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The resilience of juvenile Acropora corals when exposed to artificial light at night, commonly associated with coastal urbanisation

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Abstract
In recent years, artificial light pollution has expanded in area and concentration due to global population growth and technological advances in lighting. The impacts of artificial light at night (ALAN) are now well acknowledged within terrestrial biomes however, influences to surrounding coastal marine ecosystems are poorly recognised. Similarly, research has only just begun to reveal the extent of damage to coral reef systems, which have both substantial ecological and economic importance. However, the survival of important reef building Scleractinia within areas of ALAN is relatively unknown. Here a series of investigations underpin the extent of damage of short and long wavelength spectral street lighting to the reef building species, Acropora microclados and compared responses to coral of unlit coastal areas. A series of experiments quantified the pigmentation degradation throughout ALAN exposure and the response to recovery simulations to investigate if restoring natural solar cycles allowed pigmentation restoration. Results showed severe degradation of the green and red pigmentation within 49 days of exposure to both LED and metal halide lighting at night. While yellow ALAN caused more decline than shorter wavelength spectra in experiment 1, recovery investigations uncovered that within 28 days of exposure to ALAN, corals experienced 20% more green pigmentation decline in shorter wavelengths. Recovery experiments showed that recruitment of red and green pigmentation was reliant on previous spectral compositions during treatments and the restoration of the natural circadian rhythm. Investigations provide insight into species specific responses to real world scenarios of light pollution displayed around coastal waters. Additionally, research highlights that current reef recovery initiatives may be counterproductive in areas that are enduring the pressures of ALAN. Therefore, deeper understanding of the true impacts of ALAN is urgently needed in order to appropriately protect coral reef systems in proximity to developed coastlines.

Keywords: Coastal urbanisation, artificial light, ALAN, pollution, coral reefs, bleaching, Acropora, zooxanthellae, coral resilience
Introduction
As the global human population continues to increase at an exponential rate, migration has promoted urbanisation in coastal zones (Nicholls, 1995). Likewise, revolutionary technology advancements have generated structural mechanisms, providing economic sustenance to these regions (Marafa, 2008). 2015 was labelled as the “International Year of Light” due to advancements in artificial lighting across spectra and uses (Davies et al., 2016). However, regardless of the anthropogenic progression this grants, research has begun to shed light on the substantial consequences of economic growth, which threaten surrounding coastal ecosystems (Bird et al., 2004; Gaston et al., 2015b). In recent years, research has emphasised artificial light at night, also known as ALAN, as an emerging yet substantial anthropogenic stressor (Gaston et al., 2015a). On a global scale, modernisation in developing countries is promoting regional expansion of artificial lighting, whereas in developed countries increased concentration of the sensory pollution is becoming visible (Bird et al., 2004). Consequently, increases in sensory pollution exposure can stimulate negative responses which threaten productivity of key ecosystems, including coral reefs (Zantke et al., 2013).

Emerging research into the global decline of coral reefs, have highlighted their ecological and economic importance (Chadwick et al, 2011; Graham and Nash, 2013; Ayalon et al., 2021). Anthropogenic stressors including pollutants, dredging, land reclamation, and over exploitation of species and habitats have shifted key environmental stimuli (Dubinsky & Stambler, 2011; Lam et al., 2015; Hughes et al., 2018). Scleractinia rely on these stable parameters for adequate building of the three-dimensional calcium carbonate structure (Hughes et al., 2018). Therefore, ecosystem shifts are altering key relationships between coral and associated flora and fauna, and as a result, reef systems are displaying degradations globally (van Woesik et al., 2011; Ayalon et al., 2019). Consequences throughout the food chain emerge, as lack of nurseries, food, and shelter, shift biodiversity in these regions (Moberge and Folke, 1999). Equally, economic viability is threatened as coastal defences, fisheries and tourism are likewise vulnerable.

The use of artificial lighting has transformed over the last century as economic revolution and global migration drives anthropogenic activity after dusk (Gaston et al., 2015a). Concentrated yellow glows are visible from space due to sky glow (reflection and dispersion of lighting through the atmosphere) and direct lighting (found via shipping, ocean surveying and most commonly inland street lighting) (Henderson et al., 2011; Davies et al., 2014; Falchi et al., 2015). Furthermore, the use of satellite imagery and in situ monitoring techniques revealed that 22% of coastlines were exposed to ALAN in 2010 and this area is expanding at a rate of 2.2% annually (Davies et al., 2016; Kyba et al., 2017) (Figure 1A and 1B). Alongside this, in recent years there has been a global transition from narrow spectrum yellow lighting to broader blue lighting (Gaston et al., 2012). This included the switch from Low-Pressure Sodium (LPSs) and High-Pressure Sodium (HPSs) lighting to Light Emitting Diodes (LED). While these advancements stimulate economic drivers, improves human field of vision, and provides more technological control, it exposes regions that theoretically would previously only have received lunar illuminance between dusk and dawn (Gaston et al., 2015b; Tidau et al., 2021). As a result, altered natural light cycles supply an impending pressure to local ecosystems (Schröer and Hölkner, 2017; Tidau et al., 2021).
Figures 1A and 1B: Original maps displaying global distribution of the sensory pollution and coral reefs, respectively. ALAN is highlighted as yellow areas, comparable to coral reef sites as blue. This aims to portray proximity of sensory pollution to keystone coral reef ecosystems. Data from these maps were derived from ArcGIS (2021).
Though there are extensive investigations into impacts to terrestrial environments, research has only just begun to question the scale of pressures to marine systems (Ayalon et al., 2021). Examination of artificial lighting through the water column has uncovered penetration up to one hundred metres deep (Rosenberg et al., 2019) and the true dispersal of artificial light across oceans is now being mapped globally (Smyth et al., 2021). This has shed light on the global presence of artificial light penetration through the water column, and has revealed that at 1m depth, 1.9 million square km of coastal ecosystems are exposed to the influences of ALAN. Moreover, at 10m depth, 1.6 million square km are exposed (Smyth et al., 2021). This research suggests alterations to essential sensory stimuli in coastal pelagic ecosystems and also presents enquiries into the influences on shallower photic zone ecosystems, including coral reefs surrounding the Indo-Pacific regions (Sevilla et al., 2019).

