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

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Authors accepted version

10 **Abstract**

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

26

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

## 28 Introduction

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

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

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

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

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

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

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

## 86 **Methods**

### 87 ***Lighting setup***

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

94 system which captures variability in night-time lighting as the moon transits the sky (38). Moonlight  
95 was simulated using a bank of 2700-3500K 1.2 cd LEDs housed within diffusing spheres. Lunar cycles  
96 were simulated using a pulse width modulated signal (scale 0–100%) applied to the 5 V output of  
97 Raspberry Pi 3 model B+, with maximum lunar brightness set to 0.5 lux. Lunar brightness was  
98 adjusted every minute from a look up table of Zenith Sky Brightness values modelled for Plymouth,  
99 UK, accounting for lunar phase, altitude, opposition, parallax and atmospheric scattering (39). ALAN  
100 was simulated between sunset and sunrise in Plymouth using Aquaray cool white Flexi-LED strips  
101 (Tropical Marine Centre, UK), with brightness controlled using voltage dimming. We kept larvae  
102 under one of six ALAN treatments: 0, 0.2, 0.5, 1, 10, 50 lux (measured in irradiance as  $W\ m^{-2}$ : 0.00,  
103 0.18, 0.47, 2.94, 23.34, 124.80). ALAN brightness (lux) was measured using the SpectroSense2 Lux  
104 sensor, irradiance ( $W\ m^{-2}$ ) with the multispectral irradiance sensor (Skye Instruments, UK).

### 105 **Experimental design**

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

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

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

### 126 **Barnacle *Austrominius modestus***

127 **Adult collection and larval husbandry**

128 Adult barnacles were collected from South Milton Sands, UK (50°15'40" N, 3°51'33" W), which is a  
129 light naïve area (41), in March 2022. Intact barnacles were carefully removed from the substratum  
130 using a knife and inspected for egg masses. Adults were transported to the laboratory at the  
131 University of Plymouth, where they were placed in individual vials containing 100ml UV-irradiated,  
132 0.5µm filtered seawater (FSW) to spawn (33). After an hour, most parents had released larvae. We  
133 selected adults that had released a large proportion of their brood and whose larvae were actively  
134 swimming (established by a directional light source). Animals were kept in incubators set at 15°C and  
135 reared in 100ml plastic vials filled to 80ml with FSW (33). Every two days, we changed water and fed  
136 larvae with live diatom *Skeletonema marinoi* (CCAP 1077/5) culture at a density of  $4 \times 10^5$  cells ml<sup>-1</sup>  
137 (33).

138 **Data collection**

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



149

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

153

154 **Blue mussel *Mytilus edulis***

155 **Adult collection, fertilisation, and larval husbandry**

156 Adult mussels were supplied by Offshore Shellfish Ltd., a rope culturing mussel farm in Lyme Bay, UK  
157 (50°41'59" N, 2°53'59" W) with three sites five to ten kilometres offshore and away from the highly  
158 ALAN lit region of Torbay (see ALAN underwater atlas for the North Atlantic by Smyth et al. 16).  
159 Mussels were landed in Brixham Harbour (50°23'39" N, 3°30'57" W) in April 2022 and directly  
160 transported to the laboratories at the University of Plymouth, UK (50°22'34" N, 4°8'37" W). The  
161 brood stock of around 100 animals was maintained in a recirculating system supplying four aquaria  
162 of ca. 60l each with FSW, at a salinity of 33/34ppt, temperature of 10°C and 12:12 light dark cycle (in  
163 accordance with seasonal daylight times for Plymouth). Animals were fed every other day with  
164 *Isochrysis* 1800 according to manufacturer's instructions (Reed Mariculture Inc., USA) until  
165 fertilisation. Fertilisation was initiated by thermal shock method to induce gamete release (32, 37).  
166 Mussels were exposed to air in a fridge at 3±1°C for up to 48 hours before being immersed in FSW in  
167 individual glass vials, which were moved between 10°C and 25°C water baths every 45-60 minutes.  
168 Only the most recently released spermatozoa and eggs were used for fertilisation. Numbers of  
169 released eggs were estimated from five small aliquots counted under a microscope on a Sedgewick  
170 Rafter Cell chamber whilst sperm concentration was assessed by fixing with Lugol's solution before  
171 counting individual cells on a haemocytometer. Sperm from a single male was used to fertilise eggs  
172 from two females at a ratio of 200:1 in 500 ml cylinders. After 45 minutes, over half of the eggs were  
173 observed to have a polar body indicating a good fertilisation rate (32). The developing embryos were  
174 pipetted into 100ml culture vials filled to 80ml with UV-irradiated, 0.5µm FSW at a density of 200  
175 fertilised eggs cm<sup>-2</sup> surface area of the vial and kept under one of the six ALAN treatments in  
176 incubators set at 15°C (32). Embryos were left undisturbed for 72 hours to develop from  
177 trochophore to the early veliger stage (32). Once the veliger stage was reached, density was reduced  
178 to high-density (approximately 30 larvae ml<sup>-1</sup>) and low-density (10 larvae ml<sup>-1</sup>) treatments (37).  
179 Water was changed three times a week by carefully sieving larvae onto a 30 µm mesh. Larvae were  
180 returned to a clean vial containing fresh FSW and fed with live *Isochrysis galbana* (CCAP 927/1)  
181 cultures at a density of 5x10<sup>4</sup> cells ml<sup>-1</sup> (32).

