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Philosophical Transactions of The Royal Society B: Biological Sciences

Cyswllt i'r cyhoeddiad / Link to publication

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.

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

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).

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.

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 4×10^5 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.
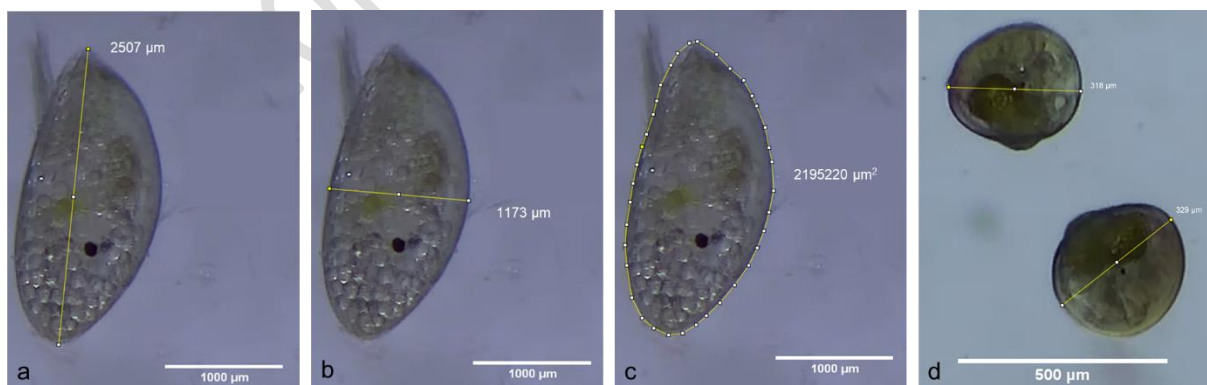
Figure 1 Example of larvae size measurements taken in ImageJ software. Carapace length (a), width (b), and area (c) measurements for *Austrominius modestus* cyprid. Maximum shell length measured parallel to the hinge line on Day 60 for *Mytilus edulis* (d). The black dots inside the larvae are the eyes. 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)

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

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).

Statistical analysis

194 The relationship between each response variable, ALAN and Parent (~Irradiance*Parent), and for

195 mussels also density (~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.

Results

Barnacle *Austrominius modestus*

234 Increasing ALAN intensity reduced survival of barnacle larvae (Figure 2a; χ^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^2),

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 (χ^2 = 224.69, df = 68, p

240 < 0.001; see Supplement S1 for additional figures) without showing a significant interaction with

241 irradiance (χ^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).
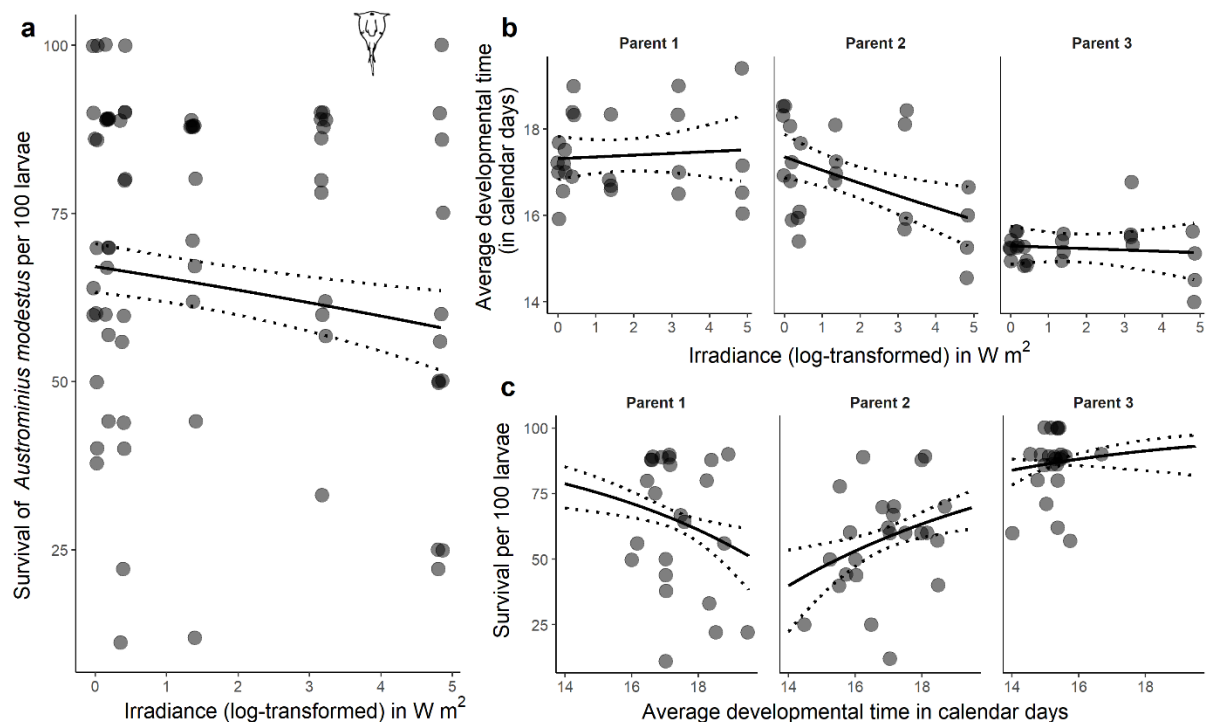
Figure 2a) Influence of irradiance on the barnacle *Austronimius modestus* survival between nauplii and cyprid per 100 animals. b) Influence of irradiance and parent on the average developmental time of barnacle larvae from nauplii to cyprid. c) Relationship between of the average developmental time 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**