Examination of coral reef systems in proximity to urbanisation and coastal development (Gorbunov and Falkowski, 2002; Levy et al., 2003; Kaniewska et al., 2015; Rosenberg et al., 2019) have established fundamental relationships between daily light cycles and the key biological functioning of reef building corals; this includes mass spawning events of Acropora species and polyp contraction feeding methods (Roth et al., 2010; Boch et al., 2011; Hilton et al., 2012; Craggs et al., 2017; Ayalon et al., 2021). It is now known that extensive periods of light exposure, or fluctuating spectrums of light, result in unsynchronised spawning and distorted feeding mechanisms (Rosenburg et al., 2019). Although there is increasing investigations into reef system photobiology, research into individual coral response is limited. Key papers have discovered the influences of spectral variation to host coral growth (Gorbunov et al., 2002; Levy et al., 2003; Ayalon et al., 2019). This highlights that further investigations must be completed on an individual level, in order to understand the true impacts of modern light on the survival of reef systems.

The existence of blue light in the water column influences chlorophyl \( a \) concentration and photosynthesis determination (Rosenburg et al., 2019). Invasive coral photobiology research has supported this, showing that coral photoreception sensitivity becomes critical at smaller wavelengths, from 400-520nm (Gorbunov et al., 2002; Levy et al., 2003). However, while these methods have highlighted the potential negative consequences of prolonged alterations to spectral composition, they were not able to provide details of coral health changes overtime. This was because the investigations involved before and after examinations. Therefore, the progressive shifts in coral functioning, while enduring ALAN exposure, remains poorly understood. This research includes non-invasive methods to holistically evaluate signs of inadequate functioning, by including visual representation of pigment degradation, (i.e.) bleaching. This is with the aim of providing longitudinal data, visualising coral health.

Vast research has investigated the recovery of coral reef systems following other anthropogenic induced bleaching events; however, is yet to consider the recovery capability of corals after termination of exposure to ALAN (McClanahan et al., 2005; Burt et al., 2008; van Woesek et al., 2011). This raises questions regarding the recovery of biological and physiological processes within individuals and reef systems in a modernising world transitioning to light of shorter wavelength. Previous research has presented the impacts of anthropogenic light, which threaten the reef building Scleractinia. This guides further enquiries into the influences of the different spectrums of light, which are being displayed during the revolutionization of modern
lighting technology along highly populated coastlines. The aim of this research is to observe the development of reef building corals when enduring enhanced ALAN at blue and white spectra. Longitudinal analysis of pigmentation and coral health will be examined, followed by similar examinations of recovery in natural cycles compared to enhanced aquarist lighting simulations.

Methodology

Coral Fragging and Dipping

An *Acropora microclados* (common name, strawberry cheesecake) mother coral was selected to represent reef building corals within the Indo-Pacific region. This area was chosen as it represents multiple emerging countries which display intense ALAN in proximity to concentrated areas of coral reef systems. Additionally, the economies of these regions are heavily reliant on reef tourism. The mother coral was sampled from the Biozone 7 exhibit of the National Marine Aquarium, Plymouth, United Kingdom. Using a set of Aquamedic stainless steel coral cutters, forty juvenile corals were fragged from the mother coral, measuring at <5cm each. To acclimatise and sanitize, a series of coral dipping techniques were used. Corals were submerged in 50ml Coral Revive cleaner: 5L tank water solution for 5 minutes. This was followed by 2-3minutes in 100% tank water solution. Gloves were used to avoid contamination between solutions. Using Betta Coral Glue, each coral frag was ‘planted’ to a coral plug to provide a stable surface for the coral to adhere to and promote healthy growth and survival. It was ensured that the bases of corals and plugs were dry before setting.

