Red light mitigates ALAN-induced memory impairment in the clownfish *Amphiprion ocellaris*

Jan IJ van Straaten[1], Lior Appelbaum[2], Fabio Cortesi[1]

[1]School of the Environment, The University of Queensland, St Lucia, Brisbane, 4072, Australia
[2]The Faculty of Life Sciences and the Multidisciplinary Brain Research Center, Bar Ilan University, Israel

Abstract

Artificial light at night (ALAN) is an increasingly pervasive anthropogenic pollutant in coastal ecosystems. However, its effects on brain function and behaviour in reef fishes remain poorly understood. Here, we investigated the impact of ALAN on sleep-dependent learning and memory in the clownfish, *Amphiprion ocellaris*, a diurnal reef fish with well-characterised photoreceptive systems. Cognitive performance was assessed using a colour discrimination task following exposure to ALAN of varying intensities and spectral compositions. ALAN impaired learning and memory after only two consecutive nights of exposure, with effects emerging at intensities as low as 0.5 lux. At higher intensities (5–50 lux), memory was consistently disrupted under white-cool, white-warm, blue, and green LED light, whereas red light had no detectable effect. Together, these findings demonstrate that ALAN rapidly alters cognitive performance in clownfish, and that these effects are strongly wavelength-dependent. Our results highlight the vulnerability of diurnal reef-associated fishes to nocturnal light pollution and underscore the need for ecologically informed lighting strategies, using longer-wavelength red-emitting light sources, that reduce both the intensity and spectral impact of ALAN in coastal environments.

Introduction

Artificial light at night (ALAN) is an emerging terrestrial and marine anthropogenic pollution worldwide (Tidau et al., 2021). Approximately 22% of the global near-shore marine environment and 35% of marine protected areas are directly exposed to ALAN (Davies et al., 2016). The increased use of energy-efficient, short-wavelength 'white-cool' LEDs further intensifies the environmental impact, as the emitted shorter blue to green wavelengths penetrate the water more deeply and disrupt the circadian rhythms, physiology, and behaviour of marine organisms (Brüning et al., 2018; Li et al., 2024).

While the effect of ALAN on marine invertebrates, such as corals and crustaceans (Marangoni et al., 2022), is well studied, how it affects brain function and sleep in marine vertebrates, specifically reef fishes, is poorly understood. In the freshwater zebrafish (*Danio rerio*) model, ALAN alters learning and has task-specific effects on cognitive flexibility (De Russi et al., 2024; Lucon-Xiccato et al., 2023). Reef fishes rely on cognitive functions such as memory and learning to support critical behaviours, including social interactions, foraging, and predator avoidance (Kieffer & Colgan, 1992). Consequently, ALAN-induced disruptions to cognitive processes may impair these essential behaviours, ultimately affecting reproduction and survival. This is supported by a recent study, which found that ALAN impaired learning in the cleaner wrasse *Labroides dimidiatus* (Sowersby et al., 2025). However, the aspects of ALAN that caused the impairment and the underlying mechanism were not investigated. Understanding the specific mechanisms by which ALAN affects brain function and health has implications for conservation-oriented strategies, such as implementing ecologically safer light wavelengths in coastal environments (Ferretti et al., 2024). Considering that sleep benefits brain function and is essential for neuronal maintenance in animals ranging from jellyfish to fish and mammals (Aguillon et al., 2026; Eban-Rothschild et al., 2018; Zada et al., 2021), ALAN-induced sleep disruption may represent a key mechanism underlying cognitive impairment in reef fish.

As for other vertebrates, teleost fishes possess multiple photoreceptive structures to mediate responses to light. Light is captured by photoreceptors, either rods or cones, which transmit an electrical signal that initiates the phototransduction cascade, allowing downstream processing (Lamb et al., 2016). Retinal photoreceptors support visually guided behaviours such as foraging, predator avoidance, and colour discrimination (Marshall et al., 2019), while extra-retinal photoreceptors, particularly those in the pineal gland, entrain circadian rhythms (Ben-Moshe Livne et al., 2016; Ben-Moshe et al., 2014).

Light-mediated circadian rhythms are well-characterised in zebrafish, where they regulate processes such as melatonin secretion, opsin gene expression, retinal activity, and sleep-wake cycles (Li, 2019; Ben-Moshe Livne et al., 2016). However, their function in reef fishes remains poorly understood. Recent studies on clownfishes (subfamily Amphiprioninae) have described the diversity and distribution of photoreceptors in the retina and pineal gland, highlighting this species as a valuable model for examining light sensing and circadian regulation in a reef fish context (Cortesi et al., 2022; Mitchell et al., 2021). Moreover, clownfishes are emerging model organisms for marine science that can be readily raised in captivity (Laudet & Ravasi, 2023). They are amenable to training and learning (Powell et al.,

2021) and are capable of performing complex visual and cognitive tasks (Mitchell et al., 2024), making them ideal for studying the effects of ALAN on cognitive performance.

Here, we examine the effects of ALAN on memory and learning in the false percula clownfish, *Amphiprion ocellaris*. Cognitive performance was assessed using a colour discrimination task (as per Triki et al., 2023), following the acute exposure over two consecutive nights to different intensities of white-cool LED ALAN (0.5, 5, and 50 lux) and to different colours of ALAN (all at 50 lux). These treatments were selected to compare modern (white-cool LED) versus older-style (white-warm LED) ALAN, and to test a range of wavelengths (blue, red, and green) to identify the spectrum-dependent effects on the brain. We found intensity-dependent effects of ALAN on memory and learning. In addition, while all other tested spectral compositions of ALAN negatively impacted the fish's cognitive performance, red light had no effect.

Material & Methods

Animal husbandry

Darwin morphs (black) false percula clownfish, *Amphiprion ocellaris*, were captive-bred as part of the Marine Sensory Ecology Group's breeding colony at the Institute of Molecular Biosciences, University of Queensland. Approximately one-year-old individuals were housed in pairs, with the larger fish as the female and the smaller fish as the male, allowing us to distinguish the individuals in each tank by their size. Fish were kept under laboratory conditions (broad white-warm LED Lights, 12L:12D, artificial seawater at 1.025 ppm salinity, 27-28 °C) in 80 L tanks and provided with a small terracotta pot as shelter. Before training, fish were acclimated to their experimental aquarium for two weeks. Sixteen fish (eight females and eight males) were used in the intensity experiment, and 16 different fish (eight females and eight males) were used in the experiment testing different spectral compositions of ALAN. In each experiment, four pairs served as controls and four as treatments; no pair was used in both conditions. Animal care and euthanasia followed procedures approved by the University of Queensland's Animal Ethics Committee (2022/AE000569).

Experimental setup and paired-choice colour discrimination test

The experimental setup and paired-choice colour discrimination paradigms were conducted as described in detail by Triki et al. (2023). Briefly, experimental aquaria were partitioned into separate housing and testing compartments using opaque plexiglass (Figure 1). Using this physical and visual separation between a pair of fish prevented social learning during trials

(Figure 1). Prior to each test trial, individual fish were confined to a smaller housing compartment within the test compartment using a pair of acrylic barriers—one transparent and one opaque (dimensions: 24 × 22 cm). The removal of the opaque barrier, followed by the transparent barrier, enabled the fish to visually inspect the test compartment before engaging with the task.

Before training, fish were habituated to isolation in the test compartment, then to feeding shrimp paste (approx. 0.5 cm in diameter) smeared onto grey plastic plates (5cm x 5cm 0.5cm), and subsequently to feeding from the designated colour cue (coloured plastic plates with the exact dimensions as the grey ones). During training and trials, fish were presented with a yellow and green cue, taped onto the grey plastic support (Figure 1). We used yellow as the rewarding cue, with shrimp paste stuck to the back of the support, invisible to the fish, and green as the control. Following cue placement, the opaque barrier was removed to allow visual inspection of the test compartment. After two seconds, the acrylic sheet was lifted, permitting the fish to engage in the task (Fig. 1). To claim their reward, fish had to swim to the back of the yellow cue. A fish approaching the yellow cue and claiming the reward was scored a "success", while a fish that first explored the green cue was scored a "failure", which resulted in cues being removed. Fish were trained for three weeks to learn the task, and testing began when both the treatment and control groups exceeded an 85% success rate over ten trials. These learning criteria correspond to a probability of correct responses that is greater than the chance level of 50% ($p < 0.05$, binomial test). During the testing phase, each fish underwent ten trials per day over a period of three consecutive days (two nights of ALAN exposure). After three days of testing, the fish were left to recover under normal dark-light conditions for two days. During this time, reinforcement training in the colour discrimination task was conducted to the 85% threshold before the next testing phase began. Then, the fish were left to recover for two days without training before a new experimental round began.

