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Impacts of artificial light at night on the early life history of two ecosystem engineers

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Abstract

Sessile marine invertebrates play a vital role as ecosystem engineers and in benthic-pelagic coupling. Most benthic fauna develop through larval stages and the importance of natural light cycles for larval biology and ecology is long-established. Natural light-dark cycles regulate two of the largest ocean-scale processes that are fundamental to larvae's life cycle: the timing of broadcast spawning for successful fertilisation and diel vertical migration for foraging and predator avoidance. Given the reliance on light and the ecological role of larvae, surprisingly little is known about the impacts of artificial light at night (ALAN) on the early life history of habitat-forming species. We quantified ALAN impacts on larval performance (survival, growth, development) of two cosmopolitan ecosystem engineers in temperate marine ecosystems, the mussel *Mytilus edulis* and the barnacle *Austrominius modestus*. Higher ALAN irradiance reduced survival in both species (57% and 13%, respectively). ALAN effects on development and growth were small overall, and different between species, time-points, and parentage. Our results show that ALAN adversely affects larval survival and reiterates the importance of paternal influence on offspring performance. ALAN impacts on the early life stages of ecosystem engineering species have implications not only for population viability but also the ecological communities these species support.

Keywords: development, growth, larvae ecology, light pollution, supply-side ecology, survival

Introduction

Sessile marine invertebrates like corals, bivalves, and polychaetes play a vital role as ecosystem engineers and in benthic-pelagic coupling. Ecosystem engineering species modify, maintain, and create habitats and thereby drive the availability of resources to other species (1-4). Sessile marine invertebrates can facilitate recruitment of conspecifics and other species and provide refugia by creating three-dimensional structures that enhance habitat complexity (2-4). As suspension feeders, they form an important link between primary producers (mainly phytoplankton and bacteria) and upper level consumers that prey on them, a function that contributes to benthic-pelagic coupling (5). Most sessile marine invertebrates develop through pelagic and dispersive larval stages (6, 7).

Marine larvae possess remarkable sensitivity to low intensity light and the importance of solar and lunar light cycles for their biology and ecology is long-established (6, 8-11). Light-dark cycles drive large scale ocean processes including broadcast spawning and diel vertical migration (DVM). Broadcast spawning is a dominant reproductive strategy in marine ecosystems, often synchronised around specific nights within the (annual) lunar cycle (12, 13). Alternatively, internally fertilising parents release larvae synchronously, often in the night to avoid predation (7). DVM plays a crucial role in the trade-off of feeding and predator avoidance for planktonic larvae. Commonly described as the largest daily movement of biomass, discrete changes in solar and lunar light cycles drive DVM down to 300m, even during the Arctic Winter (11, 14). Larval dispersal and subsequent settlement drives recruitment in ecological networks and communities and shapes marine biodiversity (7, 15). Consequently, larval performance (survival, development, growth, settlement) not only regulates the health and distribution of ecosystem engineering populations but biogenic reefs and the ecological communities they facilitate.

Surprisingly little is known about the impacts of artificial light at night (ALAN) on larval stages despite their light sensitivity and role in marine ecosystems. The first global atlas of ALAN underwater shows that 1.9 million km^2 of the world's coasts are exposed to ALAN (16), many of which host global megacities where ALAN exceeds natural moonlight nearly all year round (17). Extensive offshore development such as oil and gas platforms, windfarms, and island development contribute to ALAN exposure of pelagic ecosystems (16). Due to predicted expansion of human societies, ALAN is expected to increase exponentially in coastal regions (18). The global transition towards energy efficient Light Emitting Diodes (LEDs), a technology rich in short wavelengths that penetrate deep in the water column, means that ever more marine species and ecosystems are exposed to ALAN (19).

Evidence of ALAN impacts on larvae and early life stages is fragmentary. For example, exposure to artificially lit nights decreases survival in zebrafish *Danio rerio* embryos even before neural light

detecting structures are present (20). Larvae in aquaculture settings often grow larger and faster at the expense of increased mortality under ALAN (21-24). In contrast, pond snail *Lymnaea stagnalis* hatched under ALAN grew larger but mortality was not altered (25). Tadpoles of the American toad *Anaxyrus americanus* showed reduced body mass and growth under ALAN (26) as did fry of the Atlantic salmon *Salmo salar* (27). ALAN impacts also differ between developmental stages. While pre-settlement in the barnacle *Semibalanus balanoides* was not affected by ALAN (28), it reduced settlement of the barnacles *Notochthamalus scabrosus* and *Jehlius cirratus* and hence population recruitment (28, 29). The presence of such diverse responses of organisms during early life stages to ALAN makes prediction beyond the focal species difficult.

To address this major gap in understanding of ALAN impacts, we experimentally quantify larval performance (survival, growth, and development) of two cosmopolitan temperate marine ecosystem engineers under ALAN. Both, the blue mussel *Mytilus edulis* and the barnacle *Austrominius modestus*, are known for the light sensitivity during their larvae phase (8, 10) and are acknowledged habitat-forming species (2-4, 30). Mussels are also ecologically important as they accumulate significant amounts of calcium and carbon and are of global commercial value (2, 31). While mussels reproduce via broadcast spawning and thus external fertilisation (32), barnacles fertilise eggs internally (33). *A. modestus*, native to subtropical and temperate Australasia and widespread in the Atlantic since the 1940s, is now considered a naturalised European species (34).

