

2022-11-09

Under the influence of light: How light pollution disrupts personality and metabolism in hermit crabs

Velasque, M

<http://hdl.handle.net/10026.1/19965>

10.1016/j.envpol.2022.120594

Environmental Pollution

Elsevier BV

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

1 **Under the influence of light: how light pollution disrupts personality and metabolism in**
2 **hermit crabs**

3 Velasque, M.^{1,2,3*} Denton, J.A.⁴, & Briffa, M.¹

4 1. School of Biological and Marine Sciences, University of Plymouth, Plymouth, Devon, United
5 Kingdom

6 2. Genomics & Regulatory Systems Unit, Okinawa Institute of Science & Technology, Okinawa,
7 Japan

8 3. The Experimental Evolutionary Biology Lab, School of Biological Sciences, Monash
9 University, Clayton, Victoria, Australia

10 4. The World Mosquito Program, Institute of Vector-borne Disease, Monash University,
11 Clayton, Victoria, Australia

12 * mari.velasque@monash.edu

13

14

15 **PRE-PRINT**

16

17 Version of record: <https://doi.org/10.1016/j.envpol.2022.120594>

18

19 Published in *Environmental Pollution*.

20

21 **Highlights**

- 22 • Coastal zones are highly affected by light pollution
- 23 • Constant light reduced boldness and increased metabolism in hermit crabs
- 24 • Behavioural and metabolic changes can affect population stability
- 25 • Light pollution might disrupt hermit crab populations changing intertidal diversity

26

27 **Abstract**

28 Anthropogenic disturbances are known to cause significant physiological and behavioural
29 changes in animals and, thus, are the critical focus of numerous studies. Light pollution is an
30 increasingly recognised source of disturbance that has the potential to impact animal physiology
31 and behaviour. Here, we investigate the effect of constant light on a personality trait and
32 metabolic rate in the European hermit crab *Pagurus bernhardus*. We used Bayesian mixed
33 models to estimate average behavioural change (i.e. sample mean level behavioural plasticity)
34 and between- and within-individual variation in boldness in response to laboratory light. Hermit
35 crabs experiencing constant light were consistently less bold and had a higher metabolic rate than
36 those kept under a standard laboratory light regime (12:12h light/dark). However, there was no
37 effect of light on individual consistency in behaviour. As boldness is associated with coping with
38 risk, hermit crabs exposed to light pollution at night may experience increased perceived
39 predation risk, adjusting their behaviour to compensate for the increased conspicuousness.
40 However, reduced boldness could lead to lower rates of foraging and this, in combination with
41 elevated metabolic rate, has the potential for a reduction in energy balance.

42 **Keywords**

43 Light pollution, artificial light at night, animal personality, environmental change

44 Introduction

45 In natural environments, light regimes follow predictable and cyclic patterns of change. This
46 predictability favoured the evolution of mechanisms that allow animals to anticipate and react to
47 changes in light conditions (Burgess and Marshall, 2014; Lines et al., 2012; Tuomainen and
48 Candolin, 2011), such as adapting their behaviour and physiology according to the duration of
49 the day (Gaston et al., 2013; Hut and Beersma, 2011). For intertidal animals, light availability
50 and brightness carry additional information: changes in lunar brightness caused by the moon
51 cycle help animals to anticipate changes in current velocity and tidal height (Davies et al., 2014;
52 Naylor, 2010; Truscott et al., 2017). However, artificial light at night (ALAN) can mask seasonal
53 and monthly fluctuations in sky brightness regimes and thus interfere with these cues.

54 There is increasing evidence that ALAN can alter physiology (Forsburg et al., 2021;
55 Luarte et al., 2016; Raap et al., 2016a; Zubidat et al., 2018), metabolism (Finch et al., 2020;
56 Nelson, 2019; Raap et al., 2018a; Welbers et al., 2017), foraging (Davies et al., 2013; Farnworth
57 et al., 2018) reproduction and mating behaviour (Ayalon et al., 2021; Botha et al., 2017; Degen,
58 2015; Touzot et al., 2019), often in a species specific manner (Amadi et al., 2021; Baskir et al.,
59 2021; Brisbane and van den Burg, 2020; Polak et al., 2011), making understanding ALAN's
60 impacts challenging. However, changes in physiology and behaviour could alter interspecific
61 dynamics, having significant ecological consequences disrupting entire ecological communities
62 (Bennie et al., 2018; Sanders et al., 2018, 2015). In addition, ALAN was also shown to disrupt
63 metabolic rate, increasing organisms' energy requirement and food consumption, which can
64 further alter the stability of the food web (Hillyer et al., 2021; Manríquez et al., 2019; Marangoni
65 et al., 2022).