Tank Setup

In the coral propagation laboratory of the National Marine Aquarium, Plymouth, UK, three simulation tanks were constructed. This consisted of three pre-built tanks with the dimensions 50cm X 53cm X 110cm and filled to 250L with system water. Three treatments were set up to simulate a sunrise and sunset cycle (group A, control group), Light Emitting Diode (LED) artificial light pollution (group B) and a metal halide artificial light pollution (group C). The forty coral frags were split between the three simulations and labelled A1-A10, B1-B5 and C1-C5, using a Sharpie Fine Point Marker. Each group then underwent 49 days of treatment.

Experiment 1: Treatment

Artificial Illumination (AI) Hydra TwentySix HD LED lighting systems were set up in each tank to manipulate the lighting spectra. This system enabled the alterations of 26 LEDs to produce different wavelengths of light typically shown in natural daylight, lunar light, LED street lighting and metal halide street lighting. The lighting system had a peak PAR of 174, however it averaged 88 PAR over the area of coverage when at 100% intensity and overhanging 24 inches above water level. The total light coverage across the surface was 91cm. However, coral fragments were kept within the focal point of 61cm. Each experimental group experienced a daylight simulation, however following sunset at 1800hrs, group B and C were subjected to low level light pollution simulations. In comparison, group A experienced natural lunar phases. The simulations’ spectra and timings were as follows:
• **Group A**: Control Group. Group A experienced a natural sunrise and sunset cycle at 6500K and 5% intensity at 0600hrs, rising to 80% intensity from 1000-1400hrs and setting at 1800hrs at 5% intensity. From 1810-0550hrs, a lunar illuminance was synchronised to monthly lunar cycles of the Indo-Pacific region for the period of March-August. The intensity maximized at 5% at a full moon and decreasing to 0% at a new moon (Table 1).

• **Group B**: LED simulation. Group B included a sunrise and sunset cycle at 6500K and 5% intensity at 0600hrs, followed by 80% intensity at 1000-1400hrs; decreasing back to 5% at 1800hrs. At 1810hrs, group B experienced a low-level LED simulation at 10000K and 5% intensity (Figure 2A).

• **Group C**: Metal Halide simulation. Group C experienced a sunrise and sunset cycle at 6500K and 5% intensity at 0600hrs, followed by 80% intensity at 1000-1400hrs; lowering to 5% intensity again at 1800hrs. At 1810hrs, group C experienced a low-level metal halide ALAN simulation at 5000K at 5% intensity (Figure 2B).

**Table 1**: The calculation for lunar phase intensity with corresponding fraction of illumination at each lunar phase. The intensity calculations are shown in column three, where 100% is equal to 88 PAR at 24 inches from the light source. The maximum intensity at a full moon is 5%, therefore the output intensities at column four are given, ranging from 0% at a new moon to 5% at a full moon.

<table>
<thead>
<tr>
<th>Lunar Phase</th>
<th>Lunar Illumination Fraction</th>
<th>Intensity Calculation</th>
<th>Output intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Moon</td>
<td>0.1</td>
<td>5% x .10</td>
<td>0%</td>
</tr>
<tr>
<td>Waxing Crescent</td>
<td>0.2</td>
<td>5% x .20</td>
<td>1%</td>
</tr>
<tr>
<td>First Quarter</td>
<td>0.4</td>
<td>5% x .40</td>
<td>2%</td>
</tr>
<tr>
<td>Waxing Gibbous</td>
<td>0.6</td>
<td>5% x .60</td>
<td>3%</td>
</tr>
<tr>
<td>Full Moon</td>
<td>1</td>
<td>5% x 1.0</td>
<td>5%</td>
</tr>
<tr>
<td>Waning Gibbous</td>
<td>0.6</td>
<td>5% x .60</td>
<td>3%</td>
</tr>
<tr>
<td>Third Quarter</td>
<td>0.4</td>
<td>5% x .40</td>
<td>2%</td>
</tr>
<tr>
<td>Waning Crescent</td>
<td>0.2</td>
<td>5% x .20</td>
<td>1%</td>
</tr>
</tbody>
</table>

**Corresponding Parameters**
To correctly simulate reef action, two VorTechMP40 Propellor Pumps were fitted at opposite ends of the tanks to provide adequate water movement. The pumps were set to ‘reef crest random’ mode, which consisted of irregular influxes of motion, followed by lower water movement intensity. Pumps were examined weekly to ensure quality maintenance.

**Data Analysis**
Following the data collection, analysis included studies of the weekly photographs in the image processing software, ImageJ. Other non-invasive methods have included an optical coherence tomography (OCT) (Spicer et al., 2019) to study optical properties of colonies. However, as the specialist cellular analysis equipment was not available, photo imagery allowed representative assessment of coral optical properties and zooxanthellae response, as a measurement of pigment concentration.
and composition throughout the host. Current research uses the “Greyscale Model” to quantify coral bleaching (McLachlan & Grottoli, 2021), however during this research, the green and red band wavelengths were examined to provide an observation of chlorophyll \( a \) density and zooxanthellae, respectively. Pixel coverage across the corals’ surfaces provided numerical and longitudinal visualisations of the coral pigmentation within these bands, throughout the treatments.