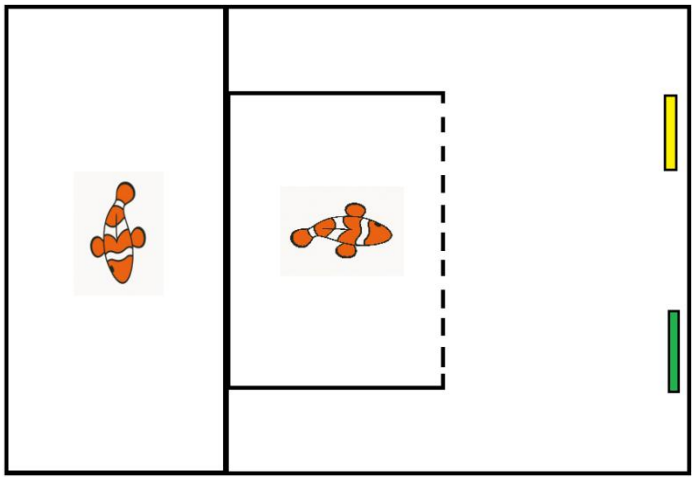

Figure 1: Overview of the experimental setup. Fish were divided into the holding compartment (left fish) and the housing compartment within the test compartment (right fish) using a removable opaque acrylic barrier. For the two-choice experiment, a yellow (reward) and green cue were placed at the far end of the test compartment. The test fish was kept in the test compartment, with the dotted line representing two acrylic barriers, one transparent and one opaque. The opaque barrier was initially lifted to allow the fish to become accustomed to the test arena before the transparent barrier was lifted.

Light treatment

For the intensity experiment, ALAN was generated using broad-spectrum white-blue LED lights (VIPARSPECTRA, Model V165) at three intensities: 0.5, 5 and 50 lux as measured with a lux meter. For 0.5 lux, the LED light was set at an intensity of 1/100, for 5 lux, this was 4/100, and for the 50 lux treatment, the light was set to 15/100. The light intensity was measured at the water surface in the centre of the tank, and fish were exposed to ALAN from 7 p.m. to 7 a.m. Based on the effects of these three intensities (Fig. 2), 50 lux was chosen as the standard intensity for comparing the impact of different spectral compositions of ALAN on memory and learning in *A. ocellaris.*

To test the effects of ALAN with different spectral compositions, we used broad-spectrum LED lights (VIPARSPECTRA, Model V165) combined with various LEE colour filters (https://leefilters.com/). Four different light treatments were created: white-cool LED, blue LED, red LED, and green LED. White-cool LED was produced in the same manner as described above. For the blue LED treatment, broad-spectrum LED light (VIPARSPECTRA, Model V165) was set to full intensity (100/100) at the cool LED setting, and the light was passed through a 0.3 neutral density filter (LEE 209), a diffusing sheet, and a blue colour filter (LEE 201). For the green LED treatment, the cool LED intensity was reduced to 70% of its original value, and the light was filtered through a 0.3 neutral density filter, a diffusing sheet, and a green colour filter (LEE 244). The red LED condition was produced by setting the cool

LED to an intensity of 0/100, using a diffusing sheet, and a red colour filter (LEE 106). An additional white-warm LED treatment was produced by using a broad-spectrum white-warm LED light strip without further filtering.

The absolute irradiance in photons/count at 50 lux for each ALAN source was measured using a 1000 µm optical fibre connected to a USB2000 spectrometer (Ocean Optics) and OceanView software. The fibre was pointed at a 45^0 angle at a diffusion reflectance standard (WS-1, Ocean Optics) placed at the bottom, in the middle of the experimental section of the tank. This way, the light in the tank was collected from the semisphere above the reflectance standard, representing the overall light intensity experienced in the tank. For each light treatment, the absolute irradiance was measured ten times and averaged (Fig. 3A–E). The light environment during the day, consisting of a mixture of white-warm LED and fluorescent light sources, was measured in the same way (Fig. 3F).

Statistical analysis of the colour discrimination test

We analysed the binary outcome variable, choice, with 0 indicating failure and 1 indicating success, across multiple trials over several days to assess 1) the effect of different white-cool ALAN intensity and 2) ALAN with different spectral composition on success rates over time. The merged datasets consisted of 2,600 observations from 31 individuals, with repeated measurements per fish.

To account for repeated-measures binomial data, we compared Generalised Linear Mixed Models with a binomial distribution and individual ID as a random factor using the glmer() function from the lme4 package (Boeck et al., 2011) in R v.4.3.3 (Posit Team, 2025). Nested models, including a null model, models with day or treatment alone, an additive model, and a full model with their interactions, were compared using Akaike Information Criterion (AIC). Model selection was based on comparing ΔAIC values and likelihood ratio tests, with the simplest model having $\Delta AIC < 2$ and a non-significant LRT ($p > 0.05$) chosen as the best-fitting model.

Results

The effect of light intensity on memory and learning

Using the white-cool LED treatment at different intensities (0.5, 5 and 50 lux), we first assessed the effect of ALAN light intensity on memory and learning in *A. ocellaris.* For the 0.5 lux treatment, the full interaction model (days × treatment) was selected based on the lowest AIC ($\Delta AIC = 0$), indicating that treatment effects varied over time (Fig. 2A; Tables S1-3). At

baseline, success rates were slightly higher in the ALAN treatment (~88%) than in the control group (~85%; estimate = 0.30, 95% CI = −0.73 to 1.33). However, while the control group improved over the experiment (~99% success at the end; estimate = 0.93, 95% CI = 0.27 to 1.59), no improvement was observed in the treatment group (~87%; interaction estimate = −0.93, 95% CI = −1.74 to −0.12), indicating that learning was impeded in the treatment group.

For the 5 lux treatment, the additive model (days + treatment) was selected as the best-supported model (ΔAIC < 2 and non-significant LRT), indicating that days and treatment independently influenced success (Fig. 2B; Tables S1-3). The effect of experimental duration was negligible for both groups (estimate = −0.03, 95% CI = −0.38 to 0.32). The control group consistently achieved a very high success rate (~95%). Conversely, the ALAN treatment group consistently exhibited lower success (~85%; estimate = −1.02, 95% CI = −2.19 to 0.23), indicating that no improvement due to learning occurred in the treatment fish.

In the 50 lux treatment, the interaction model (days × treatment) was selected (ΔAIC < 2 and non-significant LRT), suggesting that ALAN modulated the temporal dynamics of success (Fig. 2C; Tables S1-3). At the start of the experiment, treatment fish had a lower success rate than control fish (~86% vs ~ 94%; estimate = −0.98, 95% CI = −2.19 to 0.23). Days had a small positive effect on controls, increasing to ~98% success at the end (estimate = 0.15, 95% CI = −0.60 to 0.89). Importantly, for the treatment group, 50 lux ALAN greatly reduced success over time (~69% success at the end; interaction estimate = −0.72, 95% CI = −1.56 to 0.12), indicating a strong adverse effect on memory and learning at higher light intensity.