To enhance real-world application of our experiments, we included two of the principal factors affecting larval performance (survival, growth, and development): 1) parental influences (33, 35) and 2) rearing density as a proxy for food availability or competition (33, 36, 37). Our experiments aim to quantify: 1) ALAN impacts on larval survival, growth, and development; 2) whether effects of ALAN are consistent among larvae from different parents, and 3) the interaction between ALAN and rearing density. Finally, our experimental design allows us to test for relationships between larval performance parameters.

Methods

Lighting setup

We fitted incubators for rearing larvae with a unique, custom-built lighting system, which simulates ALAN (in form of LEDs), solar and lunar light cycles (for details see 38). Briefly, daylight was simulated with an Aquaray Natural Daylight Tile set at 5000 lux diffused with 3mm frosted Perspex to avoid bright spots (19) and powered by the BioLumen Control Unit (Tropical Marine Centre, UK) timed to match natural variation in sunrise and sunset at Plymouth, UK (50°22'34'' N, 4°8' 37'' W). The intensity and timing of natural night-time conditions were simulated using our novel moonlight

system which captures variability in night-time lighting as the moon transits the sky (38). Moonlight was simulated using a bank of 2700-3500K 1.2 cd LEDs housed within diffusing spheres. Lunar cycles were simulated using a pulse width modulated signal (scale 0–100%) applied to the 5 V output of Raspberry Pi 3 model B+, with maximum lunar brightness set to 0.5 lux. Lunar brightness was adjusted every minute from a look up table of Zenith Sky Brightness values modelled for Plymouth, UK, accounting for lunar phase, altitude, opposition, parallax and atmospheric scattering (39). ALAN was simulated between sunset and sunrise in Plymouth using Aquaray cool white Flexi-LED strips (Tropical Marine Centre, UK), with brightness controlled using voltage dimming. We kept larvae under one of six ALAN treatments: 0, 0.2, 0.5, 1, 10, 50 lux (measured in irradiance as W m^2: 0.00, 0.18, 0.47, 2.94, 23.34, 124.80). ALAN brightness (lux) was measured using the SpectroSense2 Lux sensor, irradiance (W m^2) with the multispectral irradiance sensor (Skye Instruments, UK).

Experimental design

Larvae of both species were exposed to six light treatments in experimental laboratory systems. The lighting setup was the same for both species, but the different reproductive strategies and life cycles of barnacles and mussels meant that we tailored the experimental design for each species (see specific section below). *A. modestus* is a hermaphroditic species, undergoes internal fertilisation and releases multiple broods of larvae over the spring, summer, and autumn period (33, 40). Larvae progress through multiple naupliar stages before reaching the final cyprid stage prior to settling (8). We collected parents from the field and brought them to the lab to release larvae. Three adults were randomly selected from which 10 larvae were randomly cultured in 100ml vials at a replication of four vials per each of the three parent for each of the six ALAN treatments (n = 72).

Mussels reproduce via broadcast spawning. Females release gametes into the water column where fertilisation occurs from early spring to late summer (9, 32). Fertilised embryos develop into trochophores and after 72 hours into veligers (various stages) before settling (10). Due to the challenges in facilitating spawning in the laboratory (32), we had the sperm from a single male to fertilise eggs from two females. Fertilised eggs were reared in 100ml vials at two density treatments, high-density (approximately 30 larvae ml^{-1}) and low-density (10 larvae ml^{-1}) (37). Each of the two density treatments was replicated in four 100ml vials for each of the two mothers across all six ALAN treatments (n = 96).

In both species larval development varies from two to several weeks depending on temperature and food supply (9, 33, 40). Larvae were reared in incubators set at 15°C to maintain temperature. Vials were placed in the centre of the incubator to ensure an even light field across all vials.

Barnacle *Austrominius modestus*

Adult collection and larval husbandry

Adult barnacles were collected from South Milton Sands, UK (50°15'40" N, 3°51'33" W), which is a light naïve area (41), in March 2022. Intact barnacles were carefully removed from the substratum using a knife and inspected for egg masses. Adults were transported to the laboratory at the University of Plymouth, where they were placed in individual vials containing 100ml UV-irradiated, 0.5µm filtered seawater (FSW) to spawn (33). After an hour, most parents had released larvae. We selected adults that had released a large proportion of their brood and whose larvae were actively swimming (established by a directional light source). Animals were kept in incubators set at 15°C and reared in 100ml plastic vials filled to 80ml with FSW (33). Every two days, we changed water and fed larvae with live diatom *Skeletonema marinoi* (CCAP 1077/5) culture at a density of $4x10^5$ cells ml^{-1} (33).