**Figure 2A:** Spectral makeup (Kelvin, (k)) and intensity (%) of Simulation B (LED ALAN treatment) shown in blue.  **2B:** Spectral makeup (Kelvin (k)) and intensity (%) of Simulation C (metal halide ALAN treatment) shown in green. 100% light intensity provides an output of 88 PAR at 24 inches from the light source.

**Figure 3:** Photograph collection using a 1cm x 1cm square grid stand, to retrieve longitudinal photographic data of coral pigmentation. Using reference centre alignments, the camera was centred to the base of the stand and images were cropped to reveal the final raw JPG File image.
Using ImageJ, the selected coral and data file were chosen and opened. The colour of the image was split into three channels: green, red and blue, and focussing on the green and red bands individually, the colouring threshold was set as 100 as a constant across all coral images (see Step 1 of Figure 4). Measurements were set to include perimeter, area (to provide a pixel count) and area fraction (to provide a percentage of pigmentation coverage). Each coral image was outlined using the Star G430S graphics drawing table (see Step 2 of Figure 4). The threshold was applied, and measurements were analysed (Step 3 of Figure 4).

**Step 1.**

**Step 2.**

**Step 3.**

**Figure 4:** A progression chart of coral pigmentation image analysis, within the green colour channel, using ImageJ. Step 1 shows the threshold applied to quantify the pixels covered by the focussed pigmentation. The coral fragment is outlined in Step 2 and application of the threshold converts the image to black and white in step 3. From here, measurements are taken to provide the pigmentation pixel count and area coverage of the selected area.

**Statistical Testing**

Data was collated in excel which organised the pixel percentage of the green and red pigments at each collection date for groups A, B and C. Using the statistical analysis software, Minitab 19, variances between results were tested to examine if the means of groups A, B and C were statistically different to each other. A test for normality was completed first, followed by a mixed effect model ANOVA. This was
completed following the “Stats, “ANOVA” and “Mixed Effects Model” within the drop-down menu. Fixed terms included “Treatment”, random effects included “Date” and the response was “Pixel Coverage (%)”, representing either the green or red bands. In all statistical test, the 95% significance level was implemented ($\alpha = 0.05$). The hypotheses being tested were as follows:

$H_0$: There is no significant difference between the pigmentation responses of Groups A, B and C in the green or red band.

$H_1$: There is a significant difference between the resulting pigmentation of Groups A, B and C in the green or red band.

**Experiment 2: Recovery**

Following treatment, experiment 2 considered the recovery of pigment across the coral fragments. Twenty coral fragments were cut from the mother *Acropora microcladus*, using the same methods previously mentioned in “2.1 Coral Fragging and Dipping”. These frags were split into the same two ALAN simulation as experiment 1 and labelled B6-B15 and C6-C15. Following 21 days of ALAN exposure, corals were placed into two recovery simulations for 21 days. This was to consider if enhanced LED aquarist lighting would improve pigment recovery in terms of time and percentage cover, compared to, simply, the restoration of circadian rhythms to natural solar light cycles. The recovery simulation groups were as follows:

1. **Restoration using natural light cycles.** This consisted of the same lighting simulations as Group A of experiment 1, including monthly lunar cycles.

2. **Restoration using enhance aquarist lighting.** The spectral makeup stayed consistent throughout the daily cycle, however, intensities ranged from 0% at 0730hrs increasing to 100% at 1030hrs. From 1430hrs, this decreased again to 0% by 1630hrs (Figure 5).

Two Radion Spectrum XR30W Gen 4’s were used to enhance lighting, which consisted of a series of LEDs including: 8 “cool white”, 6 “deep blue”, 8 “blue”, 4 “green”, 4 “photo red” and 4 “UV” lights. At 100% intensity, the spectral makeup is as follows: 100% UV, 100% royal blue, 100% blue, 24% green, 24% red, 24% cool white and had an average PAR reading of 214.

Following data collection, data analysis included the mixed effects model ANOVA using Minitab 19, at a 95% significance level. The hypotheses were as follows:

$H_0$: There is no significant difference in the use of enhanced artificial lighting compared to natural lighting, on the recovery of corals after ALAN exposure.

$H_1$: There is a significant difference in the use of enhanced artificial lighting in comparison to natural solar cycles, when considering the recovery of corals after ALAN exposure.
Data sets were split into groups to compare recovery response after ALAN simulations:

- **Group 1**: LED ALAN treatment, followed by natural cycle recovery simulation.
- **Group 2**: LED ALAN treatment, followed by enhanced aquarist lighting recovery simulation.
- **Group 3**: Metal halide ALAN treatment, followed by natural cycle recovery simulation.
- **Group 4**: Metal halide ALAN treatment, followed by enhanced aquarist lighting recovery simulation.

**Figure 5**: The daily lighting intensities of two ALAN simulation tanks between the hours of 06:00 and 18:00. The natural cycle is displayed using orange lines and the enhanced aquarist lighting simulation is displayed using blue lines. Spectral makeup is consistently at 6500K in the natural cycle, whereas the spectral makeup in the aquarist lighting tank is measured using intensities of 6 LED lights. At 100% intensity, the spectral makeup is as follows: 100% UV, 100% royal blue, 100% blue, 24% green, 24% red, 24% cool white. Lighting intensity is measured using percentage (%) and at 100% intensity the average PAR is 214. This is represented as a solid line.

