number of fights as the dependent variable.

<table>
<thead>
<tr>
<th>Model</th>
<th>Chi-squared Result</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main Effects</td>
<td>Species</td>
<td>14.7</td>
</tr>
<tr>
<td></td>
<td>Intensity</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>Spectrum</td>
<td>23.8</td>
</tr>
<tr>
<td>Pairwise Effects</td>
<td>Species x Intensity</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>Species x Spectrum</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Intensity x Spectrum</td>
<td>18.7</td>
</tr>
<tr>
<td>Full Model</td>
<td>Species x Intensity x Spectrum</td>
<td>15.1</td>
</tr>
</tbody>
</table>

**Figure 2.** Mean (± SEM) number of fights across treatments for each species under different light intensities and variation of light spectrum. The variety of line patterns is related to individual light treatment and species. Squares represent *F. rusticus*, whereas circles represent *F. virilis*. Closed symbols represent the presence of UV and semi-open symbols represent the absence of UV. An asterisk indicates a significant difference between all light treatments and species. All statistical differences were identified through a Tukey-HSD post-hoc test.

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significantly less time fighting one another given the presence of UV than in the absence of UV (Tukey-HSD, p < 0.002).

**Maximum intensity of fights**

There was a significant interaction of species, light intensity, and light spectrum on the maximum intensity of fights (p < 0.0001; Table 4; Figure 4). *F. rusticus* under a Low, UV+ treatment, as well as *F. virilis* under any low light conditions, exhibited the highest average fight intensities (8.16 ± 0.14, 7.48 ± 0.14, and 8.11 ± 0.13 for *F. rusticus* Low, UV+, *F. virilis* Low, UV-, and *F. virilis* Low, UV+, respectively). These values were significantly higher than all other treatments (Tukey-HSD, p < 0.0001). *F. rusticus* under a Low, UV-treatment displayed the second highest fight intensity (5.15 ± 0.14).

### Table 3. Statistical results from the linear mixed models using duration of fights as the dependent variable.

<table>
<thead>
<tr>
<th>Model</th>
<th>Chi-squared Result</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main Effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>8.0</td>
<td>0.004</td>
</tr>
<tr>
<td>Intensity</td>
<td>44.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Spectrum</td>
<td>15.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pairwise Effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species x Intensity</td>
<td>6.0</td>
<td>0.014</td>
</tr>
<tr>
<td>Species x Spectrum</td>
<td>0.4</td>
<td>0.51</td>
</tr>
<tr>
<td>Intensity x Spectrum</td>
<td>0.7</td>
<td>0.40</td>
</tr>
<tr>
<td>Full Model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species x Intensity x Spectrum</td>
<td>1.8</td>
<td>0.18</td>
</tr>
</tbody>
</table>

**Figure 3.** Mean (± SEM) duration of fights (in seconds) across treatments for each species under different light intensities and variation of light spectrum. The variety of line patterns is related to individual light treatment and species. Squares represent *F. rusticus*, whereas circles represent *F. virilis*. Closed symbols represent the presence of UV and semi-open symbols represent the absence of UV. A repeated measures mixed model was ran to assess the effect on duration of fights. The black bolded circle indicates a significant difference between light level treatments and species. All statistical differences were identified through a Tukey-HSD post-hoc test.
and this was significantly higher than all high light intensity treatments (Tukey-HSD, p < 0.01). *F. rusticus* under a High, UV-treatment, as well as *F. virilis* under a High, UV-treatment, had the third highest fighting intensities (4.67 ± 0.09 and 4.48 ± 0.08 for Rusy High, UV-, *F. virilis* High, UV-, respectively). These values were significantly higher than all high light intensity treatments with UV (Tukey-HSD, p < 0.05), which had values of 3.7 ± 0.08 for *F. rusticus* and 3.59 ± 0.09 for *F. virilis*.

**Serotonin concentrations**

There was no significant interaction of species, light intensity, and light spectrum on the serotonin concentrations within the crayfish (p = 0.69; Table 5; Figure 5). A significant difference in the serotonin concentration was determined across species (p = 0.038;
The highest serotonin concentrations were found in *F. virilis* regardless of light treatment.