Data collection

Every two days, during the water changes, barnacle larvae were counted (alive, dead), checked for development stage (nauplii, cyprid), and pipetted into a clean vial containing fresh FSW (33). We collected data for five responses: (i) mortality (vial averaged number of dead individuals scaled up to a 100 animals, *n* = 72), (ii) average developmental time (vial averaged number of calendar days from freshly released nauplii to cyprid, *n* = 72), and size measured as (iii) area (in μm^2), (iv) length (in µm) and (v) width (in µm) using the opensource software imageJ (Figure 1a-c). While we aimed to measure 10 cyprids for each vial, the naturally staggered development and mortality led to an unbalanced number of replicates for all size measurements and hence observations were averaged per vial (*n* = 63). The experiment was terminated after 32 days when only four nauplii had failed to develop into cyprids and were deemed unlikely to do so.

Figure 1 Example of larvae size measurements taken in ImageJ software. Carapace length (a), width (b), and area (c) measurements for *Austrominius modestus* cyprid. Maximum shell length measured parallel to the hinge line on Day 60 for *Mytilus edulis* (d). The black dots inside the larvae are the eyes. Image credit: Fraser Brough.

Blue mussel *Mytilus edulis*

Adult collection, fertilisation, and larval husbandry

Adult mussels were supplied by Offshore Shellfish Ltd., a rope culturing mussel farm in Lyme Bay, UK (50°41'59" N, 2°53'59" W) with three sites five to ten kilometres offshore and away from the highly ALAN lit region of Torbay (see ALAN underwater atlas for the North Atlantic by Smyth et al. 16). Mussels were landed in Brixham Harbour (50°23’39” N, 3°30’57” W) in April 2022 and directly transported to the laboratories at the University of Plymouth, UK (50°22'34'' N, 4°8'37'' W). The brood stock of around 100 animals was maintained in a recirculating system supplying four aquaria of ca. 60l each with FSW, at a salinity of 33/34ppt, temperature of 10°C and 12:12 light dark cycle (in accordance with seasonal daylight times for Plymouth). Animals were fed every other day with *Isochrysis* 1800 according to manufacturer’s instructions (Reed Mariculture Inc., USA) until fertilisation. Fertilisation was initiated by thermal shock method to induce gamete release (32, 37). Mussels were exposed to air in a fridge at 3±1°C for up to 48 hours before being immersed in FSW in individual glass vials, which were moved between 10°C and 25°C water baths every 45-60 minutes. Only the most recently released spermatozoa and eggs were used for fertilisation. Numbers of released eggs were estimated from five small aliquots counted under a microscope on a Sedgewick Rafter Cell chamber whilst sperm concentration was assessed by fixing with Lugol’s solution before counting individual cells on a haemocytometer. Sperm from a single male was used to fertilise eggs from two females at a ratio of 200:1 in 500 ml cylinders. After 45 minutes, over half of the eggs were observed to have a polar body indicating a good fertilisation rate (32). The developing embryos were pipetted into 100ml culture vials filled to 80ml with UV-irradiated, 0.5µm FSW at a density of 200 fertilised eggs cm^{-2} surface area of the vial and kept under one of the six ALAN treatments in incubators set at 15°C (32). Embryos were left undisturbed for 72 hours to develop from trochophore to the early veliger stage (32). Once the veliger stage was reached, density was reduced to high-density (approximately 30 larvae ml^{-1}) and low-density (10 larvae ml^{-1}) treatments (37). Water was changed three times a week by carefully sieving larvae onto a 30 µm mesh. Larvae were returned to a clean vial containing fresh FSW and fed with live *Isochrysis galbana* (CCAP 927/1) cultures at a density of $5x10^4$ cells ml^{-1} (32).

Data collection

To record data, larvae were carefully sieved from the vials into a petri dish for inspection and image-taking under a microscope and afterwards returned to vials with FSW. Four response variables were extracted using imageJ (Figure 1d): (i) survival on Day 60 (expressed as counts of live individuals per vial per 10,000; *n* = 96); (ii) length on Day 9 (in µm for a subset of 10 individuals per vial; *n* = 960); (iii)

length on Day 60 (in µm due to high mortality averaged per vial; *n* = 77); (iv) and vial averaged growth (in µm averaged per vial, *n* = 77) calculated as follows:

$$(Vial\ averaged\ length\ Day\ 60 - Vial\ averaged\ length\ Day\ 9)/\ Vial\ averaged\ length\ Day\ 9.$$

Survival of mussel larvae was expressed per 10,000 individuals because of overall low number of survivors. A lower scale would lead to the loss of important data (many small values rounding to zero).