**Results**

**Experiment 1: Treatment**

Pixel cover within the green and red channel declined significantly when corals underwent ALAN in both treatments. However, there was also some, but less severe
pigmentation loss within the control group. Table 2 provides a visual representation of the difference in coral pigmentation after 49 days of ALAN exposure. Within the green channel, group A showed a mean pixel cover loss of 31.98%, compared to groups B and C, at 52.9% and 59.92%, respectively (Figure 7). After 49 days of exposure to artificial light pollution, the mean green pixel cover was 40.48% for groups B and 33.1% in group C. However, some showed more sensitivity than others; the lowest pixel coverages in the ALAN simulations were C5, at 5.88%, and B3, at 7.48%.

Table 2: An illustration of the decline in pigment within the green band of three example corals. A10 represents the control group, B4 represents the LED ALAN simulation and C3 represents the metal halide ALAN simulation. Green band pigmentation is displayed using red pixels and photographs of before treatments (14/04/2021) and after treatments (02/06/2021).

<table>
<thead>
<tr>
<th>Sample of coral fragment green band pigmentation degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Treatment 04/04/2021</td>
</tr>
<tr>
<td>A10</td>
</tr>
<tr>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>After Treatment 02/06/2021</td>
</tr>
<tr>
<td><img src="image4.png" alt="Image" /></td>
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</tbody>
</table>

Measurements across the red channel demonstrated greater decline of pixel coverage than green pigmentation. Group A showed an average decline of 64.37% within 49 days (Figure 6). Alternatively, the ALAN simulations displayed a mean pigmentation loss of 75.79% (Group B) and 77.53% (Group C). After 49 days of exposure to ALAN, the average red coverage was 11.91% in group B and 6.73% in group C.

Longitudinal analysis of the optical properties showed clear differences in coloration of both channels between groups within 14 days of exposure to ALAN (Figure 7). There was a 3.94% difference between the average green pigmentation colour between groups A and B, and 6.33% differences between A and C after 14 days of exposure. Within the green channels, the differences between the average green pigmentation compared to the control group was 3.12% between groups A and B and 13.3% between groups A and C.
Pigmentation loss occurred at areas closest to the lighting, before gradually spreading throughout the remainder of the host (see table 3). Group B included excessive bleaching of one coral, B1, which led to excess turf algae growth (Figure 8). This led to a distinct line in which bleaching prevented recruitment of zooxanthellae and the recovery of pigmentation. In contrast, the weight results showed no significant changes or associations with treatments throughout the duration of experiment. However, when weighing, corals from both ALAN simulations produced substantially less, or no mucus, compared to the control group.

**Figure 6**: The mean green and red channel pigmentation loss of 40 coral frags, within a 49 days period of exposure to artificial light at night. The control group consisted of a natural day night cycle (Group A, in green), Group B were exposed to LED ALAN pollution (highlighted in blue) and Group C, Metal Halie ALAN pollution (highlighted in yellow).
Figures 7A and 7B: The trend of mean pixel area coverage (%) of corals over a forty-nine-day treatment of different light exposures. This shows the changes in average pigmentation when exposed to a natural light cycle (solid line), LED artificial light at night (dashed line) and metal halide artificial light at night (dotted line). The green band wavelength is highlighted in green (A) and the red band wavelength is highlighted in red (B).
Table 3: A timeline of pigmentation loss from coral B1 in the red band wavelength. Raw photographs are compared alongside the analysed pixel photographs. Bleaching occurs from the tips, at the points nearest to the exposed light, and gradually spreads throughout the coral to the end of the treatment period.

<table>
<thead>
<tr>
<th>Date</th>
<th>Photograph</th>
<th>Photograph</th>
<th>Photograph</th>
<th>Photograph</th>
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<td><img src="image15" alt="Photo" /></td>
<td><img src="image16" alt="Photo" /></td>
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</tbody>
</table>

Figure 8A and 8B: The impacts of bleaching to coral B1 that underwent LED ALAN exposure. 8A shows the raw discolouration, whilst 8B shows the analysed pigmentation pixel count. The impacts of bleaching are highlighted in orange circles, whereas turf algal production is highlighted using green circles.
Experiment 1 Statistical Testing
Table 4 lists the outputs from the stats model, comparing differences between the means of groups A, B, and C across the green and red wavelength. Comparing all three simulation treatments across the green band gave a p value of p= 0.013. As p<0.05, there is sufficient evidence to suggest the treatment of lighting significantly impacts the mean coral pigmentation coverage within the green band (Figure 7). Comparison of both A to B and A to C in the green band showed a significant difference in output data; p=0.025 and p=0.037, respectively. This suggests that both ALAN simulations alter the pigmentation of corals within green channel. A comparison of groups B and C provided an output p value of p=0.452, suggesting that there was no significance difference between the green channel data between ALAN treatments.

