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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 km² 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 4×10^5 cells ml⁻¹
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 µm²), (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⁻² 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⁻¹) and low-density (10 larvae ml⁻¹) 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 5x10⁴ cells ml⁻¹ (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 μm due to high mortality averaged per vial; $n = 77$); (iv) and vial averaged
188 growth (in μm 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 Irradiance*Parent), and for
195 mussels also density (\sim Irradiance*Mother + 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

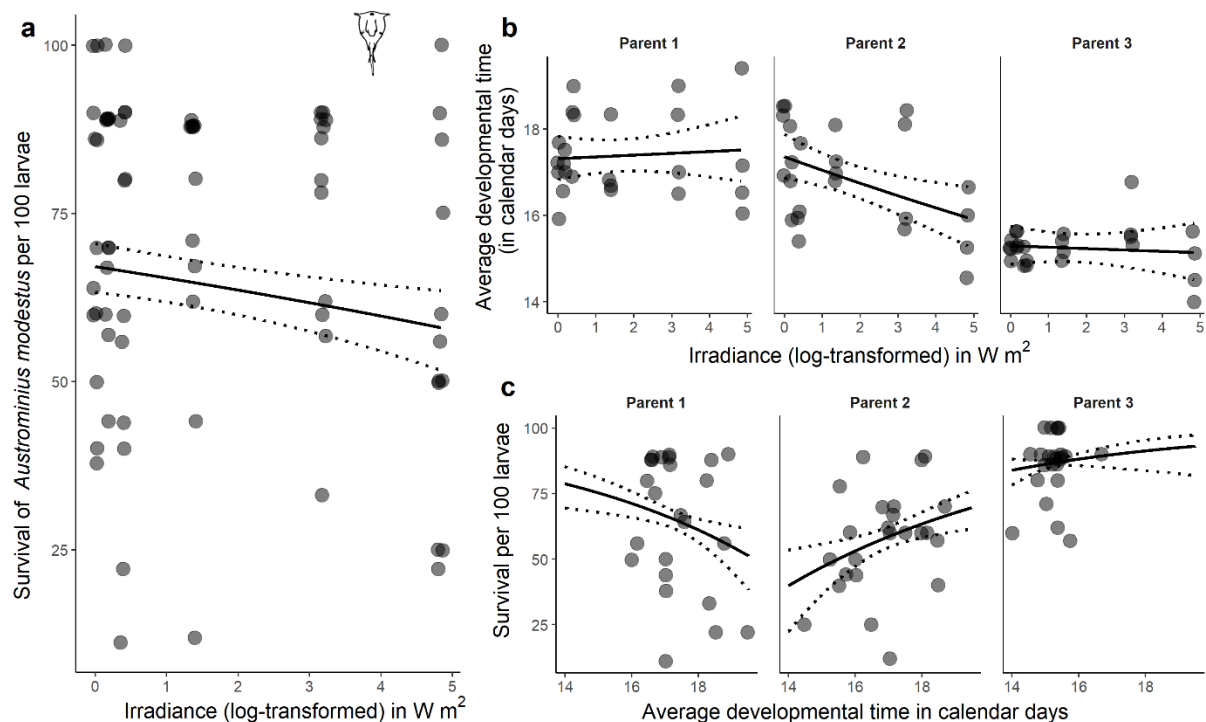
220 nestedness of 10 random observations per vial. Due to low survival at Day 60, observations were
221 averaged over vial and analysed using a linear model. Growth of mussel larvae between Day 9 and
222 Day 60 was calculated as an average per vial due to low survival on Day 60. Growth was analysed
223 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 m²),
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 (μm^2), length (in μm)
 261 and width (in μm) 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 ^a	Irradiance * Parent	0.30	66	0.861
	Parent	224.69	68	< 0.001
	Irradiance	5.97	70	0.015
Average developmental time ^b	<i>Irradiance * Parent</i>	3.09	66	0.052
	Parent	39.29	68	< 0.001
	Irradiance	1.25	70	0.268
Size as area (in μm^2) ^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

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

266 ^b Gamma distribution Analysis of Deviance

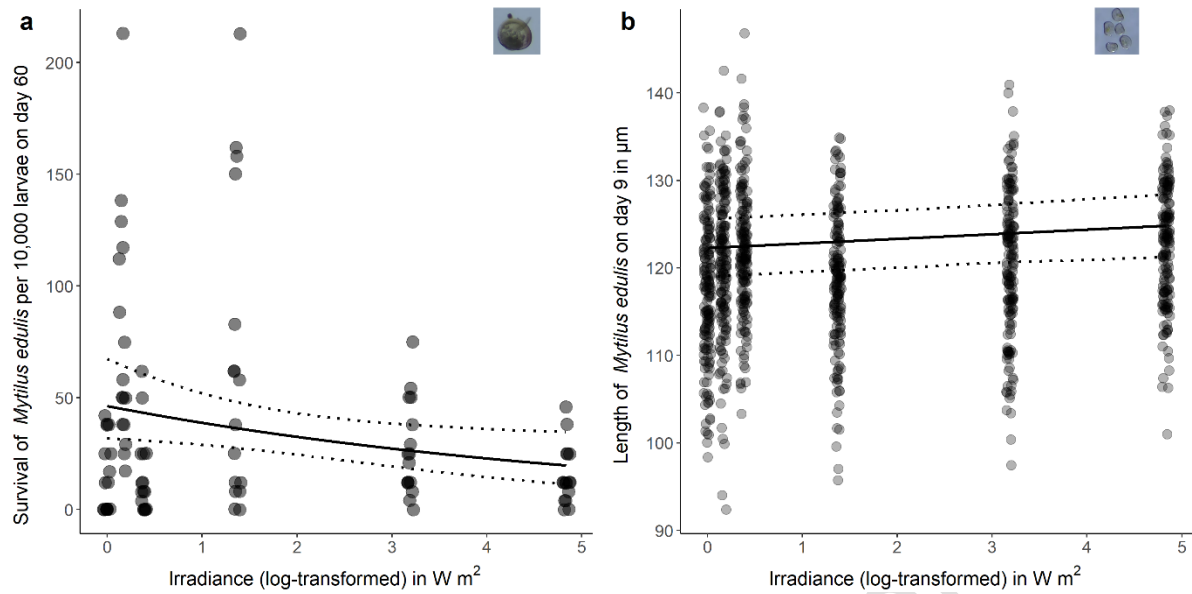
267 ^c Gaussian distribution Analysis of Deviance

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 m²) 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 μm) 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 μm). 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 ^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

300

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

301

302 ^a Likelihood ratio tests of Negative Binomial Models

303 ^b Linear mixed effects model

304 ^c 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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