Statistical analysis

The relationship between each response variable, ALAN and Parent (~Irradiance*Parent), and for mussels also density (~Irradiance*Mother + Irradiance*Density), was modelled in an Analysis of Covariance. ALAN was analysed as a continuous response variable (irradiance) and log transformed to reduce the leverage introduced by high intensity treatments. Parent was modelled as a factor (individual ID). Density was a factor with two treatment levels (low/ high). The significance of model parameters was quantified using the Type III ANOVA approach of stepwise parameter removal starting with the full model and removing interactions before main effects (42). The significance of all responses was assessed at the 95% confidence level, unless explicitly reported otherwise. Where Gaussian errors were fitted to the response, model fit was visually assessed to comply with assumption of residual normality. Significant differences between treatment levels of factors (Parent, Density) and confidence intervals were quantified by pairwise comparisons using the emmeans function (CRAN: emmeans). The predicted relationships of irradiance and their 95% intervals for generalized linear models (GLMs) were modelled using the add_ci function (CRAN: ciTools). The predicted relationship of irradiance and their 95% intervals for (generalised) linear mixed effects models (GLMMs) were modelled using the predictInterval function (CRAN: merTools).

Mortality of barnacles was expressed as a count of the number dead per vial per 100 individuals and analysed using a negative binomial error distribution (dispersion variance/ mean > 1). The model was weighted by the number of individual larvae in each vial at the start of the experiment. The vial averaged developmental time of barnacles was continuous but non-normally distributed, hence it was analysed using the Gamma error distribution. The vial averaged size of barnacle larvae measured as area and width were normally distributed and modelled using a linear relationship. Length was non-normally distributed, hence analysed using the Gamma error distribution.

Survival of mussel larvae, counts of survivors scaled up to 10,000 animals per vial, was analysed using a negative binomial error distribution (dispersion variance/ mean > 1). Length of mussel larvae after 9 days and 60 days were fitted with Gaussian error distributions. Length at Day 9 was analysed using a linear mixed effects model (CRAN: lme4) with vial ID as a random factor to account for

nestedness of 10 random observations per vial. Due to low survival at Day 60, observations were averaged over vial and analysed using a linear model. Growth of mussel larvae between Day 9 and Day 60 was calculated as an average per vial due to low survival on Day 60. Growth was analysed using a generalised linear model fitted using a Gaussian error distribution. All models were implemented in the R version 4.2.2.

Note that, while barnacle mortality was analysed, to better compare responses between barnacle and mussel larvae, the data for both species will be displayed and discussed as survival. This was necessary to account for the different reproductive strategies and life cycles between barnacles and mussels described above and data distribution. The size difference of mussel and barnacle larvae at the beginning of the experiment (i.e. mussel larvae stem from eggs fertilised in the laboratory and larvae too small to count dead individuals) meant that only survival data could be recorded for mussels.

Results

Barnacle *Austrominius modestus*

Increasing ALAN intensity reduced survival of barnacle larvae (Figure 2a; $\chi^2 = 5.97$, $df = 70$, $p = 0.015$). The predicted relationship between survival and ALAN shows a survivorship of 67 individuals per 100 (lower 95% prediction interval 63; upper: 71; numbers extracted from prediction model described above) at no ALAN irradiance (control). However, at the highest irradiance (50 lux or 124.80 W m^2), survivorship drops to 58 individuals (lower 95% prediction interval 52; upper 64); a reduction by 13%. Survival differed considerably between offspring from different parents ($\chi^2 = 224.69$, $df = 68$, $p < 0.001$; see Supplement S1 for additional figures) without showing a significant interaction with irradiance ($\chi^2 = 0.30$; $df = 66$, $p = 0.861$). The interactive effect of irradiance and parent on the average developmental time from nauplii to cyprid was significant at the 90% confidence level (Figure 2b; $F = 3.09$, $df = 66$, $p = 0.052$). The influence of the average developmental time on survival was modulated by parent (Figure 2c; $F = 20.57$, $df = 66$, $p < 0.001$). In offspring from parent 1, longer development time coincided with lower survival, but in offspring from parents 2 and parent 3 this pattern was reversed. Here larvae with the shorter developmental time showed the lowest survival (Figure 2c). The size of barnacle cyprids was not affected by the interaction between irradiance and parent (area: $F = 0.35$, $df = 57$, $p = 0.706$; length: $F = 0.34$, $df = 57$, $p = 0.712$; width: $F = 0.30$, $df = 57$, $p = 0.743$) or by irradiance (area: $F = 0.01$, $df = 61$, $p = 0.917$; length: $F = 0.03$, $df = 61$, $p = 0.865$; width: $F = 0.06$, $df = 61$, $p = 0.805$). Larvae from different parents varied in size i.e., in area ($F = 3.41$, $df = 59$, $p = 0.040$), length ($F = 3.46$, $df = 59$, $p = 0.040$) and width ($F = 3.20$, $df = 59$, $p = 0.048$).