Conclusions from red channel data comparing all groups gave p=0.016. Therefore, as p<0.05, the null hypothesis is rejected and there is sufficient evidence to suggest that the treatment of lighting significantly impacts the red pigmentation coverage of the corals (Figure 7). Comparing groups A and B gave an output value of p=0.260, suggesting no statistical difference between the pigmentation data. Comparisons of groups A to C and groups B to C produced p values of p=0.013 and p=0.057 respectively. This suggests there are significant differences between the mean output pigmentations of group C compared to the control group and shorter wavelength ALAN simulation.

Table 4: The outputs of the mixed effects model to compares the mean red and green pigmentation pixel cover groups between coral fragments that underwent artificial light pollution (B and C) compared to a natural circadian rhythm (A). ALAN simulations include LED artificial light pollution (B) and metal halide artificial light pollution (C). The output degrees of freedom (df), f-value (f-stat) and p-values are included.

<table>
<thead>
<tr>
<th>Band</th>
<th>Groups Included</th>
<th>df</th>
<th>f-stat</th>
<th>p-value</th>
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<tr>
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<td>5.95</td>
<td>0.013</td>
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<tr>
<td></td>
<td>A, B</td>
<td>7.00</td>
<td>8.14</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>A, C</td>
<td>7.00</td>
<td>6.61</td>
<td>0.037</td>
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<tr>
<td></td>
<td>B, C</td>
<td>7.00</td>
<td>0.64</td>
<td>0.452</td>
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<tr>
<td></td>
<td>A, B, C</td>
<td>14.00</td>
<td>5.61</td>
<td>0.016</td>
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<tr>
<td></td>
<td>A, B</td>
<td>7.00</td>
<td>1.50</td>
<td>0.260</td>
</tr>
<tr>
<td></td>
<td>A, C</td>
<td>7.00</td>
<td>10.94</td>
<td>0.013</td>
</tr>
<tr>
<td>Red Band</td>
<td>B, C</td>
<td>7.00</td>
<td>5.16</td>
<td>0.057</td>
</tr>
</tbody>
</table>

Experiment 2: Recovery
Optical data analysis showed pigmentation restoration of all corals throughout the green and red channels in both natural cycle and enhance lighting simulations within 42 days. Moreover, longitudinal analysis displayed an increase in pigmentation
within 21 days in all groups. Corals that underwent LED ALAN showed more red pigmentation recruitment in enhanced aquarist conditions; group 1 displayed 61.1% increase, whereas group 2 showed 67.8% red band recruitment (Figure 9). However, restoration of the natural cycle provided increased recruitment of green pigmentation. Group 1 presented a 34.9% increase compared to 33.9% within group 2. Corals that endured metal halide ALAN indicated increased red and green pigmentation recruitment if the natural circadian rhythm was restored. For example, within the green channel, group 3 displayed a 15.8% increase compared to 13.5% in group 4. Also, within the red band, group 3 increased 71.6%, in comparison to 41.5% in group 4.

![Figure 9](image)

**Figure 9:** The mean green and red channel pigmentation recruitment of 20 coral fragments during recovery simulations after 21 days of ALAN exposure. Group 1 (navy blue) endured LED ALAN followed by restoration of the natural solar cycle; group 2 (light blue) experienced LED ALAN followed by an enhanced aquarist lighting recovery simulation; group 3 (orange) experienced metal halide ALAN followed by restoration for the natural light cycle and group 4 (yellow) underwent low level metal halide ALAN followed by an enhanced aquarist lighting recovery simulation.

Within experiment 2, corals that experience LED ALAN showed more recruitment within 42 days of recovery than metal halide. Although, Figure 10 displays the extended decline in pigmentation of group 1 and 2 corals within the treatment phase between 23/06/2021- 04/08/2021, LED simulation had a mean percentage pigment of 17.28% in red and 58.5% in green, compared to metal halide: 21.1% in red and 79.9% in green. This highlights a 3.82% difference in red and 21.4% difference in green. In some cases, recruitment of pigments exceeded that of when corals were initially fragged. For example: within green, initial LED mean was 87.29%, within group 1 final pigmentation was 93.42% and group 2 92.42%. Metal halide initial
mean was 93.48%, after treatment and recover simulation group 3 was 95.72% and 93.35%.