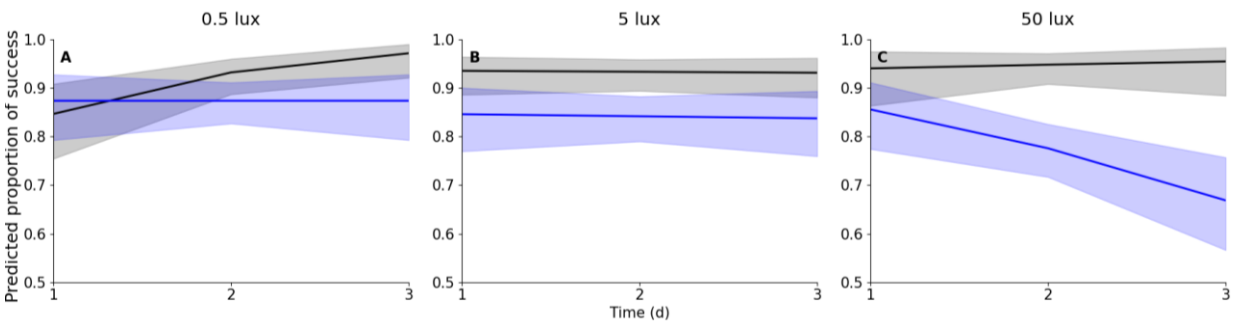

Figure 2: Predicted success rates of *Amphiprion ocellaris* exposed to varying intensities of white-cool LED ALAN. Each panel shows the model-predicted mean (solid line) and 95% confidence interval (shaded area) for fish exposed to ALAN over two consecutive nights at 0.5 (A), 5 (B), or 50 lux (C), compared to the dark control group (black) (n = 8 fish per treatment). The y-axis starts at 0.5, representing random chance.

The effect of spectral composition on memory and learning

The behavioural responses of coral reef fishes to ALAN may be dependent on its spectral composition, as different wavelengths penetrate the water column with varying efficiency and trigger distinct physiological pathways (Holles et al., 2016; Schligler et al., 2021). With ALAN intensity fixed at 50 lux, we assessed the effect of different spectral compositions (Fig. 3) on memory and learning in *A. ocellaris* (Fig. 4).

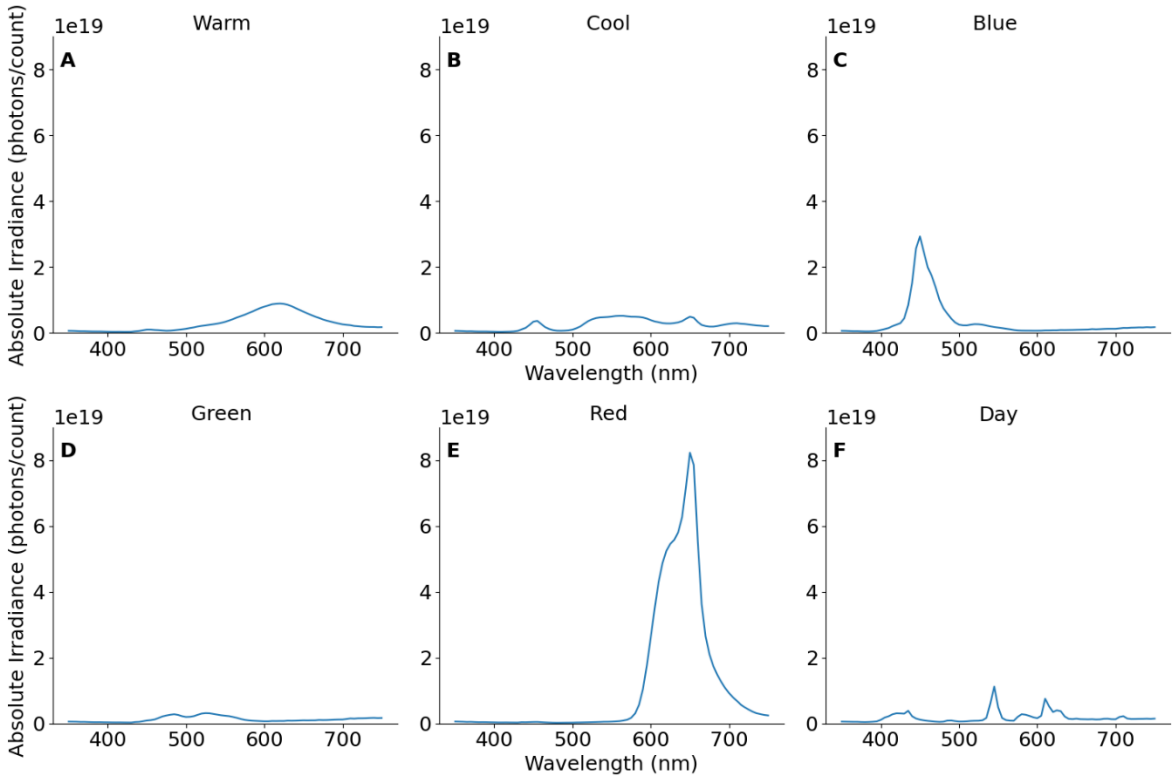

Figure 3: Spectral composition of each light treatment in the discrimination experiment at 50 lux. A – D) ALAN LED-based light sources with: white-warm A), white-cool B), blue C), green D), and red light E). F) Standard laboratory light conditions during the day (a mixture of white-warm LEDs and white fluorescent lights). Note that, because lux meters are optimised for human vision, at 50 lux, red and blue exhibited higher light emissions within their respective spectral ranges compared to the other tested colours. For detailed spectral composition graphs, see Figure S1.

Using a new cohort of fish, we initially repeated the white-cool LED experiment. As per the findings from the intensity experiment (Fig. 2), the full interaction model (days × treatment) had the highest support (ΔAIC < 2 and non-significant LRT) (Fig. 4; Table S4-6). At baseline, success was lower in the white-cool LED treatment (~86%) than in the control (~94%; estimate = –0.98, 95% CI = –2.19 to 0.23). The control group showed modest improvement over the course of the experiment, with a success rate of ~98% at the end (estimate = 0.15, 95% CI = –0.60 to 0.89). Conversely, the white-cool LED treatment greatly

reduced success over time (~68%; interaction estimate = –0.72, 95% CI = –1.56 to 0.12), repeating and strongly supporting the results from the intensity experiment.

Based on the strong adverse effect on learning and memory that white-cool LEDs had, we then tested the impact of white-warm LED lights. These lights had reduced emission in the blue-green spectrum, but increased emission in the yellow-red range compared to white-cool LEDs (Fig. 3A). The full interaction model (days × treatment) was again selected (ΔAIC < 2 and non-significant LRT) (Fig. 4; Table S4-6). At the start of the experiment, treatment fish (~91%) had a slightly lower success rate than controls (~94%; estimate = –0.38, 95% CI = –1.47 to 0.71). Days had little effect on control, with success rates staying consistently high (~95%; estimate = –0.17, 95% CI = –0.75 to 0.40), but the interaction between days and treatment did show a substantial reduction in success over time in the white-warm LED treatment with a success rate of ~70% at the end of the experiment (interaction estimate = –0.56, 95% CI = –1.30 to 0.18). Hence, both white-LED treatments greatly reduced memory and learning in *A. ocellaris*, with white-cool LEDs showing a slightly more pronounced effect.

Next, we tested the different spectral ranges that make up human-visible white light separately. Starting from the shorter wavelength end, we first tested the effects of blue and green LEDs, as they penetrate the water the furthest (Jerlov, 1976). For blue LEDs, the highest support was for the additive model (days + treatment) (ΔAIC < 2 and non-significant LRT), while for green LEDs, the highest support was for the full interaction model (days × treatment) (ΔAIC < 2 and non-significant LRT) (Fig. 4, Tables S4-6). In the blue LED experiment, success rates at baseline were similar between the treatment and control groups (90-95%; estimate = 2.37, 95% CI = 1.80 to 2.94). The level of success for both groups dropped slightly over time (estimate = –0.23, 95% CI = –0.59 to 0.12). However, while the correct choice in the control group remained well above 90%, in the treatment group it dropped to slightly below 85% (Fig. 4), which we considered to be the minimum level of success beyond random chance for our experiments (see Methods above).