**Table 5.** Statistical results from the linear mixed models using serotonin levels as the dependent variable.

<table>
<thead>
<tr>
<th>Model</th>
<th>Chi-squared Result</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main Effects</td>
<td></td>
<td></td>
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<tr>
<td>Species</td>
<td>4.3</td>
<td>0.038</td>
</tr>
<tr>
<td>Intensity</td>
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<td>0.15</td>
</tr>
<tr>
<td>Spectrum</td>
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<td>0.60</td>
</tr>
<tr>
<td>Pairwise Effects</td>
<td></td>
<td></td>
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<tr>
<td>Species x Intensity</td>
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<td>0.13</td>
</tr>
<tr>
<td>Species x Spectrum</td>
<td>0.08</td>
<td>0.77</td>
</tr>
<tr>
<td>Intensity x Spectrum</td>
<td>0.03</td>
<td>0.85</td>
</tr>
<tr>
<td>Full Model</td>
<td>Species x Intensity x Spectrum</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Figure 5.** Mean (± SEM) serotonin concentrations (ng/g) across the treatments for each species under the presence or absence of ALAN. The solid line and squares represent *F. rusticus*, whereas the dotted line and circles represent *F. virilis*. Statistical differences were identified through a mixed model and are found in the text.

Table 5). The highest serotonin concentrations were found in *F. virilis* regardless of light treatment.

**Discussion**

This study demonstrates that both the intensity and spectrum of light significantly affects the agonistic interactions within two crayfish species (Figures 2–4). Higher intensities of light elicited significant decreases in the number and maximum intensity of fights (p < 0.0001, Figure 2; p < 0.0001, Figure 4), while increasing the duration of fights (p < 0.05, Figure 3). Furthermore, the addition of UV decreased the number of fights and increased the duration of interactions. Under the low light intensity treatment, UV heightened the maximum intensity of fights, but UV decreased the maximum intensity of fights under the high light intensity treatment (p < 0.05, Figure 4). Higher intensities of light decreased serotonin concentrations altogether, whereas serotonin levels were generally higher in UV treatments within species (p = 0.038, Figure 5).
Overall, higher light intensities and the addition of UV had the greatest effect on the social behaviour of crayfish. Through shifts in the light intensity and light spectrum of the surrounding environment, ALAN impacts the social processes of both *F. rusticus* and *F. virilis* crayfish.

ALAN is considered to affect the social network structure of organisms by altering the frequency, duration, and strength of social interactions (Kurvers et al. 2014; Kurvers and Hölker 2015). Nocturnal organisms are predicted to have weaker social networks from exposure to ALAN (Kurvers et al. 2014; Kurvers and Hölker 2015). In addition, animals are expected to have opportunities to exchange social information with ALAN, which can influence agonistic interactions (Perkin et al. 2011; Davies et al. 2013; Kurvers and Hölker 2015). The results from this study support the prediction that nocturnal organisms will have fewer and weaker interactions, as the crayfish had less intense and a decreased number of fights with higher light intensities and UV-added light spectrum. Crayfish engage in fights with conspecifics to gain information about their opponent’s dominance ranking and secure resources (Hazlett 1969; Bruski and Dunham 1987; Bergman and Moore 2003; Fero et al. 2007; Moore 2007; Martin III and Moore 2008; Jurcak et al. 2016). As crayfish win more fights and become dominant, they gather less information about their opponents resulting in shorter, more intense fights (Goessmann et al. 2000). The duration of fights was observed to increase, while the maximum intensity of fights decreased under higher light intensities and added UV. This may reflect that the presence of ALAN decreases the ability for the crayfish to obtain social information about their conspecifics.

These underlying changes in behaviour may also be a result in the physiological alterations the crayfish undergo due to exposure to ALAN. When serotonin is injected into crayfish, subordinates will readily engage in fights after the dominance hierarchy is formed (Huber et al. 1997a, 1997b). While the duration and intensity of fights has been shown to increase with serotonin injections (Huber et al. 1997b), other studies have noted higher doses of serotonin diminish the aggressive behaviours of crayfish possibly through discontinuation of the behaviour (Peeke et al. 2000; Tierney and Mangiamele 2001). Although not statistically different, serotonin concentrations were greater with added UV and decreased in higher light intensities. The relationship between these concentrations and the social behaviour could be varied due to species. It has been also hypothesized that serotonin also functions to regulate the circadian rhythms within the photoreceptors in the eyestalks of the crayfish and other crustaceans (Beltz and Kravitz 2002; Wildt et al. 2004; Calderón-Rosete et al. 2006; Strauss and Dickerson 2010). Thus, it is possible that ALAN levels are potentially changing serotonin physiology of the crayfish via input from their eyes.