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

Blue mussel *Mytilus edulis*

270 Survival of mussel larvae declined with increasing ALAN irradiance (Figure 3a; χ^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^2) 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 (χ^2 = 0.02, *df* = 91, *p* = 0.887), the

276 interaction between irradiance and mother (χ^2 = 0.36, *df* = 90, *p* = 0.547) or either of the remaining

277 main effects, namely rearing density (χ^2 = 0.62, *df* = 93, *p* = 0.432) or mother (χ^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 (Figure3b; *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
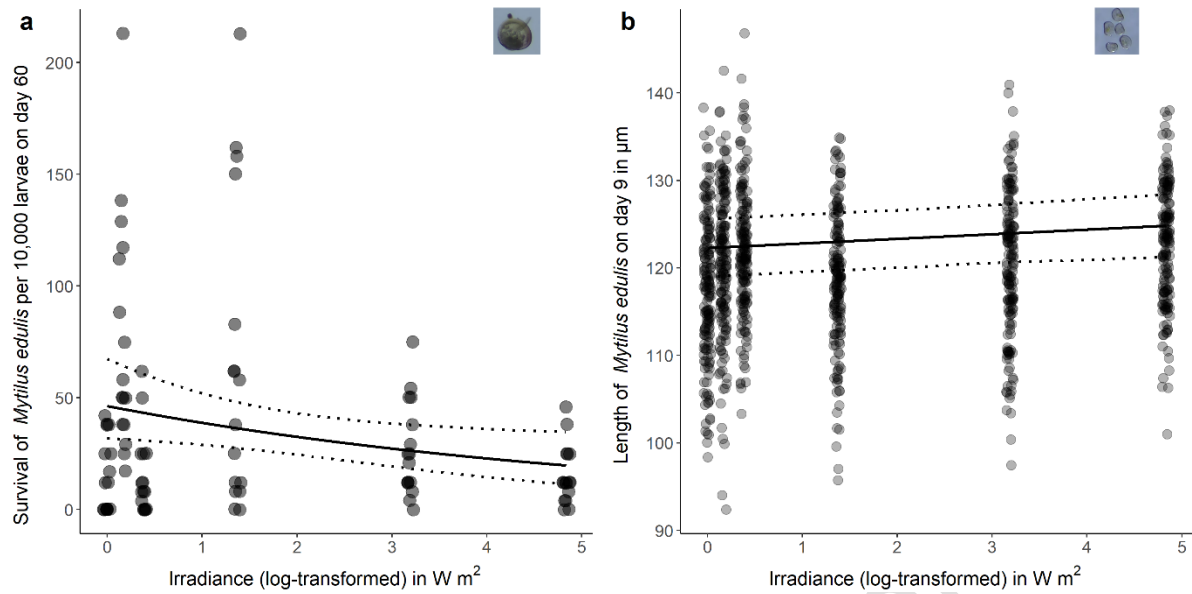
Figure 3 Influence of irradiance on a) the survival of blue mussel *Mytilus edulis* larvae per 10,000 animals (from
fertilisation until Day 60) and b) the length of mussel larvae (measured in µm) on Day 9. The figure shows the raw data
(dots are jittered, darker areas represent overlapping data points), 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**

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

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

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   foetuses (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.

Data accessibility

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

421 Material.

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.

Competing interests

427 We have no competing interests.

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