66 Consistent between-individual differences in behaviour, known as 'animal personality',
67 are present in a wide variety of taxa (Bergmüller, 2010; Briffa and Weiss, 2010; Carter et al.,
68 2013). Risk-taking behaviour, or 'boldness', is one of the most studied aspects of animal
69 personality as it has direct fitness consequences (Bevan et al., 2018; Oosten et al., 2010;
70 Westneat et al., 2013). For instance, consistent differences in boldness are linked with
71 differences in survival (Bubac et al., 2018; White et al., 2013), reproductive success (Gasparini
72 et al., 2019; Smith and Blumstein, 2008), predation pressure (Biro et al., 2006; Stamps, 2007),
73 parental care (Reddon, 2012), foraging (Sneddon, 2003) and even to different life-history traits,
74 such as growth (Finstad et al., 2007; Lantová et al., 2011). Thus, more active individuals also
75 tend to be more aggressive, bolder and more explorative compared to less active individuals
76 forming a pace-of-life syndrome (POLS; Careau et al., 2008; J. Galliard et al., 2013; Réale et al.,
77 2010). Individuals with a faster POLS (i.e. higher activity, boldness and aggressiveness) also
78 tend to have a higher energetic demand and consequently higher metabolic rates (Dammhahn et
79 al., 2018; J.-F. L. Galliard et al., 2013).

80 Despite being one of the best-studied personality traits, little is known about how risk-
81 taking behaviour (i.e. boldness) changes in response to environmental disturbances. This is
82 particularly important as environmental changes, such as ALAN, can alter multiple aspects of

83 predator-prey dynamics. Their impact also varies with the intensity of environmental
84 disturbance, organism physiology and personality (i.e. pace of life; Belgrad et al., 2017; Pamela
85 Delarue et al., 2015). In addition to affecting an individual's mean boldness (and hence that of
86 the population; Kurvers et al., 2018; O'Connor et al., 2019), night-time light may also alter
87 behavioural plasticity itself (i.e. the extent to which individuals adjust their behaviour to match a
88 change in circumstance) and consistency, further increasing the variability across individuals in
89 how they respond to this stressor (Raap et al., 2018b, 2015).

90 In addition to boldness, light may also alter behavioural plasticity (i.e. mean level
91 adjustments to a change in circumstance) and consistency, increasing the variability with
92 individuals' respond to this stressor. For example, light can hamper the ability to assess risk,
93 which they might counter by behaving in more risk-averse ways, for example, by increasing
94 their behavioural variability (i.e. within or intra-individual variation and residual variance; see
95 Stamps et al., 2012; Westneat et al., 2015). Alternatively, it could alter how different individuals
96 within a population respond to risk, changing not only the population mean level response but
97 also the among-individual variation around such a mean (i.e. between individual variation or
98 behavioural reaction norms see Dingemans et al., 2010). Alternatively, it could alter how
99 different individuals within a population respond to risk, changing a population level response
100 (i.e. between individual variation or behavioural reaction norms (Dingemans et al., 2010)).
101 These effects would alter the repeatability of behaviour, the key marker for animal personalities.
102 However, the way in which ALAN alters individual and population-level behavioural expression
103 is not well known.

104 In arthropods, oxygen is transported by haemocyanin, analogous to haemoglobin in
105 vertebrates (Mangum 1985). Changes in the concentration of this protein are directly correlated
106 to an organism's changes in energetic demands and, thus, can provide a robust estimation of
107 long-term response to stressors (Spicer and Baden, 2000). For instance, an increase in
108 haemocyanin concentration could be associated with adaptations to an increased energetic
109 demand caused by an environmental stressor. Therefore, patterns of oxygen consumption can be
110 used as a proxy for energetic demand and activity to determine environmental factors that shape
111 metabolism (Hillyer et al., 2021; Manríquez et al., 2019; Marangoni et al., 2022; Spicer and
112 Baden, 2000)

113 Here, we investigated how light at night can disrupt animal personality and its variance
114 components (among and within individual variation in behaviour). As a model system, we used
115 *Pagurus bernhardus*. *P. bernhardus* is a diurnal species (Mitchell, 1973) and one of Europe's
116 most common intertidal decapods (Benedict, 1901; Lancaster, 1990). Although they can be
117 found in a wide range of environments and depths, younger individuals primarily inhabit the
118 intertidal, where they are extremely common in north-western Europe (Benedict, 1901; Elwood,
119 n.d.; Lancaster, 1990). Therefore, *P. bernhardus* is likely to be the species more frequently
120 affected by light pollution, especially in terms of its youngest and largest cohorts.