182 **Data collection**

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



187 length on Day 60 (in  $\mu\text{m}$  due to high mortality averaged per vial;  $n = 77$ ); (iv) and vial averaged  
188 growth (in  $\mu\text{m}$  averaged per vial,  $n = 77$ ) calculated as follows:

189  $(\text{Vial averaged length Day 60} - \text{Vial averaged length Day 9}) / \text{Vial averaged length Day 9}$ .

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

### 193 **Statistical analysis**

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

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

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

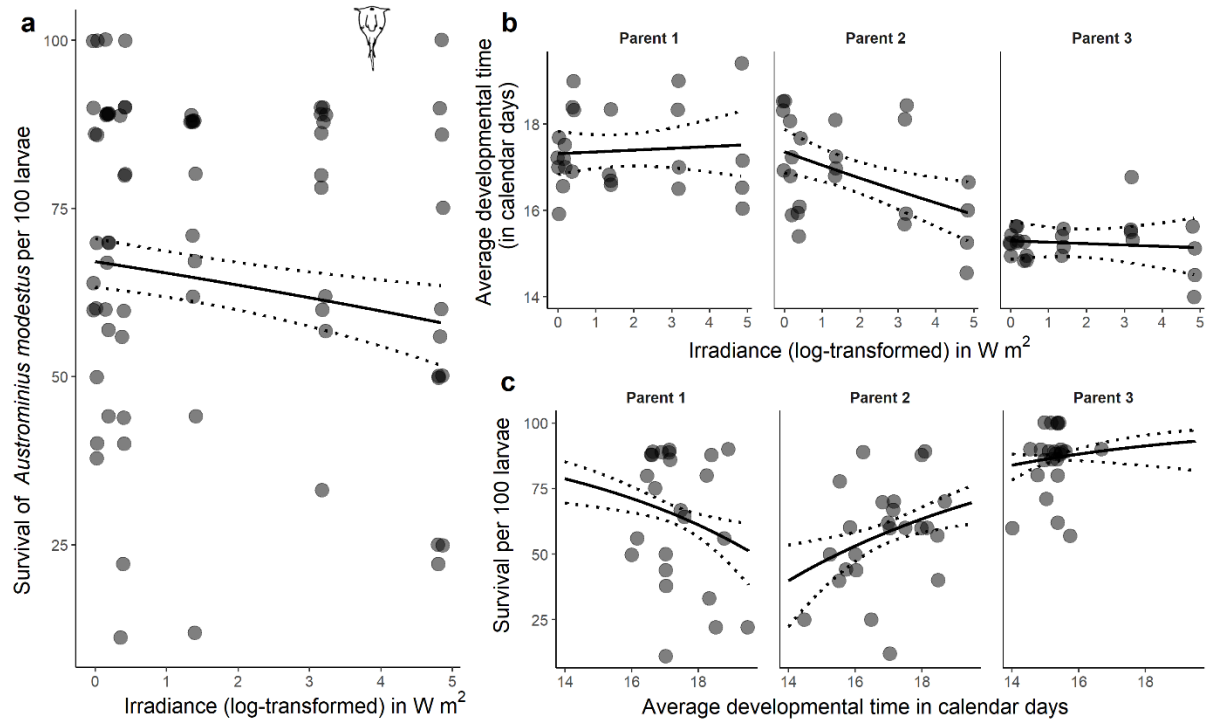
220 nestedness of 10 random observations per vial. Due to low survival at Day 60, observations were  
221 averaged over vial and analysed using a linear model. Growth of mussel larvae between Day 9 and  
222 Day 60 was calculated as an average per vial due to low survival on Day 60. Growth was analysed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