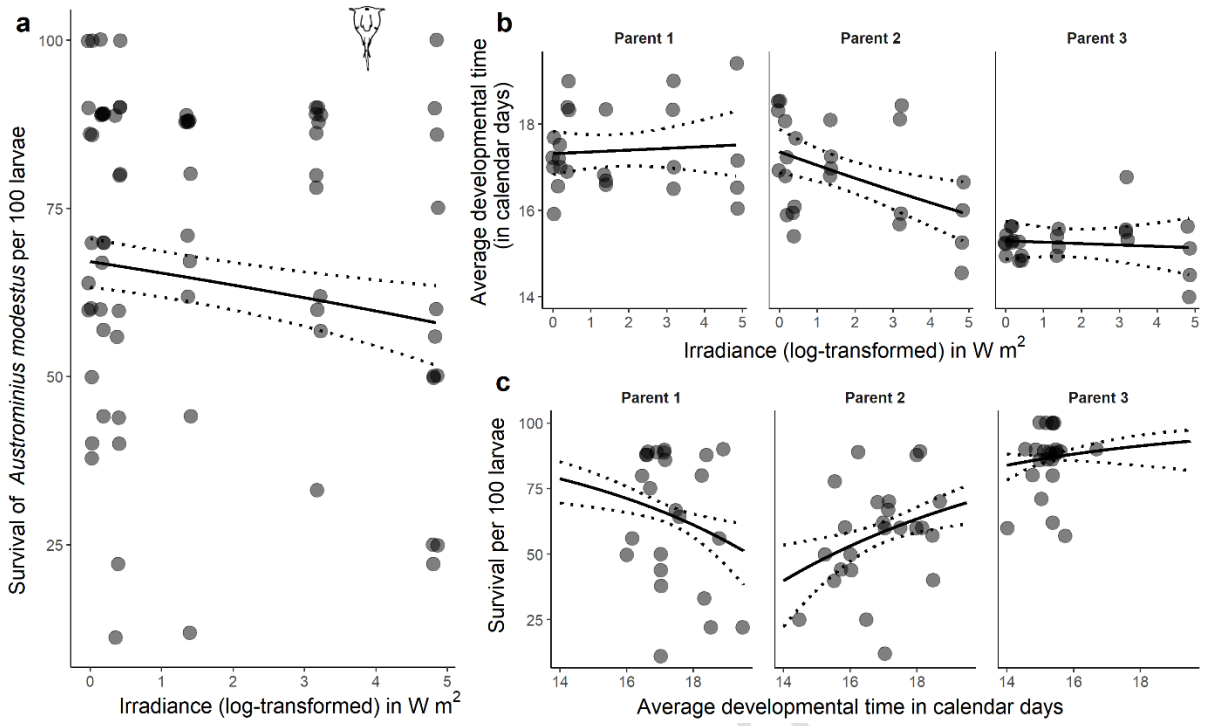

Figure 2a) Influence of irradiance on the barnacle *Austronimius modestus* survival between nauplii and cyprid per 100 animals. b) Influence of irradiance and parent on the average developmental time of barnacle larvae from nauplii to cyprid. c) Relationship between of the average developmental time and survival of barnacle from different parents. The figures show the raw data (dots are jittered, darker areas represent overlapping data points), predicted relationships (solid line) and 95% prediction intervals (dotted lines). Note that barnacle data is displayed as survival to allow better comparison between species. Image credit: Harms *et al* (1987).

Table 1 The impact of artificial light at night (irradiance) and parent on survival (as number dead per 100 individuals), average developmental time from nauplii to cyprid (as calendar days), cyprid size measured as area (µm²), length (in µm) and width (in µm) in larvae of the barnacle *Austrominius modestus*. The last response variable shows the relationship between survival, average developmental time, and parent. Significant main effects and interactions are in bold. Significant effects at the 90% confidence level are in bold and italics. Note that barnacle data is displayed as survival to allow better comparison between species.

Response	Predictor	Test Statistic	Residual Df	*P*
Survival [a]	Irradiance * Parent	0.30	66	0.861
	Parent	**224.69**	**68**	**< 0.001**
	Irradiance	**5.97**	**70**	**0.015**
Average developmental time [b]	***Irradiance * Parent***	***3.09***	***66***	***0.052***
	Parent	**39.29**	**68**	**< 0.001**
	Irradiance	1.25	70	0.268
Size as area (in µm²) [c]	Irradiance * Parent	0.35	57	0.706
	Parent	**3.41**	**59**	**0.040**
	Irradiance	0.01	61	0.917
Size as length (in µm) [b]	Irradiance * Parent	0.34	57	0.712
	Parent	**3.46**	**59**	**0.040**
	Irradiance	0.03	61	0.865
Size as width (in µm) [c]	Irradiance * Parent	0.30	57	0.743
	Parent	**3.20**	**59**	**0.048**

	Irradiance	0.06	61	0.805
Survival [a]	**Average developmental time * Parent**	**20.57**	**66**	**< 0.001**
	Parent	**175.20**	**68**	**< 0.001**
	Average developmental time	**45.72**	**70**	**< 0.001**