121 We were particularly interested in how both within-individual variances in boldness and
122 population average boldness behaviour might be affected by exposure to consistent light at night.
123 Personality traits are often correlated with energy consumption and metabolism (i.e. fast-slow
124 life-history continuum; see Holtmann et al., 2017; Réale et al., 2010), a trait well known to be
125 disrupted by ALAN (O'Connor et al., 2019; Pulgar et al., 2019) metabolic rate. We focused on
126 two aspects of behavioural variation: between individuals (i.e. behavioural plasticity in response
127 to a change in conditions) and within-individuals (i.e. behavioural consistency). We used
128 haemocyanin concentration of hermit crabs and oxygen concentration as proxies for metabolism.
129 As ALAN's effects on behaviour and physiology seem to be dependent on the organism's
130 circadian rhythm (i.e. nocturnal vs diurnal; Dickerson et al., 2022; Le Tallec et al., 2013;
131 Spoelstra et al., 2018), it is possible that any effects also influence day-time behaviour.
132 Therefore, we investigated behavioural changes at two different time periods, day (i.e. during
133 daylight hours) and night (i.e. during night-time hours). We predicted that hermit crabs would be
134 aware of their conspicuousness (Briffa and Twyman, 2011) and adjust their behaviour, by
135 decreasing boldness, in response to light at night. Furthermore, as *P. bernhardus* is a diurnal
136 species (Mitchell, 1973), we expect a decrease in boldness during the night and an increase
137 during the day when exposed to ALAN. In addition, we also expect that the increased perceived
138 predation risk caused by ALAN would increase individual variation (i.e. decrease predictability)
139 while decreasing overall variation within a population (i.e. among individual variation). We also
140 predict that such a pattern would be conditioned to the time period, with day measurements being
141 less variable than nights.

142 **Methods**

143
144 We collected hermit crabs from Hannafore Point, UK., an area with low light pollution
145 levels and transported them to the lab. We removed crabs from their shells by cracking in a
146 bench vice in the laboratory. This stage is necessary because hermit crabs' behaviour, including
147 the startle response's duration, could be affected by the shell mass (Briffa and Bibost, 2009). All
148 crabs thus received a new *Littorina littorea* shell with 100% of its preferred mass, calculated
149 from a relationship between crab mass and preferred shell mass established during a previous
150 shell-selection experiment (Briffa and Elwood, 2007). We only used adult male crabs (mean
151 mass = 0.76 g ± S.E. = 0.34 g) as females may change their shell preferences in unpredictable
152 ways during the breeding season (Benedict, 1901; Lancaster, 1990). All hermit crabs were free
153 from apparent parasites (such as Rhizocephalans) that generally attach to the abdomen or are
154 present in the gill chambers, causing noticeable bulging of the carapace, appendage damage, or
155 recent moult. Crabs were individually housed in white plastic dishes of 16 cm in diameter, filled
156 to 4 cm depth with seawater, with continuous aeration, and fed daily ad libitum with cubes of
157 white fish at the end of night-time measurements (i.e. there was always excess food available in
158 the housing dishes, outside of the observation periods) and water was replaced daily.

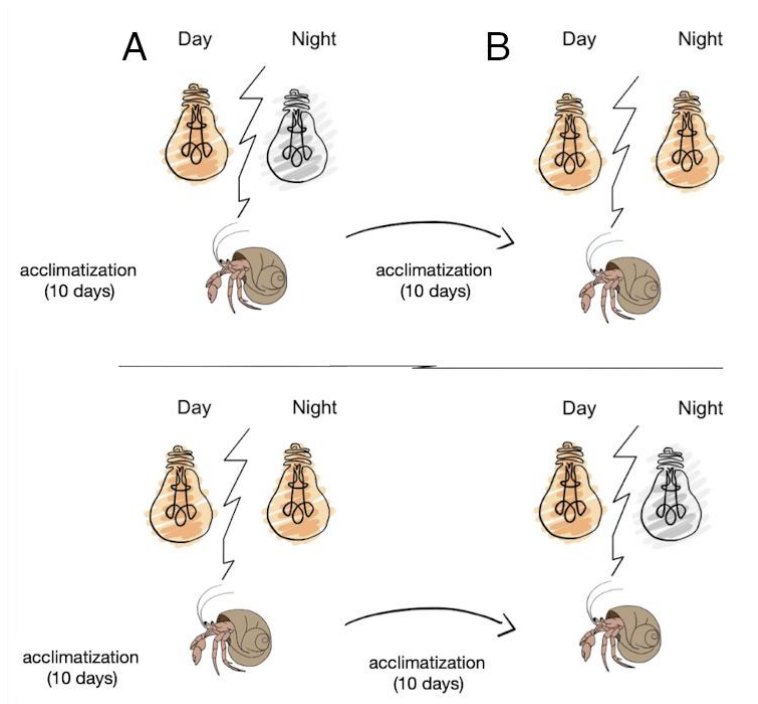
159 Before all observations, all animals were housed in separate chambers with controlled
160 light conditions to simulate either constant light or a 'standard light' regime. Standard light

161 treatment employed a 12:12h (7 am:7 pm) light: dark treatment, whereas constant light used the
162 same intensity light for 24 hours. Those animals were left in either condition for a 10-day
163 acclimation period, followed by ten days of observations (twice a day as described below). The
164 startle response was measured on the 11th day following the acclimation, and on the 21st day, we
165 reversed light conditions experienced by animals. Thus, all animals initially undergoing standard
166 light treatment were transferred to constant light treatment and vice versa. We restarted
167 observations after a 10-day acclimation period, followed by ten days of observations (Briffa and
168 Bibost, 2009). We applied a crossover design (Briffa et al., 2013), as it is the standard approach
169 in behavioural experiments involving repeated measures, where individuals are subject to more
170 than one treatment.