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

## 232 **Results**

### 233 **Barnacle *Austrominius modestus***

234 Increasing ALAN intensity reduced survival of barnacle larvae (Figure 2a;  $\chi^2 = 5.97$ ,  $df = 70$ ,  $p = 0.015$ ).  
235 The predicted relationship between survival and ALAN shows a survivorship of 67 individuals per 100  
236 (lower 95% prediction interval 63; upper: 71; numbers extracted from prediction model described  
237 above) at no ALAN irradiance (control). However, at the highest irradiance (50 lux or 124.80 W m<sup>2</sup>),  
238 survivorship drops to 58 individuals (lower 95% prediction interval 52; upper 64); a reduction by  
239 13%. Survival differed considerably between offspring from different parents ( $\chi^2 = 224.69$ ,  $df = 68$ ,  $p$   
240  $< 0.001$ ; see Supplement S1 for additional figures) without showing a significant interaction with  
241 irradiance ( $\chi^2 = 0.30$ ;  $df = 66$ ,  $p = 0.861$ ). The interactive effect of irradiance and parent on the  
242 average developmental time from nauplii to cyprid was significant at the 90% confidence level  
243 (Figure 2b;  $F = 3.09$ ,  $df = 66$ ,  $p = 0.052$ ). The influence of the average developmental time on survival  
244 was modulated by parent (Figure 2c;  $F = 20.57$ ,  $df = 66$ ,  $p < 0.001$ ). In offspring from parent 1, longer  
245 development time coincided with lower survival, but in offspring from parents 2 and parent 3 this  
246 pattern was reversed. Here larvae with the shorter developmental time showed the lowest survival  
247 (Figure 2c). The size of barnacle cyprids was not affected by the interaction between irradiance and  
248 parent (area:  $F = 0.35$ ,  $df = 57$ ,  $p = 0.706$ ; length:  $F = 0.34$ ,  $df = 57$ ,  $p = 0.712$ ; width:  $F = 0.30$ ,  $df = 57$ ,  
249  $p = 0.743$ ) or by irradiance (area:  $F = 0.01$ ,  $df = 61$ ,  $p = 0.917$ ; length:  $F = 0.03$ ,  $df = 61$ ,  $p = 0.865$ ;  
250 width:  $F = 0.06$ ,  $df = 61$ ,  $p = 0.805$ ). Larvae from different parents varied in size i.e., in area ( $F = 3.41$ ,  
251  $df = 59$ ,  $p = 0.040$ ), length ( $F = 3.46$ ,  $df = 59$ ,  $p = 0.040$ ) and width ( $F = 3.20$ ,  $df = 59$ ,  $p = 0.048$ ).



252

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

259 **Table 1** The impact of artificial light at night (irradiance) and parent on survival (as number dead per 100 individuals),  
 260 average developmental time from nauplii to cyprid (as calendar days), cyprid size measured as area ( $\mu\text{m}^2$ ), length (in  $\mu\text{m}$ )  
 261 and width (in  $\mu\text{m}$ ) in larvae of the barnacle *Austrominius modestus*. The last response variable shows the relationship  
 262 between survival, average developmental time, and parent. Significant main effects and interactions are in bold.  
 263 Significant effects at the 90% confidence level are in bold and italics. Note that barnacle data is displayed as survival to  
 264 allow better comparison between species.