[a] χ^2 -squared Likelihood ratio tests of Negative Binomial Models

[b] Gamma distribution Analysis of Deviance

[c] Gaussian distribution Analysis of Deviance

Blue mussel *Mytilus edulis*

Survival of mussel larvae declined with increasing ALAN irradiance (Figure 3a; χ^2 = 3.85, *df* = 94, *p* < 0. 0.0498). The predicted relationship for mussel larvae survival and ALAN shows that at the highest irradiance (50 lux or 124.80 W m^2) mussel survival falls below 50% i.e., ~ 20 larvae per 10,000 (lower 95% prediction interval: 11; upper: 35) compared to ~46 larvae per 10,000 (lower 95% prediction interval: 32; upper: 67) without ALAN. This represents a drop in survival by 57%. Survival was neither affected by the interaction between irradiance and rearing density (χ^2 = 0.02, *df* = 91, *p* = 0.887), the interaction between irradiance and mother (χ^2 = 0.36, *df* = 90, *p* = 0.547) or either of the remaining main effects, namely rearing density (χ^2 = 0.62, *df* = 93, *p* = 0.432) or mother (χ^2 = 0.13, *df* = 92, *p* = 0.715; see Supplement S1 for additional figures). After 9 days of exposure to ALAN, larvae under higher irradiance had grown longer than conspecifics under dimmer conditions (Figure3b; *F* = 5.28, *df* = 958, *p* = 0.022). Length at Day 9 was not influenced by interactions between irradiance and density (*F* = 0.03, *df* = 955, *p* = 0.957) or irradiance and mother (*F* = 1.11, *df* = 954, *p* = 0.292) or density as a main factor (*F* = 0.23, *df* = 957, *p* = 0.633). The length on Day 9 had no effect on survival (*F* = 0.07, *df* = 94, *p* = 0.800) nor did mother (*F* = 1.06, *df* = 93, *p* = 0.303) or an interaction between length Day 9 and mother (*F* = 1.99, *df* = 92, *p* = 0.158).

Any effect of ALAN irradiance on the length of mussel larvae was negated after 60 days (*F* = 0.153, *df* = 958, *p* = 0.697) by which point differences in length were quantifiable only between individuals from different mothers (*F* = 30.85, *df* = 956, *p* < 0.001). Similarly, growth was only influenced by mother (*F* = 16.51, *df* = 956, *p* < 0.001). None of the interactions or main effects other than mother influenced the length of mussel larvae on Day 60 and their growth between Day 9 and 60 (see Table 2 for all test results).

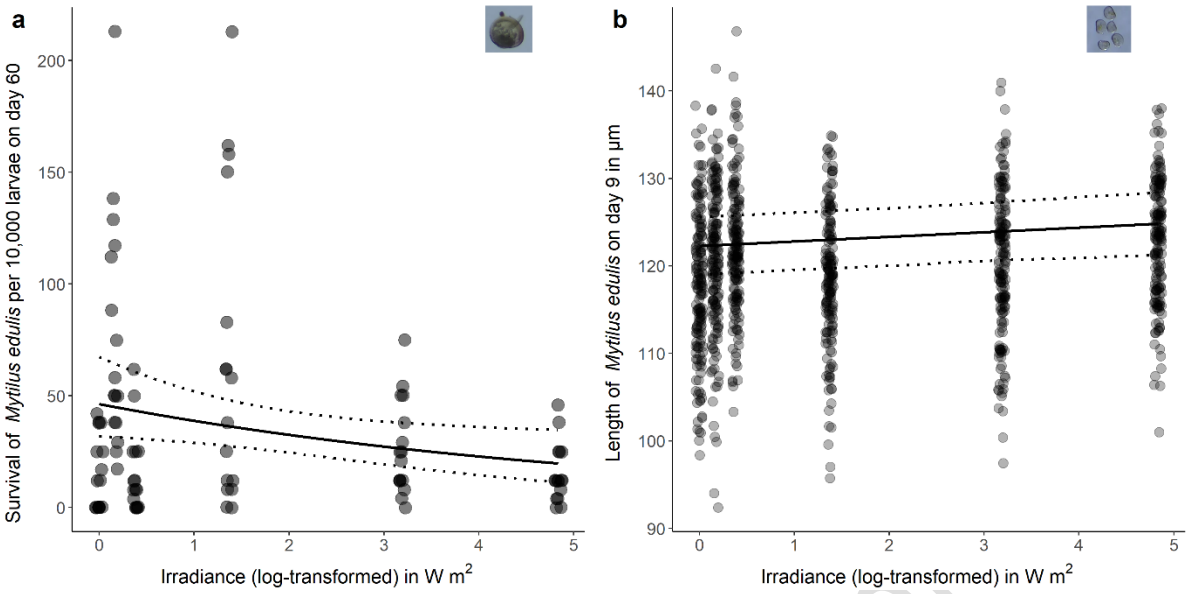

Figure 3 Influence of irradiance on a) the survival of blue mussel *Mytilus edulis* larvae per 10,000 animals (from fertilisation until Day 60) and b) the length of mussel larvae (measured in µm) on Day 9. The figure shows the raw data (dots are jittered, darker areas represent overlapping data points), predicted relationships (solid line) and 95% prediction intervals (dotted lines). Image credit: Fraser Brough.

Table 2 The impact of artificial light at night (irradiance), mother and rearing density on *Mytilus edulis* survival (measured as count of survivors in every 10,000 individuals), size at Day 9, size at Day 60 and growth between Day 9 and 60 (measured as length in µm). The last response variable shows the relationship between survival, length on Day 9 and mother. Significant main effects and interactions are in bold. Borderline none-significant effects in bold and italics.