171 Hermit crabs were kept under fluorescent lights (fluorescent tubes, white colour) with
172 similar light conditions during the day (unpaired t-test $t_{24} = 0.11$, $p = 0.92$) and were on average
173 38.554 lux (range = 30.1- 44.6, $n_{\text{locations}} = 25$). In Hannafore Point, street lighting used high-
174 pressure sodium lamps and varied across the shore, averaging 0.127 lux (range = 0 - 1.4, $n_{\text{locations}}$
175 = 25). Although light conditions at the laboratory were higher and less variable when compared
176 to Hannafore Point, the light intensity of 30-40 lux can also be considered conservative relative
177 to developed coastal areas with high human influence (60-210 lux; Luarte et al., 2016; Pulgar et
178 al., 2019).

179 *Behavioural assays*

180 We used a startle response as a proxy for boldness. We induced startle response (standard light,
181 $N = 15$; constant light, $N = 15$, $N_{\text{total}} = 30$) using a handling protocol, where crabs were lifted
182 out of their tank and replaced in an inverted position on the tank's base. This causes them to
183 withdraw into their gastropod shell. We timed the recovery latency from the point at which the
184 crab was replaced in the tank to when the walking legs were re-contacted with the tank's base
185 (Briffa et al., 2008). Although many marine animals are assumed to be more active at night, *P.*
186 *bernhardus* under standard light conditions (12:12h light: dark) is more active during the day
187 than at night (peak of activity at 9:00 and lower activity at 22:30h(Mitchell, 1973). Therefore, we
188 induced startle responses twice daily at 9:00 (day-time observations) and 21:00 (night-time
189 observations) for 10 days in each experimental period. We made night-time observations under
190 the 12:12h light: dark cycle with low levels of red light to avoid influencing crab behaviour
191 (Hazlett, 1966; Sinn and Moltschaniwskyj, 2005).



193

194

195

196

197

198

199

200

201

202

203

204

205

206

207

208

209

210

211

212

213

Figure 1: Schematic representation of the experiment's timeline, showing the experimental groups (constant and standard light) and the experimental crossover design. Incandescent light bulbs are used for illustrative purposes only, experiment was conducted using fluorescent light bulbs.

Metabolic rate measurements

To investigate whether metabolic rate varies in response to the light conditions, we measured the routine metabolic rate (routine MR) of 10 individuals in each group (constant light-A, N= 5; constant light-B, N= 5, standard light-A, N= 5; standard light-B, N= 5) exposed to the same conditions as described above. Routine MR was measured with a different cohort as startle response duration. Routine MR refers to an animal's average rate of metabolism and is measured when the animal is undergoing normal behaviours or some other specified type of activity (Dupont-Prinet et al., 2010; Speakman, 2013)

Animals used to estimate the routine MR were submitted to similar light conditions to those used in the behavioural assay. Thus, we measured each hermit crab's metabolic rate in two light regimes, constant and standard light. To minimise measurement errors, we measured routine MR with a different cohort of animals in the same room (i.e. same experimental conditions) where the animal was maintained and in parallel with the startle response duration. To ensure that changes in oxygen consumption were mediated by changes in light regime (i.e. standard or constant light treatment) and not a temporal component (i.e. duration of the

214 experiment, time in the laboratory), we performed MR measurements on alternating days on
215 each condition.

216 We measured routine MR throughout 24 hours using the oxygen uptake as a proxy in a
217 closed chamber respirometer. Due to experimental limitations, we only measured one hermit
218 crab at a time (20 measurements in total). We used an oxygen-sensitive sensor spot (PreSens
219 Precision Sensing GmbH, Regensburg, Germany) attached to the inner wall of the chamber with
220 a silicone rubber compound, as specified by the manufacturer. This approach allowed non-
221 invasive and more precise measures by preventing gas exchange during the readings.

222 Measures conducted in closed chambers are never constant due to the continuous oxygen
223 consumption by the animal. We used the difference in oxygen concentration over time to
224 estimate the oxygen consumption inside the chamber, which can be read by the sensor spot and
225 recorded by a Fibox 4 trace machine (PreSens Precision Sensing GmbH, Regensburg, Germany)
226 attached to a temperature sensor (Pt100, Bioengineering AG, Wald, Switzerland). To prevent
227 oxygen stratification and ensure enough water mixing, we placed the chamber onto a multi-
228 channel magnetic stirrer (MIX 15 eco; 2mag A.G., Munich, Germany) with a magnetic flea
229 inside. We placed a mesh between the hermit crab and the magnetic flea to prevent contact
230 between them.