Response	Predictor	Test Statistic	Residual Df	P
Survival <sup>a</sup>	Irradiance * Parent	0.30	66	0.861
	<b>Parent</b>	<b>224.69</b>	<b>68</b>	<b>&lt; 0.001</b>
	<b>Irradiance</b>	<b>5.97</b>	<b>70</b>	<b>0.015</b>
Average developmental time <sup>b</sup>	<b><i>Irradiance * Parent</i></b>	<b>3.09</b>	<b>66</b>	<b>0.052</b>
	<b>Parent</b>	<b>39.29</b>	<b>68</b>	<b>&lt; 0.001</b>
	Irradiance	1.25	70	0.268
Size as area (in $\mu\text{m}^2$ ) <sup>c</sup>	Irradiance * Parent	0.35	57	0.706
	<b>Parent</b>	<b>3.41</b>	<b>59</b>	<b>0.040</b>
	Irradiance	0.01	61	0.917
Size as length (in $\mu\text{m}$ ) <sup>b</sup>	Irradiance * Parent	0.34	57	0.712
	<b>Parent</b>	<b>3.46</b>	<b>59</b>	<b>0.040</b>
	Irradiance	0.03	61	0.865
Size as width (in $\mu\text{m}$ ) <sup>c</sup>	Irradiance * Parent	0.30	57	0.743
	<b>Parent</b>	<b>3.20</b>	<b>59</b>	<b>0.048</b>

	Irradiance	0.06	61	0.805
Survival <sup>a</sup>	<b>Average developmental time * Parent</b>	<b>20.57</b>	<b>66</b>	<b>&lt; 0.001</b>
	<b>Parent</b>	<b>175.20</b>	<b>68</b>	<b>&lt; 0.001</b>
	<b>Average developmental time</b>	<b>45.72</b>	<b>70</b>	<b>&lt; 0.001</b>

265 <sup>a</sup>  $\chi^2$ -squared Likelihood ratio tests of Negative Binomial Models

266 <sup>b</sup> Gamma distribution Analysis of Deviance

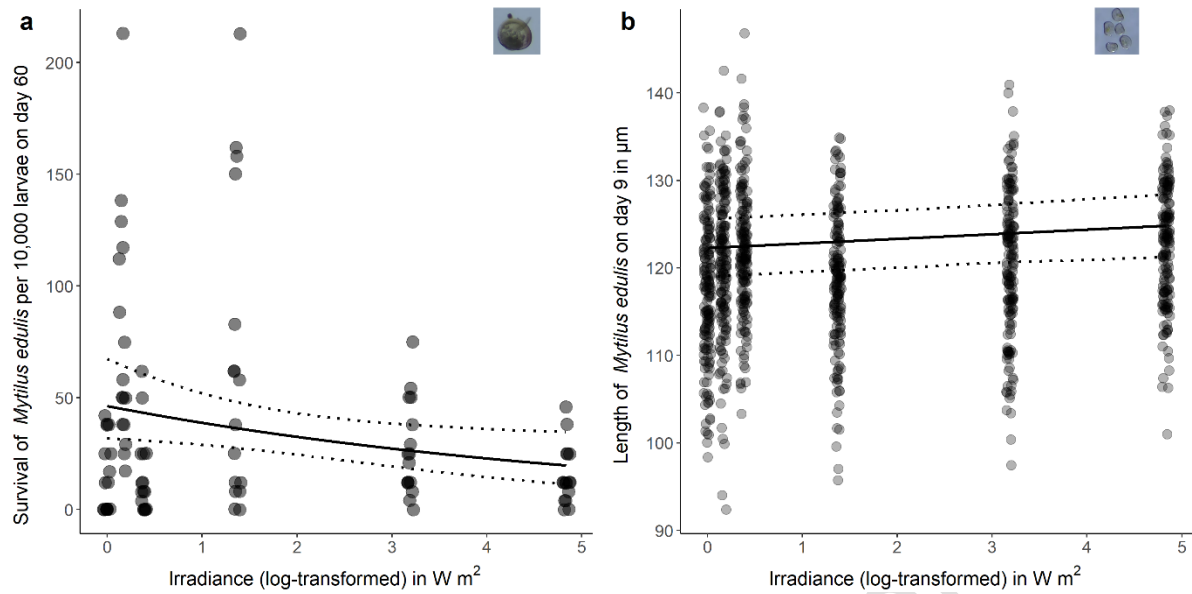
267 <sup>c</sup> Gaussian distribution Analysis of Deviance