Response	Predictor	Test Statistic	Residual Df	*P*
Survival [a]	Irradiance * Density	0.02	91	0.887
	Irradiance * Mother	0.36	90	0.547
	Density	0.62	93	0.432
	Mother	0.13	92	0.715
	Irradiance	**3.85**	**94**	**0.0498**

Response	Predictor	Test Statistic	Residual Df	*P*
Length at Day 9 (in µm) [b]	Irradiance * Density	< 0.01	955	0.957
	Irradiance * Mother	1.11	954	0.292
	Density	0.23	957	0.633
	Mother	**38.74**	**956**	**< 0.001**
	Irradiance	**5.28**	**958**	**0.022**
Length at Day 60 (in µm) [c]	Irradiance * Density	0.28	955	0.600
	Irradiance * Mother	0.78	954	0.380
	Density	< 0.01	957	0.962
	Mother	**30.85**	**956**	**< 0.001**
	Irradiance	0.15	958	0.697
Growth between Day 9 & 60 (in µm) [c]	Irradiance * Density	0.40	955	0.531
	Irradiance * Mother	0.56	954	0.458
	Density	0.01	957	0.938
	Mother	**16.51**	**956**	**< 0.001**
	Irradiance	0.81	958	0.365
Survival [a]	Length Day 9 * Mother	1.99	92	0.158
	Mother	1.06	93	0.303
	Length Day 9	0.07	94	0.800

[a] Likelihood ratio tests of Negative Binomial Models

[b] Linear mixed effects model

[c] Linear model

Discussion

Demonstrated ALAN impacts on habitat-forming species like marine sessile invertebrates are rare, except for tropical corals (43-45) and three species of barnacles (28, 29). Here, we quantified ALAN impacts on two cosmopolitan, sessile invertebrates from temperate marine ecosystems, the barnacle *Austrominius modestus* and the mussel *Mytilus edulis*, by measuring larval performance (survival, growth, and developmental time) under an ALAN gradient. Higher ALAN irradiance reduced survival in both species. While barnacle survival dropped by 13% and showed large variation in survival across ALAN intensities, i.e. larger spread of data (Figure 2A), survival of mussel larvae dropped by 57% across replicates under highest tested ALAN irradiance (Figure 3A). Direct ALAN impacts on development and growth were largely absent and if present, small. These impacts varied between the two species and between time-points (9 vs 60 days in mussels). Within the first 9 days mussel larvae grew larger at higher ALAN irradiances. This pattern was absent on Day 60 and ALAN

had no influence on growth between Day 9 and 60. There is some indication at the 90% confidence interval that ALAN impacts on developmental time in barnacles vary between offspring from different parents (some developed faster, others slower under increased ALAN intensity). Not surprisingly, we found strong parental influence on nearly all measured responses of larval performance in both species (note that due to challenges in facilitating spawning in the laboratory (32) maternal influences on mussel larvae are based on two mothers). Rearing density as a proxy for food availability did not affect any of the responses measured in mussels (survival, length at Day 9, Day 60, growth).

ALAN impacts on survival, development, and growth in early life stages have been shown across taxa. However, as in our experiments, the patterns are not uniform and can vary between species, within species between life stages and parentage. First, it should be noted that larval survival is generally low in the wild (7) and challenging in laboratory settings (32, 37). Second, extensive aquaculture research on the effects of ALAN across teleost fish commonly shows that larger and faster larval growth under ALAN comes at the expense of reduced survival, such as via malformations followed by mortality (21, 22). Exposure to artificially lit nights decreases survival as early as during embryogenesis, even before an organism possesses neural light detecting structures (20). Larvae of the tropical convict surgeonfish *Acanthurus triostegus* (46) grew larger and survival declined under ALAN. Similarly, crustacean larvae showed shorter developmental time between stages and lower survival (23, 24). Pond snail hatchlings *Lymnaea stagnalis* also grew larger under ALAN but without compromising survival (25). Contrary to those ALAN impacts, tadpoles of the American toad *Anaxyrus americanus* (26), fry of Atlantic salmon *Salmo salar* (27), and juvenile orange-fin anemonefish *Amphiprion chrysopterus* monitored in the wild (47) all grew smaller; the latter also experienced higher mortality. ALAN reduced survival in both our species, but as described above, the pattern and percentage reduction differed between species. Under the highest ALAN levels, survival dropped by 57% in mussels and 13% in barnacles suggesting that high ALAN irradiance could have a stronger effect on mussel compared to barnacle populations.