231 We sealed the chambers underwater using filtered seawater to prevent air bubbles and
232 minimise bacterial and algal activity. As a preventive measure against algal or bacterial activity,
233 we also measured the oxygen consumption in three extra chambers ('blanks') containing a single
234 *L. littorea* shell of a similar size as used by the crab. Differences in oxygen concentration in the
235 blanks indicated microbial activity and were accounted for during routine MR estimation of *P.*
236 *bernhardus*. We obtained the O₂ consumption rate using the slope of a linear regression of the
237 oxygen consumption over time minus the blank O₂ consumption rate (Calosi et al., 2013). Then,
238 we multiplied the slope by the oxygen solubility coefficient and adjusted for salinity and
239 temperature. Although we conducted the metabolic rate measurements in a temperature-
240 controlled room, there were minimal fluctuations in temperature (0.2 - 1°C), which can affect
241 oxygen solubility values (Calosi et al., 2013; Widdows et al., 2002). We accounted for such
242 minor changes in temperature in estimating the oxygen solubility coefficient (as described
243 above). We calculate the rate of O₂ consumption using:

$$\text{Rate of } O_2 \text{ uptake } (\mu\text{moles } O_2 \text{h}^{-1}) = C(t) \times (V_r) \times \left(\frac{60}{t_1 - t_2} \right)$$

244
245 Where C(t) is the O₂ consumption rate (from the linear regression of oxygen consumption over
246 time), V_r is the total volume of water inside the jar (jar volume minus the hermit crab volume),
247 and t₁- t₂, is the measurement period (in minutes; (Calosi et al., 2013; Widdows et al., 2002). To
248 estimate the metabolic rate and create a standardised measure, allowing comparisons between
249 individuals, we divided the rate of O₂ uptake by individual body mass (Porter and Brand, 1995).
250 We allowed hermit crabs to rest for 30 minutes before starting routine MR measures of oxygen
251 consumption. We kept the same individual during the 24 hours' measurement to prevent stress

252 and possible disturbances in the animal. Although the metabolic rate was estimated during 24h
253 continuous measurement, it was later divided into two experimental blocks, corresponding to day
254 and night-time measures (12 - 12 h). Oxygen levels were carefully monitored throughout the
255 experiment, and if oxygen concentration dropped below a threshold, the investigation would be
256 interrupted and restarted with a different hermit crab. However, this step was not necessary.

257

258 *Haemocyanin concentration*

259 After completing the behavioural observations, we extracted a haemolymph sample from hermit
260 crabs from both metabolic rate measurement and startle response duration (standard light; N = 20
261 and constant light; N = 20), following the protocol described by Bridger et al. (Bridger et al.,
262 2015) by inserting an insulin syringe into the infra-branchial sinus. Then, we transferred 10µl of
263 the haemocyanin recently sampled into a semi-micro cuvette containing 690 µl of double-
264 distilled water. After mixing, we measured the haemocyanin absorbance at 337 nm in a
265 spectrophotometer. We used the Nickerson & Van Holder (1971) extinction coefficient to
266 determine the haemocyanin concentration. After the haemolymph collection, we euthanised all
267 individuals by placing them into a saturated magnesium chloride solution.

268

269 *Data analysis*

270 We used three analyses to investigate the effect of constant light on the startle response. In the
271 first analysis, we quantified the impact of light and dark conditions on the duration of the startle
272 response using a hierarchical generalised linear model (HGLM) implemented within a Bayesian
273 framework (MCMC Bayesian approach implemented in the R package MCMCglmm; Hadfield,
274 2010). We then used a second HGLM to estimate treatment group and time-specific
275 repeatabilities (these could not be obtained from the primary model used to test for mean level
276 effects; see details below). In the third analysis, we determined the impact of constant light on
277 metabolic rate using repeated-measures ANOVA. Analysis, results and raw data can be viewed
278 at <https://github.com/marivelasque/Light-pollution>.

279

280 *Personality and behavioural variation*

281 In the first analysis, we fitted a model allowing a random intercept for each individual. This
282 enables the estimation of between-individual variation in startle responses (V_{BI} : between-
283 individual variance) and random slopes across the repeated observations (V_{WI} : within-individual
284 or residual variance, providing a measure of consistency) and the estimation of individual
285 variation across all observations. To account for the time the animal was in the laboratory, we
286 added the experimental period as a random effect. We assumed the residual variance to be
287 normally distributed and uncorrelated across all observations. We used the startle response
288 duration (\log_{10} transformed to improve normality) as the response variable and time at which
289 startle response duration was measured (day or night), treatment (constant or standard light),

290 occasion (day 1-20) on which the behaviour was observed, the hermit crab mass, the
291 haemocyanin concentration as fixed effects. Because startle response duration is likely to vary
292 according to the time of the day on which it was measured and the experimental treatment. We
293 also included the interactions between treatment * time as fixed effects.