**Figures 10A and 10B:** The trend of mean pixel area coverage (%) of corals over ALAN treatment and recovery simulations within the green and red channels. This includes group 1 (LED ALAN treatment to natural solar cycle recovery simulation), group 2 (LED ALAN treatment to enhance aquarist lighting), group 3 (Metal halide ALAN to natural solar cycle recovery simulation) and group 4 (Metal halide ALAN treatment to enhanced aquarist lighting recovery simulation). The influence of ALAN exposures were tested between 09/06/2021-23/06/2021 (shown as solid blue and yellow lines) followed by recovery trends between 14/07/2021-04/08/2021 (in dashed or dotted). LED ALAN treatments are highlighted in blue, whereas metal halide treatments are highlighted in yellow. Natural solar cycle recovery simulations are displayed as a dashed line, whilst artificially enhance lighting recovery simulations are highlighted using dotted lines.
Experiment 2 Statistical Testing
Table 5 displays the output values for the mixed effects model. Statistical analysis of the green band data produced a p value of p=0.011. As p<0.05 threshold, the results are considered significant and there is enough evidence to suggest that that lighting exposure influences the recovery of corals after exposure to ALAN. Comparing green pigmentation in groups 1 and 2 produced a p-value of 0.555; suggesting after exposure to LED ALAN simulations, the exposure to either natural solar cycles or enhance lighting does not influence the recruitment of green pigmentation. However, groups 1 and 3 produced a value of p=0.008. This therefore suggests that restoration of pigmentation is reliant on the previous spectral makeup exposure. Output p-values within the red channel produce similarly statistical results. Alternatively, groups 3 and 4 produced a p-value of p=0.006. As p<0.05, it therefore suggests that the response of corals exposed to metal halide ALAN is different when experiencing different recovery lighting.

Table 5: The outputs of the mixed effects model for the green and red pigmentation response to ALAN recovery simulations. This compares groups 1 (LED ALAN treatment to natural solar cycle recovery simulation), group 2 (LED ALAN treatment to enhance aquarist lighting), group 3 (Metal halide ALAN to natural solar cycle recovery simulation) and group 4 (Metal halide ALAN treatment to enhanced aquarist lighting recovery simulation). The tests the alternative hypothesis, that lighting spectra have a significant impact on the recovery of coral fragments when exposed to ALAN. The output degrees of freedom (df), f-value (f-stat) and p-values are included.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Group</th>
<th>df-den</th>
<th>f-value</th>
<th>p-value</th>
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<td>0.36</td>
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<td>16.0</td>
<td>9.27</td>
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<td></td>
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<td>17.0</td>
<td>0.39</td>
<td>0.540</td>
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</table>

Discussion
Results from this investigation support previous work completed by Levy et al., (2003) and Rosenberg et al. (2017) and has demonstrated that reef building Scleractinia, within the Acropora genera, show susceptibility to artificial light pollution. Similarly, results expand upon recent literature by Ayalon et al. (2019), revealing the influences of spectral makeup in determining coral survival along developed coastlines. This provides valuable insight into the silent pressure of artificial light at night, which exists alongside high-profile pressures to reef systems, including ocean acidification and rising sea temperatures. The process of photosynthesis relies on consistent daylight regimes to provide adequate primary production and promote coral progression (Ayalon et al., 2021). While the intensity of
ALAN is equivalent to 0.002% of daytime light (Grubisic, 2018), fluctuation to the natural rhythm of solar and lunar illuminance phases, have shown clear changes to pigmentation across coral hosts within the ALAN treatments. The depreciation of the yellow-brown pigmentation within two weeks of ALAN exposure, indicates that the Acropora microclados fragments lost over 50% of the critical photosynthetic organisms (Fitt et al., 2000; Baker, Glynn and Riegl, 2008; Schoepf et al., 2015). While the substantial damage highlighted is concerning, it also promotes enquiries into the wider reef system recovery after exposure in urbanised regions (Schoepf et al., 2015).

Within experiment 1, longer wavelength spectra had more substantial influence on coral pigmentation in terms of time and percentage cover. Statistical analysis showed variation between the red pigmentation response between the LED and metal halide simulations. However, there was no significant difference between green pigmentation decline in groups. This differs from findings presented by Ayalon et al., (2019) and Ayalon et al., (2021) which highlighted modernising shorter wavelengths as more threatening due to further penetrations and dispersal throughout the water column (Tamir et al., 2017). Similarly, it has been emphasised that blue light is major determinant in the circadian rhythm of organisms across Animalia, including Scleractinia (Hoadley et al., 2011; Schroer and Hölker, 2017). In contradiction to experiment 1, before recovery simulations began in experiment 2, there was enhanced pigmentation decline within fourteen days of LED ALAN compared to metal halide ALAN. Although spectral results show some variation within experiments 1 and 2, this adds to the growing body of evidence that highlights the importance of spectral composition. Specifically, advances in artificial lighting technology across the globe are increasing ALAN, and human beings are manipulating spectral composition and creating 24hrs of light.

Group A similarly displayed some pigmentation loss while experiencing a natural circadian and circalunar clock. While this could be as a result of stimuli including a response to fragging and acclimatisation to lighting adjustments from previous tanks to a natural solar cycle, permission was granted to frag the mother Acropora microclados as the colony was overgrowing. As a response to lack of light, areas of the colony were displaying some signs of stress, including bleaching. Although the fragments cut showed no signs of initial bleaching, the progressive depreciation in pigmentation could suggest that pre-existing stress may enhance corals sensitivity to stimuli, and likewise, sensitivity to the influences of ALAN, as shown by the severe degradation in pigment in LED and metal halide simulations. Similarly, research has shown that corals under pre-existing stress, show more sensitivity to additional pressures (Wolff et al., 2018; Ellis et al., 2019). For example, the presence of ALAN in combination with the other high-profile stressors, such as those due to climate change, will exacerbates the bleaching. This in turn suggests that bleaching events surrounding urbanised coastlines are imminent and will be persistent as multiple pressures diminish the resilience of coral colonies (Schoepf et al., 2015).