For the green LED, at the start of the experiment, success, albeit high overall, was lower in the treatment group (~92%) compared to the control (~99%; estimate = –0.99, 95% CI = –2.46 to 0.47). The success of the control dropped slightly, to ~97% at the end of the experiment (estimate = –0.55, 95% CI = –1.31 to 0.20). Conversely, the success rate of the green ALAN treatment dropped to ~78% at the end of the experiment (estimate = –0.26, 95% CI = –1.17 to 0.65). Hence, both green and blue LED lights reduced memory and learning in *A. ocellaris* to levels well below or just at the random choice level, respectively (Fig. 4).

Finally, we tested the effects of red LED ALAN on cognitive performance in the fish, as this spectral range showed the largest difference between white-cool and white-warm LED lights (Fig. 3). Interestingly, despite being much brighter than the other treatment groups, the best model fit was found for the null model (without considering days and treatment) (ΔAIC < 2 and non-significant LRT). Hence, the success rate was similar between groups at the start of the experiment (estimate = 2.13, 95% CI = 1.57 to 2.69), and it remained at ~89% throughout the entire experiment (estimate = –0.03, 95% CI = –0.36 to 0.30) (Fig. 4; Table S4-6). This suggests that, while broad-light or shorter-spectrum LEDs have an adverse effect on A. ocellaris cognition, they appear to be indifferent to exposure to red light at night.

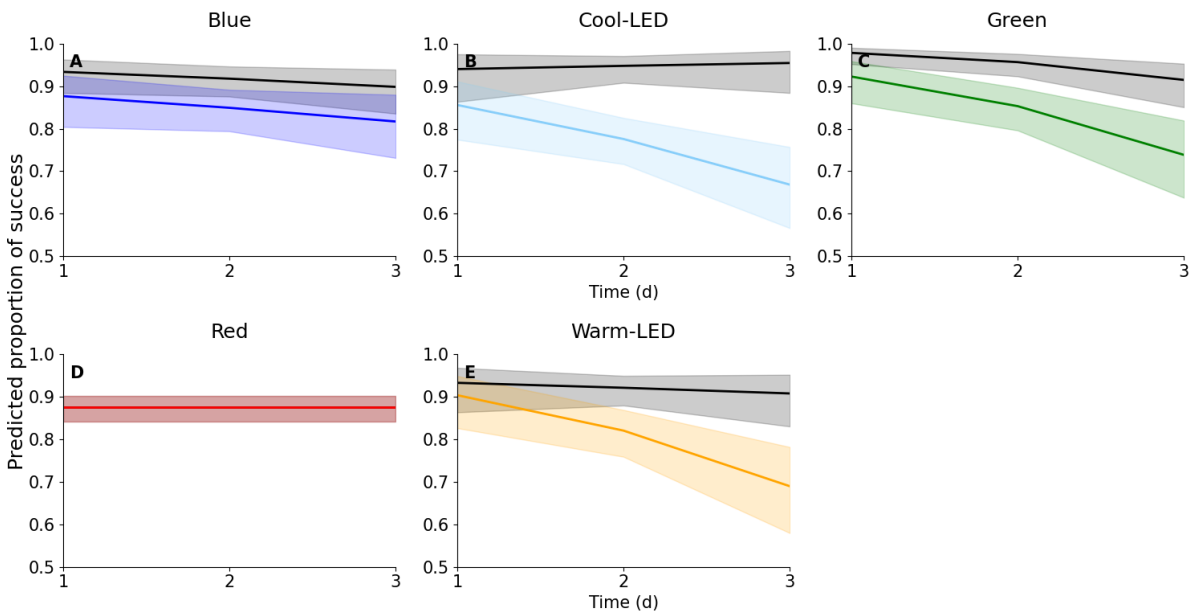

Figure 4: The impact of differently coloured LED lights at night on *Amphiprion ocellaris* memory and learning. Predicted success rates over two consecutive nights of ALAN exposure under 50 lux of blue (A), cool LED (B), green (C), red (D), and warm LED (E) compared to matched control groups (black) (n = 8 fish per group). Each panel presents the model-predicted mean (solid line) and 95% confidence interval (shaded area). For the red-light treatment, there was no difference between the control and the treatment groups. The y-axis starts at 0.5, representing random chance.

Discussion

The effects of ALAN on reef fish cognition and behaviour are poorly understood. In this study, we aimed to investigate whether different intensities and colours of ALAN impact memory in the clownfish, *A. ocellaris*. Clownfishes are representative of the many smaller, site-attached fishes on shallow tropical coral reefs, which experience a rapid increase in ALAN in their natural environment (Laudet & Ravasi, 2023). Testing contemporary white-cool LED lights at

different intensities revealed that even at very low light levels of 0.5 lux, *A. ocellaris* learning was impeded (Fig. 2A). At the highest level of 50 lux tested here, not only learning, but memory itself was clearly affected, with fish mostly resorting to random choice when looking for their food reward (Fig. 2C). Supporting the importance of shorter-wavelengths of light for the visual ecology of reef fishes (Cortesi et al., 2020), broad-spectrum white LEDs (warm and cool) and green and blue LEDs all negatively impacted learning and memory in the fish. Red LEDs, on the other hand, did not affect *A. ocellaris* cognition, even when presented at a higher intensity than the other colour treatments (Fig. 4).

Memory and learning are essential for every aspect of a fish's ecology, including migration, foraging, landmark orientation, social behaviours and adaptation to environmental change (Kieffer & Colgan, 1992; Munson & DePasquale, 2025; Overmier & Hollis, 1990). While the largest effects were observed for street-light intensities (50 lux), even low levels of ALAN at full moon intensities (0.5 lux) had an impact on learning in *A. ocellaris* (Fig. 2). Such low levels of ALAN have the potential to be far spread, especially in areas with indirect illumination via skyglow, and should be focused on more thoroughly in the future (Weschke 2024). Moreover, both chronic ALAN exposure, as found in many urbanised coastal areas (Davies et al., 2016; Tidau et al., 2021), and the natural light fluctuations at night over multiple lunar cycles should be studied to determine if low levels of ALAN cause additional stress, or whether these effects fall within the range that coral reef fishes naturally experience.

Retinal cone spectral sensitivities in *A. ocellaris* peak at 386 (ultraviolet-violet), 497 (blue-green), 515 (green), and 535 (green-yellow) nm λ_{max} (Mitchell et al., 2024). The species also uses a single rod photoreceptor with a λ_{max} at 491–499 nm for scotopic vision (Mitchell et al., 2021). These sensitivity peaks align most closely with the spectral compositions of the white-cool-, white-warm-, and green LED treatments used in our study (Figs. 3 and S1). The broader spectral output of the white LEDs likely enabled greater overlap with multiple photoreceptor sensitivities, enhancing their detectability by *A. ocellaris* (Fig. 4). The two peaks in the green LED output (Fig. S1) overlap almost perfectly with the two mid-wavelength sensitive cone photoreceptors (λ_{max} at 497 and 515 nm) as well as the spectral sensitivity of the rod photoreceptor (Mitchell et al., 2024). Accordingly, the green treatment alone was enough to replicate most of the effect found for the two broadband white-light treatments (Fig. 4). As green had the lowest intensity of all the tested light treatments (Fig. S1), increasing its intensity to match the maximum output in the other treatments would likely have caused an even more pronounced effect.

Contrary to this, despite being 2–10 times brighter at their peak emissions (Fig. S1), neither blue nor red LEDs had a strong effect on *A. ocellaris* performance. This might be explained by the fact that the LED outputs (Fig. S1) only partially overlap with the photoreceptor spectral sensitivities of *A. ocellaris* (Mitchell et al., 2024). Indeed, when testing the colour discrimination ability of this species, Mitchell and colleagues found that they do not perform well in the blue part of the spectrum (Mitchell et al., 2024). However, as far as red colour is concerned, *A. ocellaris* has high contrast sensitivity in this part of the spectrum that matches or even outperforms that of the green spectrum (Mitchell et al., 2024). This is likely due to their algal food reflecting strongly in the far-red; clownfishes also use orange and red colours for signalling, and because their symbiotic anemones often have a red colouration (Stieb et al., 2023). Hence, the indifference to the red LED ALAN is somewhat perplexing, and the underlying neurophysiological principles warrant further investigation.