294 To compare repeatability (and V_{BI} and V_{WI} components) across treatment groups, we
295 modelled another HGLM with a similar structure for fixed effects as the model described above.
296 To achieve this, we constructed a model using block-specific (i.e. treatment groups) random
297 intercepts for individuals, on which the within-group covariance was constrained to 0 and the
298 within-group (diagonals) were allowed to vary (i.e. measures within each light treatment and
299 time of the day were allowed to vary), creating a block-specific G-structure that corresponds to
300 VBI. Similar to the previous model, we also compared effect size, autocorrelation between
301 estimated samples and DIC of this model against five priors (see Supplemental Material S1) to
302 choose the best-unbiased model based on the overall results. We report the results obtained using
303 'prior 2' as it achieved convergence and had lower autocorrelation between samples (see
304 Supplemental Material S1). All models had similar DIC values. We reported the posterior mode
305 for fixed effects and 95% credible intervals (C.I.s). Additionally, we modelled separate residual
306 variances for each experimental block (R-structure, corresponding to V_{WI}).

307 We estimated the posterior modes for repeatability in each experimental block (with 95%
308 C.I.s). We also determined whether the repeatability estimates showed significant differences
309 among the experimental blocks by calculating the posterior modal differences between blocks
310 (ΔR ; see Supplemental Material S1) and the 95% CI values of these differences (Osborn and
311 Briffa, 2017; Royauté et al., 2015; White and Briffa, 2017). We estimated the difference in
312 repeatability, ΔR , between treatments within each time of day ($R_{\text{constant light}} - R_{\text{standard light}}$) and
313 between each time of day within groups ($R_{\text{constant light during day}} - R_{\text{constant light during night}}$; $R_{\text{standard light}}$
314 $\text{during day} - R_{\text{constant light during night}}$). We made similar calculations to assess the changes in the specific
315 variance components of repeatability (ΔV_{BI} and ΔV_{WI}) between treatments and times of the day.

316 In all Bayesian modules, we compared the effect size, autocorrelation between estimated
317 samples and Deviance Information Criterion (DIC) with four different priors to choose the best-
318 unbiased model based on the overall results (see Supplemental Material S1). We report the
319 results obtained using the model containing the lower autocorrelation between samples (see
320 Supplemental Material S1). All models had similar DIC values. As standard when implementing
321 Bayesian framework, significance occurs when 95% C.I.s of their posterior modes did not
322 overlap zero. We specified a Markov Chain Monte Carlo (MCMC) for both models with 5×10^6
323 interactions, a thinning interval of 100 and a burn-in of 50000. We fitted all models using
324 MCMC methods (implemented with MCMCglmm in R version 3.6.0).

325

326 *The effect of constant light on metabolic rate and haemocyanin concentration*

327 In the third analysis, we determined the effect of light on metabolic rate using a Linear Mixed-
328 Effects Model. As the metabolic data was not normally distributed, we applied Log10 +1

329 transformation before analysis. We used metabolic rate as the dependent variable and added it as
330 a fixed effect containing the time of the day and an interaction between the treatment group and
331 experiment period (i.e. crossover). To account for the crossover effect, we built a model
332 containing a random intercept (i.e. individuals) and random slopes (i.e. experimental period),
333 indicating that individual slopes vary across different experimental periods). This analysis was
334 performed using the lmer function from the lme4 package on R. As haemocyanin was only
335 measured once at the end of the experiment, we also tested the effect of treatment on its
336 concentration using Welch's t-test (Lu and Yuan, 2010).

337 **Results**

338 *Personality and behavioural variation*

339 The parameter estimates for both random and fixed effects of the HGML model and their 95%
340 credible intervals (and estimated p values) are given in Table 1. The fixed effects components of
341 the HGLM model provide strong evidence that the startle response duration changed according
342 to the treatment ($p < 0.001$), being longer in animals experiencing the constant light treatment
343 (Figure 2). We also found strong support that startles response duration did not vary between day
344 and night measures ($p = 0.87$) nor with the hermit crab mass ($p = 0.065$).

345 Although we did not find a significant change in startle response behaviour between day and
346 night-time measures, we found strong evidence that boldness changed with the interaction
347 between treatment and time ($p < 0.001$; Figure 2; Table 1). Our results suggest that the
348 difference in startle response duration between day and night was more marked for the constant
349 light than standard light treatment ($p < 0.001$; Figure 2; Table 1). In addition, we found strong
350 evidence that the decrease in boldness was greater in hermit crabs with higher haemocyanin
351 concentration ($p < 0.01$; Figure 2; Table 1). Startle response duration also decreased throughout
352 the experiment ($p = 0.036$; Table 1). We estimated the repeatability from the second HGLM (see
353 Supplemental Material S1). The repeatability estimates (Table 2) provide strong evidence that
354 the startle response duration was repeatable in all treatment blocks. There was no significant
355 difference in repeatability between treatment groups within periods. The HGLM model also
356 indicates an increased amount of variation in response to changes in light conditions, both
357 between (V_{BI^-}) and within-individuals (V_{Wi} ; Table 3). It also indicates that such response was
358 consistent across individuals (V_{BI}) in different experimental groups and times of the day.
359 Although such behavioural consistency was considerable throughout the experimental group,
360 individuals on the standard light treatment had a lower behavioural consistency during the day.