The two main differences between our species are their reproductive strategy (external vs internal fertilisation) and the onset of ALAN exposure in the larvae's life history (embryonic vs larval stage). We fertilised mussel eggs in the laboratory and consequently, animals were under ALAN during embryogenesis. In contrast, we collected adult barnacles with developed larvae from the field where embryogenesis occurred under natural light conditions. ALAN during embryogenesis has been shown not only to lower survival as mentioned earlier (20) but also embryo quality (yolk area, egg length, eye diameter; 48). The differences between the two species might also explain why we found different ALAN effects on development and growth between mussels and barnacles. Mussel larvae

grew larger with increasing ALAN irradiance by Day 9 (a transient effect since we found no effect on Day 60). For barnacles, there is some indication that ALAN induced variability in developmental time between larvae from different parents (some developed faster, others slower under increased ALAN intensity). Given that our results are significant at the 90% confidence level, further experiments are needed to examine this pattern. However, the developmental time of barnacles correlated strongly with survival and again, the direction varied with parent. Altered growth rates away from optimal patterns (faster or slower) are known to incur both short and long-term costs (49). As growth is energetically costly, larvae that grew faster in the beginning of the experiment may not have survived the weeks after.

Our study validates the well-established pattern of strong parental influence on larval performance under environmental stress such as increased temperature and lower salinity (35, 36, 50). Torres *et al* (2020) show that larval performance (here survival and development) differed between offspring of different egg-carrying mothers and the environmental conditions (temperature and salinity) they experienced. Similarly, our results suggest that the influence of ALAN might be modulated by parental influence (offspring from some parents cope better than others), which might be driven either by genotypic variation, parental phenotype, and its environment or a combination of these. Overall, our results provide two novel directions for further research on ALAN impacts. First, the response to ALAN may depend on the onset of ALAN exposure (embryonic vs larval stage). Second, evidence shows that the parental exposure to ALAN imposes transgenerational effects on human foetuses (reviewed in 51) and rat offspring (52). Future research on how parental influence changes ALAN impacts on non-human animals could disentangle the role of genotypic variation, parental phenotype, and its environment and give insight into potential selective pressure from ALAN.

Other potential mechanisms of ALAN induced mortality and changes in growth and development in offspring are manifold. Higher mortality after accelerated growth can be a result of directly incurred physiological costs, a trade-off that has been shown well beyond exposure to ALAN and various forms of environmental stress (49). During accelerated growth, fewer resources are allocated to energy reserves. Instead, organisms experience a higher metabolic rate, which can be associated with faster production of reactive oxygen species (ROS) and thus oxidative stress reducing lifespan, potentially via higher vulnerability to cellular damage. While ALAN has been shown to increase metabolic demands (53, 54), cause overproduction of ROS and increase in oxidative damage from corals (43, 44) to vertebrates, including humans (55, 56), there is plenty of evidence that ALAN does not necessarily affect organisms via this pathway (57, 58). Alternatives can be endocrine disruptions such as depressed levels of T3, an important hormone for metamorphosis (46). Animals under ALAN also show reduced melatonin level (59), which indicates a disruption of rest-like states on the

386 molecular level. ALAN alters the expression of clock genes, which time protein synthesis, DNA repair,
387 cell division and renewal and (20, 45, 60) and impairs organisms' health by affecting the circadian
388 immune system and microbiome (61). While many studies have quantified ALAN impacts across
389 biological scales within their study system, between system comparison remains difficult, including
390 our two ecosystem engineers. A recent meta-analysis shows that the direction of physiological and
391 life history trait responses to ALAN can range from strongly negative to positive (62). The diverse
392 directions of responses to ALAN for survival, growth, and development in the literature (62) together
393 with our results suggest that further research is needed to consolidate and link patterns and
394 mechanisms.

395 Here we show that ALAN clearly affects survival, and had differential impacts on development, and
396 body size in the larval stage of two marine ecosystem engineers. Impaired larval performance under
397 ALAN is not only likely to affect population health and distribution but their role as habitat-forming
398 ecosystem engineers. Sessile fauna like our model organisms, the mussel *Mytilus edulis* and the
399 barnacle *Austrominius modestus*, support other species by providing three-dimensional habitats (2-
400 4, 30). As filter feeders with a complex life-cycle involving a larval phase, they couple benthic pelagic
401 systems and contribute to energy flows through trophic chains (5) and the accumulation of calcium
402 and carbon (2, 31). While ALAN impacts have been demonstrated at most scales of biological
403 organisation (19, 62), few studies have been able to quantify ALAN effects on ecosystems and
404 ecological functioning so far (43, 45, 60, 63). Targeting ecosystem engineering species can form an
405 entry-point to eventually scale-up ALAN impacts to ecosystem level. Tools like biogeographical and
406 predictive modelling can then identify hotspots of susceptible species and habitats at a global scale
407 (15, 64), which is yet to be realised for ALAN (19, 64). Given that larval dispersal shapes marine
408 biodiversity and various ecosystem functions including the provision of nutrients for humans, ALAN
409 impacts of the early life stages of ecosystem engineers have clear implications beyond the
410 population level to the ecological communities they facilitate.

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Data accessibility

The datasets supporting this article and code will be uploaded as part of the Supplementary Material.

Author's contribution

ST, LG, SRJ and TWD designed the experiment. ST supervised the data collection, FTB collected the data. ST, FTB and TWD analysed the data. ST drafted the first version of the manuscript. All authors revised the manuscript and approved the final version.

Competing interests

We have no competing interests.

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