It is possible that cone photoreceptors are not the primary light sensors involved in perceiving ALAN, as the retinal epithelium's (black pigment layer) movement at night may protect them from exposure. Instead, both the green-sensitive rods in the eye and the likely green-sensitive pineal gland photoreceptors might be the primary sensors contributing to the behavioural disturbances observed. Both are central for the circadian rhythm, which regulates sleep in fishes, with sleep disturbance and ALAN being irreversibly tied to one another (Sigurgeirsson et al., 2013). Hence, assessing sleep in the clownfish, combined with molecular analyses of retinal and pineal gland gene expression, is likely to elucidate the mechanisms underlying the learning and memory impairments observed in this study.

In general, many shallow-living reef fish species, and also many pelagic species, have visual systems that are similar to the visual system of *A. ocellaris* (reviewed in Marshall et al., 2019; Cortesi et al., 2020). This suggests that broadband white LEDs, especially those using the newer white-cool LEDs, are likely to have a widespread adverse effect on the reef fish community. Additionally, because many reef fish species utilise a distinct blue cone photoreceptor (Cortesi et al., 2020), which is missing in *A. ocellaris*, using blue-only light sources may not be a viable alternative. At first glance, then, focusing management efforts on using longer-wavelength emitting red light sources might be the best solution. However, whether the indifference to red light ALAN is widespread in the reef (fish) community or whether chronic exposure would eventually impair *A. ocellaris* cognition remains to be investigated.

Conclusion

Our study contributes to a growing body of literature on the effects of ALAN on the cognitive abilities of reef fish. The finding that *A. ocellaris* is indifferent to red light at night adds an essential aspect to the broader discussion of marine ALAN management. Because species differ in their responses to light of different wavelengths, it is unlikely that any single "neutral" light source can minimise ecological impacts across all taxa. For example, blue light accelerates development and metamorphosis in marine shellfish larvae (Zhang et al., 2023) and reduces the bycatch of fish and sea turtles (Stanton & Cowart, 2024). However, blue light also impairs the locomotion of gastropod grazers (Maggi et al., 2020). Red and green lights are considered less disruptive to turtle nesting and hatchling orientation (Cruz et al., 2018), North Atlantic mesopelagic fish (Peña et al., 2020), and seabird navigation (Syposz et al., 2021). Yet, these same wavelengths disrupt Arctic pelagic organisms (Geoffroy et al., 2021), and increase the biomass of primary producers, leading to the selective dominance of specific diatom species known to cause harmful algal blooms (Diamantopoulou et al., 2021). Hence, although our findings suggest that replacing broadband LEDs with longer-wavelength-emitting red LEDs may minimise the effects on shallow living coral reef fishes, the species-specific responses shown across a more broadly sampled taxonomic range underscore ecological trade-offs of wavelength-based lighting mitigation. This reinforces the general recommendation for the design of light sources at night in urban and coastal planning, that reducing overall ALAN exposure or, as a bare minimum, reducing the intensity of ALAN, remains the most effective and ecologically responsible strategy to protect the marine environment.

Data Availability

All the data is available from the Supplementary Materials and Methods or upon request from the corresponding author.

Acknowledgements

We thank Angelo Guadagno and Dr Zegni Triki for their assistance with the behavioural assay and their patience in teaching us how to implement it. We also thank the members of the Marine Sensory Ecology group for their help with aquarium husbandry and for their inspiration. This work was funded by an Australian Research Council Future Fellowship (FT240100725) to FC.

Author Contributions

F.C. and L.A. designed the experiments. J.vSt. and L.A. performed the experiments and analysed the data. J.vSt. And F.C. wrote the initial version, and all authors have contributed to the final version of the manuscript.

Competing interests

The authors declare no competing interests.

References

Aguillon, R., Harduf, A., Sagi, D., Simon-Blecher, N., Levy, O., & Appelbaum, L. (2026). DNA damage modulates sleep drive in basal cnidarians with divergent chronotypes. *Nature Communications*, 17, 3. https://doi.org/10.1038/s41467-025-67400-5

Appelbaum, L., Wang, G. X., Maro, G. S., Mori, R., Tovin, A., Marin, W., Yokogawa, T., Kawakami, K., Smith, S. J., Gothilf, Y., Mignot, E., & Mourrain, P. (2009). Sleep–wake regulation and hypocretin–melatonin interaction in zebrafish. *Proceedings of the National Academy of Sciences*, 106, 21942–21947. https://doi.org/10.1073/pnas.906637106

Ben-Moshe Livne, Z., Alon, S., Vallone, D., Bayleyen, Y., Tovin, A., Shainer, I., Nisembaum, L. G., Aviram, I., Smadja-Storz, S., Fuentes, M., Falcón, J., Eisenberg, E., Klein, D. C., Burgess, H. A., Foulkes, N. S., & Gothilf, Y. (2016). Genetically blocking the zebrafish pineal clock affects circadian behaviour. *PLoS Genetics*, 12, e1006445. https://doi.org/10.1371/journal.pgen.1006445

Ben-Moshe, Z., Alon, S., Mracek, P., Faigenbloom, L., Tovin, A., Vatine, G. D., Eisenberg, E., Foulkes, N. S., & Gothilf, Y. (2014). The light-induced transcriptome of the zebrafish pineal gland reveals complex regulation of the circadian clockwork by light. *Nucleic Acids Research*, 42, 3750–3767. https://doi.org/10.1093/nar/gkt1359

Boeck, P. D., Bakker, M., Zwitser, R., Nivard, M., Hofman, A., Tuerlinckx, F., & Partchev, I. (2011). The estimation of item response models with the lmer function from the lme4 package in R. *Journal of Statistical Software*, 39, 1–28.

Brüning, A., Hölker, F., Franke, S., Kleiner, W., & Kloas, W. (2018). Influence of light intensity and spectral composition of artificial light at night on melatonin rhythm and mRNA expression of gonadotropins in roach *Rutilus rutilus*. *Fish Physiology and Biochemistry*, 44, 1–12. https://doi.org/10.1007/s10695-017-0408-6

Cortesi, F., Tettamanti, V. & de Busserolles, F. (2022). The visual ecology of anemonefishes. In: *Evolution, development and ecology of anemonefishes*, 87-94.

Cortesi, F., Mitchell, L.J., Tettamanti, V., Fogg, L.G., de Busserolles, F., Cheney, K.L. & Marshall, N.J. (2020). Visual system diversity in coral reef fishes. *Seminars in cell & developmental biology*, 106, 31-42.

Cruz, L. M., Shillinger, G. L., Robinson, N. J., Tomillo, P. S., & Paladino, F. V. (2018). Effect of light intensity and wavelength on the in-water orientation of olive ridley turtle hatchlings. *Journal of Experimental Marine Biology and Ecology*, 505, 52–56. https://doi.org/10.1016/j.jembe.2018.05.002

Davies, T. W., Duffy, J. P., Bennie, J., & Gaston, K. J. (2016). Stemming the tide of light pollution encroaching into marine protected areas. *Conservation Letters*, 9, 164–171. https://doi.org/10.1111/conl.12191

De Russi, G., Montalbano, G., Gatto, E., Maggi, E., Cannicci, S., Bertolucci, C., & Lucon-Xiccato, T. (2024). Differential impact of artificial light at night on cognitive flexibility in visual and spatial reversal learning tasks. *Animal Behaviour*, 218, 173–183. https://doi.org/10.1016/j.anbehav.2024.10.008

Diamantopoulou, C., Christoforou, E., Dominoni, D. M., Kaiserli, E., Czyzewski, J., Mirzai, N., & Spatharis, S. (2021). Wavelength-dependent effects of artificial light at night on phytoplankton growth and community structure. *Proceedings of the Royal Society B: Biological Sciences*, 288, 20210525. https://doi.org/10.1098/rspb.2021.0525

Eban-Rothschild, A., Appelbaum, L., & de Lecea, L. (2018). Neuronal mechanisms for sleep/wake regulation and modulatory drive. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, 43, 937–952. https://doi.org/10.1038/npp.2017.294

Ferretti, M., Rossi, F., Benedetti-Cecchi, L., & Maggi, E. (2024). Ecological consequences of artificial light at night on coastal species in natural and artificial habitats: a review. *Marine Biology*, 172, 5. https://doi.org/10.1007/s00227-024-04568-2

Geoffroy, M., Langbehn, T., Priou, P., Varpe, Ø., Johnsen, G., Le Bris, A., Fisher, J. A. D., Daase, M., McKee, D., Cohen, J., & Berge, J. (2021). Pelagic organisms avoid white, blue, and red artificial light from scientific instruments. *Scientific Reports*, 11, 14941. https://doi.org/10.1038/s41598-021-94355-6

Jerlov, N. G. 1976. *Marine Optics*, 231. Amsterdam, the Netherlands; Oxford, UK; New York, NY: Elsevier Scientific Publishers. Elsevier Oceanography Series, 14.