361

362 *The effect of constant light on metabolic rate and haemocyanin concentration*

363 In addition to behavioural changes, hermit crabs exposed to constant light also had significantly
364 higher MR than those in standard light conditions. ($p = 0.001$, Figure 2; Table 4). We also found
365 the metabolic rate to vary with the period, being higher during the second part of the experiment
366 ($p = 0.043$; Table 4). In addition, we also found that such crossover effect was significantly

367 higher when animals were exposed to constant light in the second experimental period instead of
368 the first (Treatment x Period - $p = 0.008$; Table 4). We did not find any changes in metabolic rate
369 between day and night-time measures ($p = 0.509$; Table 4). We also found that constant light
370 significantly increases haemocyanin concentration in hermit crabs ($t_{17,401} = -3.173$, $p = 0.005$).

371 Table 1: Posterior summary statistics for the mean effect of startle response, showing posterior
 372 mean, lower and upper 95% C.I.s and P-values (for fixed effect only). Significant values are
 373 shown in bold.

Parameter name	Posterior mean	95% CI lower	95% CI Upper	<i>p</i>
Fixed effects				
Intercept	-29.262	-3.3272	7.1733	0.12
Time	0.0134	-0.1647	0.2084	0.87
Treatment	0.573	0.3925	0.7685	< 0.001
Occasion	0.0248	0.0023	0.048	0.036
Mass	-0.2503	-0.5195	-3e-04	0.065
Haemocyanin concentration	0.0073	0.0041	0.0109	< 0.001
Treatment x Time	-0.7164	-0.9748	-0.4443	< 0.001
Random intercepts (between-individual variation, G-structure and R-structure)				
Hermit Crab ID (intercept)	9e-04	4e-04	0.0013	-
Observational period	1534349.5381	4e-04	95.2922	-
Error	1.1287	0.9702	1.2785	-

375 Table 2: Posterior modes, upper and lower 95% C.I.s (in brackets) for MCMC repeatability
 376 estimates between treatment groups within periods and repeatability of differences (Δ) between
 377 treatments ($\Delta_{\text{constant light - standard light}}$) and between the time on which the startle response was
 378 induced ($\Delta_{\text{Night-Day}}$). Significant values are shown in bold.

	Day	Night	$\Delta_{\text{Night-Day}}$
constant light	0.34 [0.248, 0.535]	0.23 [0.127, 0.374]	0.15 [-0.043, 0.347]
standard light	0.49 [0.358, 0.65]	0.35 [0.22, 0.503]	0.14 [-0.078, 0.332]
$\Delta_{\text{constant - standard light}}$	-0.12 [-0.313, 0.109]	-0.11 [-0.311, 0.073]	-

379

380 Table 3: Posterior modes, upper and lower 95% C.I.s (in brackets) for (a) between and (b)
 381 within-individual variation in startle response duration between treatment groups within periods
 382 and the difference (Δ) in variation between treatments ($\Delta_{\text{constant light - standard light}}$) and between the
 383 time on which the startle response was induced ($\Delta_{\text{Night-Day}}$). Significant values are shown in bold

(a) *Between-individual variation, V_{BI}*

	Day	Night	$\Delta_{\text{Night-Day}}$
constant light	0.55 [0.278, 0.977]	0.30 [0.139, 0.541]	0.26 [-0.131, 0.745]
standard light	0.71 [0.377, 1.249]	0.56 [0.288, 0.993]	0.22 [-0.461, 0.785]
$\Delta_{\text{constant light - standard light}}$ in VBI	-0.04 [-0.797, 0.416]	-0.23 [-0.733, 0.167]	-

(b) *Within-individual variation, V_{WI}*

	Day	Night	$\Delta_{\text{B-A}}$
constant light	0.94 [0.77, 1.05]	0.97 [0.79, 1.11]	0.06 [-0.18, 0.24]
standard light	0.73 [0.63, 0.89]	1.03 [0.85, 1.21]	0.29 [0.04, 0.48]
$\Delta_{\text{constant light - standard light}}$	-0.13 [-0.81, 0.51]	-0.11 [-0.31, 0.16]	-