268

### 269 **Blue mussel *Mytilus edulis***

270 Survival of mussel larvae declined with increasing ALAN irradiance (Figure 3a;  $\chi^2 = 3.85$ ,  $df = 94$ ,  $p < 0.$   
271  $0.0498$ ). The predicted relationship for mussel larvae survival and ALAN shows that at the highest  
272 irradiance (50 lux or 124.80 W m<sup>2</sup>) mussel survival falls below 50% i.e., ~ 20 larvae per 10,000 (lower  
273 95% prediction interval: 11; upper: 35) compared to ~46 larvae per 10,000 (lower 95% prediction  
274 interval: 32; upper: 67) without ALAN. This represents a drop in survival by 57%. Survival was neither  
275 affected by the interaction between irradiance and rearing density ( $\chi^2 = 0.02$ ,  $df = 91$ ,  $p = 0.887$ ), the  
276 interaction between irradiance and mother ( $\chi^2 = 0.36$ ,  $df = 90$ ,  $p = 0.547$ ) or either of the remaining  
277 main effects, namely rearing density ( $\chi^2 = 0.62$ ,  $df = 93$ ,  $p = 0.432$ ) or mother ( $\chi^2 = 0.13$ ,  $df = 92$ ,  $p =$   
278  $0.715$ ; see Supplement S1 for additional figures). After 9 days of exposure to ALAN, larvae under  
279 higher irradiance had grown longer than conspecifics under dimmer conditions (Figure 3b;  $F = 5.28$ ,  
280  $df = 958$ ,  $p = 0.022$ ). Length at Day 9 was not influenced by interactions between irradiance and  
281 density ( $F = 0.03$ ,  $df = 955$ ,  $p = 0.957$ ) or irradiance and mother ( $F = 1.11$ ,  $df = 954$ ,  $p = 0.292$ ) or  
282 density as a main factor ( $F = 0.23$ ,  $df = 957$ ,  $p = 0.633$ ). The length on Day 9 had no effect on survival  
283 ( $F = 0.07$ ,  $df = 94$ ,  $p = 0.800$ ) nor did mother ( $F = 1.06$ ,  $df = 93$ ,  $p = 0.303$ ) or an interaction between  
284 length Day 9 and mother ( $F = 1.99$ ,  $df = 92$ ,  $p = 0.158$ ).

285 Any effect of ALAN irradiance on the length of mussel larvae was negated after 60 days ( $F = 0.153$ ,  $df$   
286  $= 958$ ,  $p = 0.697$ ) by which point differences in length were quantifiable only between individuals  
287 from different mothers ( $F = 30.85$ ,  $df = 956$ ,  $p < 0.001$ ). Similarly, growth was only influenced by  
288 mother ( $F = 16.51$ ,  $df = 956$ ,  $p < 0.001$ ). None of the interactions or main effects other than mother  
289 influenced the length of mussel larvae on Day 60 and their growth between Day 9 and 60 (see Table  
290 2 for all test results).



291

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

296 **Table 2** The impact of artificial light at night (irradiance), mother and rearing density on *Mytilus edulis* survival  
 297 (measured as count of survivors in every 10,000 individuals), size at Day 9, size at Day 60 and growth between Day 9 and  
 298 60 (measured as length in  $\mu\text{m}$ ). The last response variable shows the relationship between survival, length on Day 9 and  
 299 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 <sup>a</sup>	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
	<b>Irradiance</b>	<b>3.85</b>	<b>94</b>	<b>0.0498</b>

300

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

301

302 <sup>a</sup> Likelihood ratio tests of Negative Binomial Models

303 <sup>b</sup> Linear mixed effects model

304 <sup>c</sup> Linear model

305

## 306 Discussion

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

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

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

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

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

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

374 Other potential mechanisms of ALAN induced mortality and changes in growth and development in  
375 offspring are manifold. Higher mortality after accelerated growth can be a result of directly incurred  
376 physiological costs, a trade-off that has been shown well beyond exposure to ALAN and various  
377 forms of environmental stress (49). During accelerated growth, fewer resources are allocated to  
378 energy reserves. Instead, organisms experience a higher metabolic rate, which can be associated  
379 with faster production of reactive oxygen species (ROS) and thus oxidative stress reducing lifespan,  
380 potentially via higher vulnerability to cellular damage. While ALAN has been shown to increase  
381 metabolic demands (53, 54), cause overproduction of ROS and increase in oxidative damage from  
382 corals (43, 44) to vertebrates, including humans (55, 56), there is plenty of evidence that ALAN does  
383 not necessarily affect organisms via this pathway (57, 58). Alternatives can be endocrine disruptions  
384 such as depressed levels of T3, an important hormone for metamorphosis (46). Animals under ALAN  
385 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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419 **Data accessibility**

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

422 **Author's contribution**

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

426 **Competing interests**

427 We have no competing interests.

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