Kieffer, J. D., & Colgan, P. W. (1992). The role of learning in fish behaviour. *Reviews in Fish Biology and Fisheries*, 2, 125–143. https://doi.org/10.1007/BF00042881

Lamb, T. D., Patel, H., Chuah, A., Natoli, R. C., Davies, W. I. L., Hart, N. S., Collin, S. P., & Hunt, D. M. (2016). Evolution of vertebrate phototransduction: cascade activation. *Molecular Biology and Evolution*, 33, 2064–2087. https://doi.org/10.1093/molbev/msw095

Laudet, V., & Ravasi, T. (Eds.). (2023). *Evolution, development and ecology of anemonefishes: model organisms for marine science*. Taylor & Francis. https://doi.org/10.1201/9781003125365

Li, L. (2019). Circadian vision in zebrafish: from molecule to cell and from neural network to behavior. *Journal of Biological Rhythms*, 34, 451–462. https://doi.org/10.1177/0748730419863917

Li, W., Zhang, D., Zou, Q., Bose, A. P. H., Jordan, A., McCallum, E. S., Bao, J., & Duan, M. (2024). Behavioural and transgenerational effects of artificial light at night (ALAN) of varying spectral compositions in zebrafish (*Danio rerio*). *Science of The Total Environment*, 954, 176336. https://doi.org/10.1016/j.scitotenv.2024.176336

Lucon-Xiccato, T., De Russi, G., Cannicci, S., Maggi, E., & Bertolucci, C. (2023). Embryonic exposure to artificial light at night impairs learning abilities and their covariance with behavioural traits in teleost fish. *Biology Letters*, 19, 20230436. https://doi.org/10.1098/rsbl.2023.0436

Marangoni, L. F. B., Davies, T., Smyth, T., Rodríguez, A., Hamann, M., Duarte, C., Pendoley, K., Berge, J., Maggi, E., & Levy, O. (2022). Impacts of artificial light at night in marine ecosystems—a review. *Global Change Biology*, 28, 5346–5367. https://doi.org/10.1111/gcb.16264

Marshall, N. J., Cortesi, F., de Busserolles, F., Siebeck, U. E., & Cheney, K. L. (2019). Colours and colour vision in reef fishes: past, present and future research directions. *Journal of Fish Biology*, 95, 5–38. https://doi.org/10.1111/jfb.13849

Mitchell, L. J., Cheney, K. L., Lührmann, M., Marshall, J., Michie, K., & Cortesi, F. (2021). Molecular evolution of ultraviolet visual opsins and spectral tuning of photoreceptors in anemonefishes (Amphiprioninae). *Genome Biology and Evolution*, 13, evab184. https://doi.org/10.1093/gbe/evab184

Mitchell, L.J., Phelan, A., Cortesi, F., Marshall, N.J., Chung, W.S., Osorio, D.C., & Cheney, K.L. (2024). Ultraviolet vision in anemonefish improves colour discrimination. *Journal of Experimental Biology*, 227, p.jeb247425.

Peña, M., Cabrera-Gámez, J., & Domínguez-Brito, A. C. (2020). Multi-frequency and light-avoiding characteristics of deep acoustic layers in the North Atlantic. *Marine Environmental Research*, 154, 104842. https://doi.org/10.1016/j.marenvres.2019.104842

Posit, T. (2025). *RStudio: Integrated Development Environment for R. Posit Software* [Computer software]. Posit. http://www.posit.co/

Powell, S. B., Mitchell, L. J., Phelan, A. M., Cortesi, F., Marshall, J., & Cheney, K. L. (2021). A five-channel LED display to investigate UV perception. *Methods in Ecology and Evolution*, 12, 602–607. https://doi.org/10.1111/2041-210X.13555

Sigurgeirsson, B., Þorsteinsson, H., Sigmundsdóttir, S., Lieder, R., Sveinsdóttir, H. S., Sigurjónsson, Ó. E., Halldórsson, B., & Karlsson, K. (2013). Sleep–wake dynamics under extended light and extended dark conditions in adult zebrafish. *Behavioural Brain Research*, 256, 377–390. https://doi.org/10.1016/j.bbr.2013.08.032

Sowersby, W., Kobayahsi, T., Awata, S., Sogawa, S., & Kohda, M. (2024). The influence of sleep disruption on learning and memory in fish. *bioRxiv*. https://doi.org/10.1101/2024.08.28.610197

Stanton, D. L., & Cowart, J. R. (2024). The effects of artificial light at night (ALAN) on the circadian biology of marine animals. *Frontiers in Marine Science*, 11, p.1372889. https://doi.org/10.3389/fmars.2024.1372889

Stieb, S.M., Cortesi, F., Jardim de Queiroz, L., Carleton, K.L., Seehausen, O., & Marshall, N.J. (2023). Long-wavelength-sensitive (*lws*) opsin gene expression, foraging and visual communication in coral reef fishes. *Molecular Ecology*, 32, 1656-1672.

Syposz, M., Padget, O., Willis, J., Van Doren, B. M., Gillies, N., Fayet, A. L., Wood, M. J., Alejo, A., & Guilford, T. (2021). Avoidance of different durations, colours and intensities of artificial light by adult seabirds. *Scientific Reports*, 11, p.18941. https://doi.org/10.1038/s41598-021-97986-x

Tidau, S., Smyth, T., McKee, D., Wiedenmann, J., D'Angelo, C., Wilcockson, D., Ellison, A., Grimmer, A. J., Jenkins, S. R., Widdicombe, S., Queirós, A. M., Talbot, E., Wright, A., & Davies, T. W. (2021). Marine artificial light at night: an empirical and technical guide. *Methods in Ecology and Evolution*, 12, 1588–1601. https://doi.org/10.1111/2041-210x.13653

Triki, Z., Fong, S., Amcoff, M., Vàsquez-Nilsson, S., & Kolm, N. (2023). Experimental expansion of relative telencephalon size improves the main executive function abilities in guppy. *PNAS Nexus*, 2, pgad129. https://doi.org/10.1093/pnasnexus/pgad129

Weschke, E. (2024). *The nighttime ecology of coral reef fishes in a changing world.* Doctoral Thesis, University of Bristol.