The resilience of Acropora microclados showed pigmentation recovery within twenty-one days if light pollution at night was removed and a period where light is absent was provided. This supports current recovery initiatives, which acknowledge “dark sky” locations as an integral mitigation approach to tackling the disturbance of artificial light pollution (Davies et al. 2014). Similarly, other recovery schemes have used coral propagation and “outplanting” methods to minimise the anthropogenic
impacts to juvenile frags to help recovery (Lirman et al., 2016; Ware et al., 2020). However, this brings into question whether some of this work may be counterproductive in highly light polluted areas, as the light penetrations will still provide pressure and limit survival of juvenile frags. Results suggested that the recruitment of red and green pigmentation in recovery simulations was reliant on the previous spectral composition. Although individual fragments in groups B and C showed recovery, reef restoration is not guaranteed. Exposure to ALAN has recently manipulated a series of unsynchronised spawning events, resulting in unsuccessful reproduction of colonies (Kaniewska et al., 2015). Moreover, a bleaching threshold was estimated at 8 months, before there is disruption to key reproductive mechanisms (Mendes and Woodley, 2002). Fragments showed no indications of exoskeleton growth during all treatments due to the mass expulsion of endosymbionts, which direct photosynthetic processes (Mendes and Woodley, 2002; Schoepf et al., 2015; Swain et al., 2018). Previous investigations discovered the restoration of calcification rates proceeding the 1987 Caribbean bleaching events. However, throughout periods of induced pressures, inadequate biological functioning inhibited exoskeleton growth (Leder et al., 1991). Additionally, the reduction in mucus production in both ALAN simulations, which typically used as protective response to pressure, highlights the inadequate functioning of critical mechanisms in the presence of ALAN. Moreover, mortality of associate crustacean symbionts and susceptibility to disease is increased as coral resilience diminishes (Lesser, 1996; Shroer and Hölker, 2016). This promotes phase shifts within reef ecosystems (Spoelstra et al., 2015). From here, it is evident that further longitudinal investigations into the growth of corals, in proximity to artificially lit coastal zones, will provide insight into the true pressures of ALAN in determining biological functioning including exoskeleton growth.

Results of this study present scenarios of coral health decline due to artificial light pollution and provide application to circumstances occurring around urbanised coastlines, globally. While it is acknowledged that the species studied here was able to recover the exposure to ALAN across different spectra, it must be cautioned that ALAN exposure within this research, simulated only a short time scale in relation to those occurring continuously along urbanised coastline today. Nonetheless, the disruption to coral health in this period was substantial. The Acropora genera is currently globally listed as threatened under the Endangered Species Act. While Acropora spp. within the Indo-Pacific region are not currently considered endangered due to the quantity of populations, the results of this investigation show the ability for substantial reduction in colonies (Baker et al., 2008). This emphasises that further understanding of these reefs systems and the true influences of ALAN to these key ecosystems is urgently needed as the transition into shorter wavelength modern lighting and the light concentration increase is imminent as coastal urbanisation develops.

Conclusions
A growing body of research is providing evidence of the impacts of artificial light pollution, as it is becoming a more widely recognised pressure to surrounding marine ecosystems. Visual representation of coral health provided a non-invasive analysis which outlined the progressive decline in pigmentation to critical levels within 49 days of exposure to LED and metal halide street lighting simulations. Similarly, although during experiment 1, corals experience increased bleaching effects in metal
halide simulations compared to LED, recovery investigations uncovered that within 28 days of exposure, corals experience a 20% more decline in green pigmentation in shorter wavelengths. This highlighted the value of longitudinal research to view the progression of coral health during exposure to ALAN. Similarly, although this investigation only represents a small fraction of light exposure in comparison to real scenarios around coastal regions, it highlighted the importance of light spectra in determining coral pigmentation within the first fourteen days of low-level light at night. Corals showed sensitivity to recovery spectra after ALAN treatments, although pigmentation recovery was possible if the natural circadian and circalunar rhythms were restored. Responses to ALAN have been highlighted within the *Acropora microclados* species. This expands upon species specific understanding of the impacts of ALAN and further consequences to coral reefs surrounding urbanisation. While the incorporation of artificial lighting has permitted anthropogenic progression, the damaging pressures facing organisms, habitats, and ecosystems in surrounding coastal regions, cannot be ignored. Although the removal of artificial light at night may seem a straightforward solution, other societal and economic implications that require artificial lighting from dusk to dawn make resolving the wicked problem of ALAN, challenging. Furthermore, current recovery programmes may be counterproductive if in more highly light polluted areas. The use of ALAN in modern societies is becoming widespread and as humanity is advancing the modernisation of artificial lighting, the prevalence is being exacerbated. Therefore, a deeper understanding of the biological consequences of ALAN is urgently needed in order to sustain and restore the damaging consequences. Otherwise, the reoccurrence of coral bleaching events will continually disrupt the survival of keystone species and direct alterations in ecosystems community functioning.

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**References**