Melatonin and serotonin concentrations within the nervous system and in the eyestalks of crayfish show a circadian rhythm (Escamilla-Chimal et al. 2001). In addition, there is a physiological connection between photoreception and melatonin production (Castanon-Cervantes et al. 1999). Thus, ALAN may be altering both melatonin production in the eyestalk of the crayfish which can subsequently alter serotonin production within the central nervous system (CNS) of crayfish. The coupling between light sensitivity and serotonin production is known (Fanjul-Moles and Prieto-Sagredo 2003) and if photoreceptors are sensitive to UV light as well as ALAN, changes would occur in the serotonin levels within the hemolymph. Any alteration in the amount of serotonin or its natural cycles
within the hemolymph of the crayfish would disrupt the natural patterns of social behaviour.

The photoreceptors of crustaceans display a wide range in spectral sensitivity, from values of 300–660 nm (Goldsmith and Fernandez 1968). The spectral sensitivity found within F. rusticus and F. virilis has maximum wavelength values of about 530 nm and 565 nm (Goldsmith and Fernandez 1968; Cronin and Goldsmith 1982; Crandall and Cronin 1997) and there is not any significant differentiation in the range of sensitivity across these species. Despite this lack of difference in spectral sensitivity, serotonin concentrations were significantly different between these two species of crayfish, with F. rusticus having lower serotonin concentrations (p < 0.01). F. rusticus had a greater shift in increased serotonin levels under ALAN as opposed to F. virilis, yielding the conclusion F. rusticus may be more sensitive to lighting alterations. Future investigations on ALAN effects should consider the sensitivity to light when examining changes to both the physiology and behaviour of a species.

Ultimately, the presence of ALAN could pose a threat to the stability of crayfish dominance hierarchies. The presence of ALAN has been predicted to decrease the potential for a species to gather information about prior dominance rankings from interactions based on the extended and lower intensity fights (Taborsky and Oliveira 2012; Kurvers and Höller 2015; Thomas et al. 2016). Crayfish use olfaction, touch, and vision as three sensory modalities to form their dominance hierarchies (Acquistapace et al. 2002; Delgado-Morales et al. 2004; Horner et al. 2008; Callaghan et al. 2012). With the introduction of ALAN, the visual capabilities of crayfish could be diminished yielding difficulty in formation of the hierarchies. The population dynamics within species could not only be modified, but moreover cause cascading effects to entire aquatic ecosystems. Crayfish are classified as keystone species because of their role within ecological food webs and part in regulating the flow of energy (Crandall and Buhay 2008; Reynolds et al. 2013). With longer fights occurring under exposure to ALAN, the probability of crayfish being predated on by various fish species increases (Reynolds 2011). Furthermore, crayfish will loose out on obtaining vital resources, such foraging on macrophytes (Chambers et al. 1990; Momot 1995). Altered biological responses and agonistic behaviours resulting from enhanced exposure to ALAN could also impact other organisms throughout the aquatic environment who also compete for the acquisition of resources (Branch 1984; Cheroske et al. 2009; Carvalho et al. 2012, 2013; Ruchin 2018). These behavioural and physiological changes within aquatic organisms could moreover initiate trophic cascades and impact the dynamics within the habitat, all of which indicate that ecosystem functioning can be severely altered due to ALAN. Yet, these effects remain largely understudied within aquatic habitats (Gaston et al. 2013; Davies et al. 2014). Continuing research should progress towards investigating the threats ALAN can have on aquatic ecosystems, as well as the behaviour and physiology of the organisms that reside in these environments.

Overall, this study concluded that both light intensity and light spectrum of ALAN produce alterations in crayfish social behaviour and physiology. Higher intensities of light and added UV ultimately created the largest shift in agonistic interactions and serotonin concentrations. These changes may represent a transformation in the amount of information crayfish can obtain from fights. A lack of knowledge about their opponent can diminish the opportunities for crayfish to establish and recall dominance,
as well as decrease the ability to secure resources. Although different species may have distinctive sensitivities and consequently responses, disruptions to crayfish from ALAN can fundamentally change freshwater ecosystems. As strategies continue to be explored on how to mitigate ELP, advancing studies analysing how the properties of ALAN effects organisms and their environment should persist.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Data availability statement**

The data that support the findings will be available in University of Michigan Biological Station’s Research Gateway at [http://biostation.lsa.umich.edu/](http://biostation.lsa.umich.edu/) following a 6-month embargo from the date of publication to allow for commercialisation of research findings.

**References**