Zada, D., Sela, Y., Matosevich, N., Monsonego, A., Lerer-Goldshtein, T., Nir, Y., & Appelbaum, L. (2021). Parp1 promotes sleep, which enhances DNA repair in neurons. *Molecular Cell*, 81, 4979-4993.e7. https://doi.org/10.1016/j.molcel.2021.10.026

Zhang, M., Gao, X., Luo, Q., Lin, S., Lyu, M., Luo, X., Ke, C., & You, W. (2023). Ecological benefits of artificial light at night (ALAN): accelerating the development and metamorphosis of marine shellfish larvae. *Science of The Total Environment*, 903, 166683. https://doi.org/10.1016/j.scitotenv.2023.166683

Zhdanova, I. V., Wang, S. Y., Leclair, O. U., & Danilova, N. P. (2001). Melatonin promotes sleep-like state in zebrafish. *Brain Research*, 903, 263–268. https://doi.org/10.1016/S0006-8993(01)02444-1

Supplementary Materials and Methods

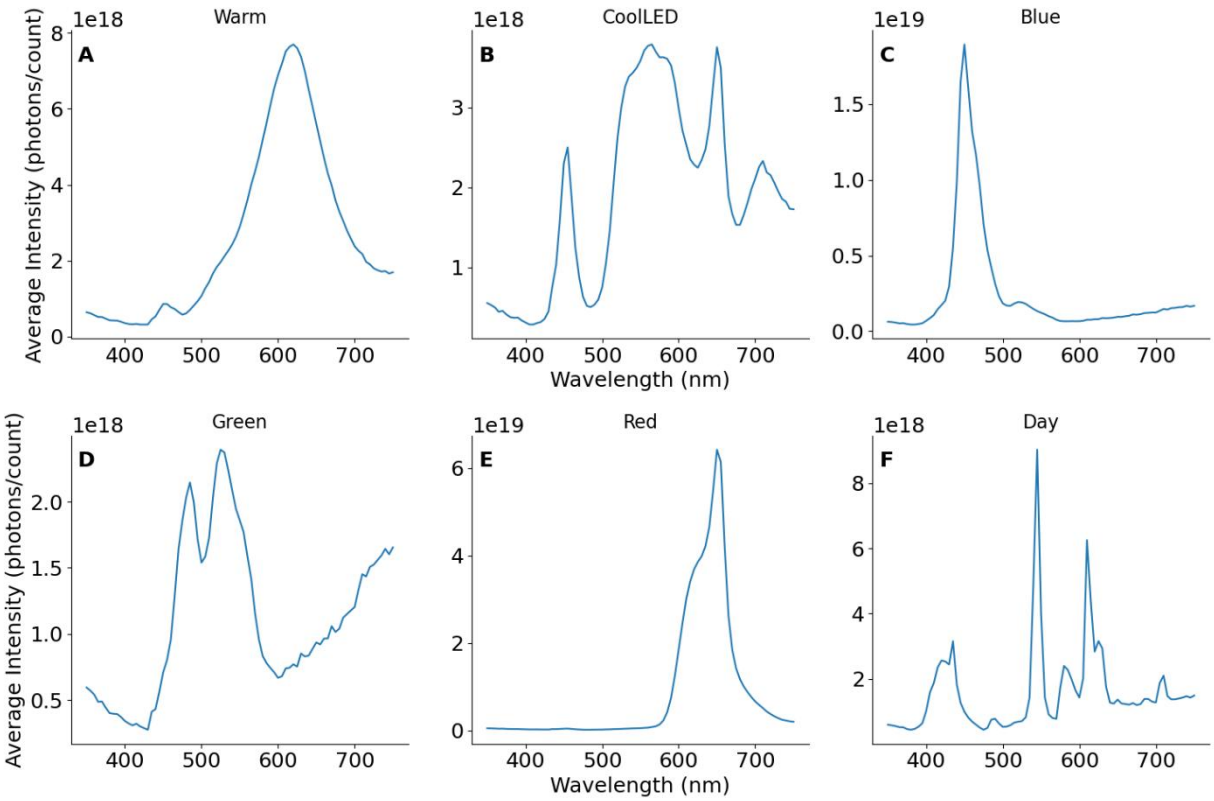

Figure S1: Absolute irradiance (photons/count) at 50 lux, and spectral composition of each light treatment in the differently coloured LED experiment, measured at the centre of the tank (averaged across 10 measurements). White-warm LED (A), white-cool LED (B), blue LED (C), green LED (D), red LED (E), standard laboratory light conditions during the day (F).

Table S1: Akaike Information Criterion (AIC) comparison of Generalised Linear Mixed Models analysing *Amphiprion ocellaris'* success in the light intensity experiment. AIC = Akaike Information Criterion; Model = model ID; ΔAIC = difference in AIC relative to the best-supported model. Models described in the next table:

m0 = "Success ~ 1 + (1 | Fish)",

m1 = "Success ~ Days + (1 | Fish)",

m2 = "Success ~ Days + Treatment + (1 | Fish)",

m3 = "Success ~ Days * Treatment + (1 | Fish)"

Degrees of freedom	AIC	Model	Treatment group	ΔAIC
2	318.87	m0	0.5 lux	3.64
3	317.36	m1	0.5 lux	2.13
4	318.49	m2	0.5 lux	3.26
5	315.22	m3	0.5 lux	0
2	333.57	m0	5 lux	1.11
3	335.54	m1	5 lux	3.08
4	332.46	m2	5 lux	0
5	333.64	m3	5 lux	1.18
2	358.05	m0	50 lux	14.60
3	354.21	m1	50 lux	10.77
4	344.18	m2	50 lux	0.74
5	343.44	m3	50 lux	0

Table S2: Likelihood Ratio Test (LRT) comparison of Generalised Linear Mixed Models analysing A. occelaris' success in the 0.5, 5, and 50 lux treatments. Term = model; AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; statistic = LRT test statistic; p-value = significance level; Models described in the next table:

m0 = "Success ~ 1 + (1 | Fish)",

m1 = "Success ~ Days + (1 | Fish)",

m2 = "Success ~ Days + Treatment + (1 | Fish)",

m3 = "Success ~ Days * Treatment + (1 | Fish)"

Term	**Number of parameters**	**AIC**	**BIC**	**Log-likelihood**	**−2 × log-likelihood**	**Statistic**	**Degrees of freedom**	**p-value**	**Treatment group**
m0	2	318.87	327.22	-157.43	314.87				Pair_0_5
m1	3	317.36	329.88	-155.68	311.36	3.50	1	0.06	Pair_0_5
m2	4	318.49	335.19	-155.24	310.49	0.86	1	0.35	Pair_0_5
m3	5	315.22	336.09	-152.61	305.22	5.26	1	0.02	Pair_0_5
m0	2	333.57	341.92	-164.78	329.57				Pair_5
m1	3	335.54	348.06	-164.77	329.54	0.03	1	0.85	Pair_5
m2	4	332.46	349.15	-162.23	324.46	5.08	1	0.02	Pair_5
m3	5	333.64	354.51	-161.82	323.64	0.81	1	0.36	Pair_5
m0	2	358.05	366.26	-177.02	354.05				Pair_50
m1	3	354.21	366.54	-174.10	348.21	5.83	1	0.01	Pair_50
m2	4	344.18	360.62	-168.09	336.18	12.02	1	0.0005	Pair_50
m3	5	343.44	363.98	-166.72	333.44	2.74	1	0.09	Pair_50

Table S3: Parameter estimates and 95% confidence intervals for the best-supported Generalised Linear Mixed Models predicting A. occelaris' success at 0.5, 5, and 50 lux. Model = selected model; Formula = model formula; Selection reason = rationale for model selection; term= model term; estimate = model coefficient; lower 95%CI/upper 95% CI = lower and upper 95% confidence intervals; effect = fixed or random effect; group = group assignment; SE = standard error; statistic = test statistic.

Treatment group	**Model**	**Formula**	**Selection Reason**	**Term**	**Estimate**	***Lower 95% CI***	***Upper 95% CI***	**Effect**	**Group**	**SE**	**Statistic**	**P-value**
0.5 lux	m3	Success ~ Days * Treatment + (1 | Fish)	Lowest AIC	(Intercept)	1.79	1.07	2.51	fixed		0.36	4.87	1.1E-06
0.5 lux	m3	Success ~ Days * Treatment + (1 | Fish)	Lowest AIC	Days	0.92	0.26	1.58	fixed		0.33	2.76	0.005
0.5 lux	m3	Success ~ Days * Treatment + (1 | Fish)	Lowest AIC	Treatment	0.29	-0.73	1.33	fixed		0.52	0.56	0.57
0.5 lux	m3	Success ~ Days * Treatment + (1 | Fish)	Lowest AIC	Days: Treatment	-0.92	-1.73	-0.11	fixed		0.41	-2.24	0.02

0.5 lux	m3	Success ~ Days * Treatment + (1 | Fish)	Lowest AIC	SD (Intercept)	0.57			random	Fish			
5 lux	m1	Success ~ Days + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	(Intercept)	2.31	1.68	2.94	fixed		0.32	7.15	8.39E-13
5 lux	m1	Success ~ Days + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days	-0.03	-0.38	0.31	fixed		0.17	-0.18	0.85
5 lux	m1	Success ~ Days + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	SD (Intercept)	0.77			random	Fish			
50 lux	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	(Intercept)	2.87	1.86	3.88	fixed		0.51	5.56	2.6E-08
50 lux	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days	0.14	-0.59	0.88	fixed		0.37	0.38	0.69

50 lux	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Treatment	-0.97	-2.18	0.23	fixed		0.61	-1.58	0.11
50 lux	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days: Treatment	-0.71	-1.55	0.12	fixed		0.42	-1.67	0.09
50 lux	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	SD (intercept)	0.54			random	Fish			

Table S4: Akaike Information Criterion (AIC) comparison of Generalised Linear Mixed Models analysing A. occelaris' success under various spectral compositions of ALAN treatment. AIC = Akaike Information Criterion; Model = model ID; ΔAIC = difference in AIC relative to the best-supported model. Models described in the next table:

m0 = "Success ~ 1 + (1 | Fish)",

m1 = "Success ~ Days + (1 | Fish)",

m2 = "Success ~ Days + Treatment + (1 | Fish)",

m3 = "Success ~ Days * Treatment + (1 | Fish)"

Degrees of freedom	AIC	Model	Treatment group	ΔAIC
2	358.05	m0	Cool LED	14.60
3	35421	m1	Cool LED	10.77
4	344.18	m2	Cool LED	0.74
5	343.44	m3	Cool LED	0
2	324.27	m0	Blue LED	2.59
3	324.63	m1	Blue LED	2.95
4	322.97	m2	Blue LED	1.29
5	321.67	m3	Blue LED	0
2	358.20	m0	Red LED	0
3	360.17	m1	Red LED	1.97
4	361.24	m2	Red LED	3.04
5	362.94	m3	Red LED	4.73
2	292.11	m0	Green LED	17.06
3	281.28	m1	Green LED	6.23
4	275.04	m2	Green LED	0
5	276.74	m3	Green LED	1.69
2	351.47	m0	Warm LED	12.95
3	344.65	m1	Warm LED	6.12
4	338.72	m2	Warm LED	0.19
5	338.52	m3	Warm LED	0

Table S5: Likelihood Ratio Test (LRT) comparison of Generalised Linear Mixed Models analysing A. occelaris' success under various spectral compositions of ALAN treatment. Term = model; AIC = Akaike Information Criterion; BIC = Bayesian Information Criterion; statistic = LRT test statistic; p-value = significance level. Models described in the next table:

m0 = "Success ~ 1 + (1 | Fish)",

m1 = "Success ~ Days + (1 | Fish)",

m2 = "Success ~ Days + Treatment + (1 | Fish)",

m3 = "Success ~ Days * Treatment + (1 | Fish)"

Term	**Number of parameters**	**AIC**	**BIC**	**log-likelihood**	**−2 × log-likelihood**	**statistic**	**Degrees of freedom**	**p-value**	**Treatment group**
m0	2	358.05	366.26	-177.02	354.05				Cool LED
m1	3	354.21	366.54	-17410	348.21	5.83	1	0.015	Cool LED
m2	4	344.18	360.62	-168.09	336.18	12.02	1	0.0005	Cool LED
m3	5	343.44	363.98	-16672	333.44	2.74	1	0.09	Cool LED
m0	2	324.27	332.49	-160.13	320.27				Blue LED
m1	3	324.63	336.96	-159.31	318.63	1.63	1	0.20	Blue LED
m2	4	322.97	339.40	-157.48	314.97	3.66	1	0.05	Blue LED
m3	5	321.67	342.22	-155.83	311.67	3.29	1	0.06	Blue LED
m0	2	358.20	366.55	-177.10	354.20				Red LED
m1	3	360.17	372.69	-177.08	354.17	0.02	1	0.86	Red LED
m2	4	361.24	377.94	-176.62	353.24	0.92	1	0.33	Red LED
m3	5	362.94	383.81	-176.47	352.94	030	1	0.58	Red LED
m0	2	292.11	300.33	-144.05	288.11				Green LED

m1	3	281.28	293.60	-137.64	275.28	12.83	1	0.0003	Green LED
m2	4	275.04	291.48	-133.52	267.04	8.23	1	0.004	Green LED
m3	5	276.74	297.28	-133.37	266.74	0.30	1	0.58	Green LED
m0	2	351.47	359.69	-173.74	347.47				Warm LED
m1	3	344.65	356.98	-169.32	338.65	8.82	1	0.002	Warm LED
m2	4	338.72	355.16	-165.36	330.72	7.93	1	0.004	Warm LED
m3	5	338.52	359.07	-164.26	328.52	2.19	1	0.13	Warm LED

Table S6: Parameter estimates and 95% confidence intervals for the best-supported Generalised Linear Mixed Models predicting A. occelaris' success at 0.5, 5, and 50 lux. Treatment group = ALAN treatment; Model = selected model; Formula = model formula; Selection Reason = rationale for model selection; term= model term; estimate = model coefficient; lower 95%CI/upper 95% CI = lower and upper 95% confidence intervals; effect = fixed or random effect; group = group assignment; SE = standard error; statistic = test statistic.

Treatment group	**Model**	**Formula**	**Selection Reason**	**Term**	**Estimate**	***Lower 95% CI***	***Upper 95% CI***	**Effect**	**Group**	**SE**	**Statistic**	**P-value**
Cool LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	(Intercept)	2.87	1.86	388	fixed		0.51	556	2.6E-08
Cool LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days	0.14	-0.59	0.88	fixed		0.37	0.38	0.699848

Cool LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Treatment	-0.97	-2.18	0.23	fixed		0.61	-1.58	0.113163
Cool LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days: Treatment	-0.71	-1.55	0.12	fixed		0.42	-1.67	0.09
Cool LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	SD (intercept)	0.54			random	Fish			
Blue LED	m1	Success ~ Days + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	(Intercept)	2.36	1.79	2.93	fixed		0.29	8.12	4.36E-16
Blue LED	m1	Success ~ Days + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days	-0.23	-0.59	012	fixed		0.18	-1.28	0.19
Blue LED	m1	Success ~ Days + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	SD (intercept)	0.46			random	Fish			
Red LED	m1	Success ~ Days + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	(Intercept)	2.12	1.56	2.68	fixed		0.28	7.43	1.02E-13

Red LED	m1	Success ~ Days + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days	-0.02	-0.36	0.30	fixed		0.17	-0.17	0.86
Red LED	m1	Success ~ Days + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	SD (intercept)	0.64			random	Fish			
Green LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	(Intercept)	3.65	2.45	4.86	fixed		0.61	5.96	2.44E-09
Green LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days	-0.55	-1.31	0.20	fixed		0.38	-1.43	0.15
Green LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Treatment	-0.99	-2.45	0.46	fixed		0.74	-1.33	0.18
Green LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days: Treatment	-0.25	-1.17	0.65	fixed		0.46	-0.55	0.57
Green LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	SD (intercept)	0.42			random	Fish			

Warm LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	(Intercept)	2.66	1.84	3.48	fixed		0.41	6.37	1.84E-10
Warm LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days	-0.17	-0.75	0.40	fixed		0.29	-0.58	0.55
Warm LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Treatment	-0.38	-1.47	0.70	fixed		0.55	-0.68	0.49
Warm LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	Days: Treatment	-0.56	-1.29	0.17	fixed		0.37	-1.48	0.13
Warm LED	m3	Success ~ Days * Treatment + (1 | Fish)	Δ AIC < 2 and LRT p > 0.05	SD (intercept)	0.30			random	Fish			