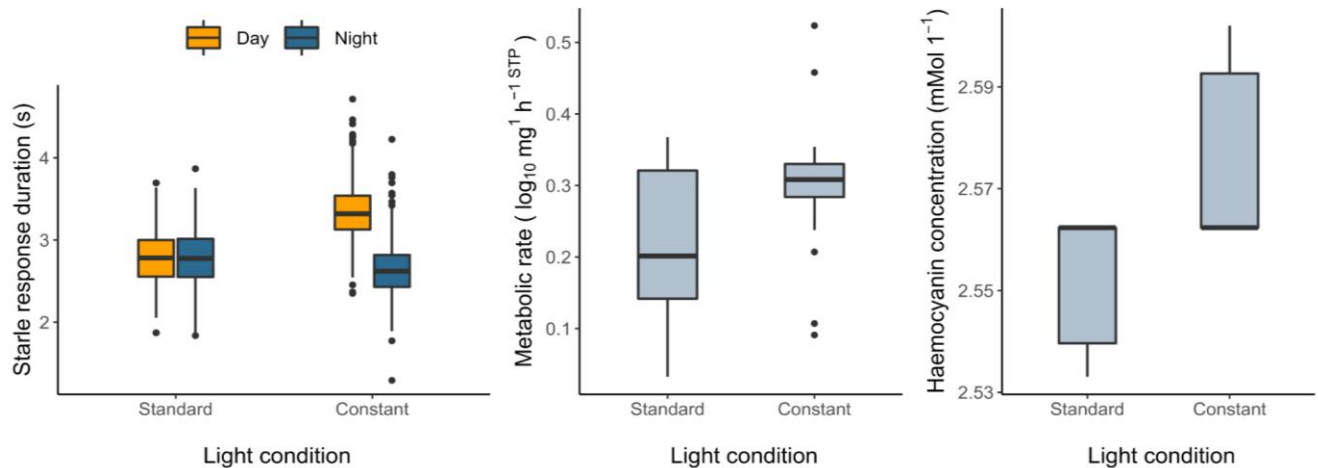
384

385

386 Table 4: Fixed-effects estimates for the Linear Mixed-Effects Models for the effect of time (day
 387 or night-time), experimental period (crossover A or B) and treatment group (constant light or
 388 standard light) on metabolic rate of hermit crabs. Significant values are shown in bold.

	Estimate	Std. Error	df	t-value	Pr(> z)
Intercept	0.477	0.166	19.559	2.87	0.01
Treatment	0.977	0.235	19.559	4.159	0.001
Period	0.52	0.245	26.801	2.122	0.043
Time	-0.142	0.212	23.999	-0.67	0.509
Treatment x Period	-1.177	0.386	15.399	-3.047	0.008
Treatment x Time	-0.395	0.299	23.999	-1.319	0.2
Period x Time	0.103	0.299	23.999	0.344	0.734
Treatment x Period x Time	0.627	0.423	23.999	1.482	0.151

389 Reference values: Treatment – constant light; Period – B; Time - night.
 390



391 Figure 2. Night-time light and time effects on the startle response duration, metabolic rate, and
 392 haemocyanin concentration of the hermit crab *Pagurus bernhardus*. A) Startle response duration
 393 was predicted using the best unbiased HGLM model (see materials and methods). B) the
 394 metabolic rate (MO₂). *Pagurus bernhardus*. Metabolic rate is expressed as log₁₀ nmol O₂ mg⁻¹
 395 h⁻¹ STP (STP - standard temperature and pressure) and haemocyanin concentration as mMol l⁻¹.
 396

397 **Discussion & Conclusions**

398 We investigated the effect of constant light on personality traits and metabolic rate in the hermit
399 crab *Pagurus bernhardus*. We show that night-time light influences mean level startle response
400 durations and repeatability estimates. Although startle responses observed during the night were
401 unaffected, those individuals kept under constant light exhibited reduced boldness during day-
402 time hours (with longer startle response duration) than individuals experiencing standard light
403 conditions. Our data also indicates that animals kept under constant light conditions showed a
404 higher metabolic rate than those under standard light conditions. The increased haemocyanin
405 concentration in constant light treated animals indicates an increased oxygen demand resulting
406 from an increased metabolic rate.

407 Night-time light increases animal visibility facilitating predator detection, and thus,
408 increases perceived predation risk (Prugh and Golden, 2014; Troscianko et al., 2009). Previous
409 studies in *P. bernhardus* have shown that hermit crabs can be aware of the shell conspicuousness
410 and predation risk associated with the shell and in turn displayed a modified startle response
411 duration (Briffa and Twyman, 2011). However, we found no overall significant difference in
412 startle response duration between day and night measures. Nevertheless, this effect depends on
413 the treatment. Hermit crabs experiencing constant light had shorter startle response duration at
414 night-time than individuals under standard light regimes. This suggests that constant light
415 treatment may increase hermit crab conspicuousness and, consequently, the perceived risk
416 (Gaston and Spicer, 2013). However, *P. bernhardus* is less active at night (Mitchell, 1973), such
417 perceived risk may be transferred, causing more severe behavioural changes when hermit crabs
418 are more active (i.e. day). Perhaps long-term adaptation may result in increased activity during
419 the daytime, but this was not observed in this work. It is also possible that constant light disrupts
420 resting and sleep behaviour (Alaasam et al., 2021; Jiang et al., 2020; Raap et al., 2016b) in
421 hermit crabs, increasing stress and modifying activity patterns and response to risk.

422 Startle response duration is a complex behaviour widely used as a proxy for defensive
423 response in several animals. In hermit crabs, gas exchange occurs in modified gills located in the
424 brachial chamber (Gerlach et al., 1976; Lancaster, 1990). When they withdraw inside their shells,
425 such gas exchange is interrupted, temporarily limiting oxygen availability. Hence, the increase in
426 startle response duration will cause a reduction in oxygen availability, creating a trade-off (Briffa
427 and Bibost, 2009). Traits with a substantial trade-off tend to vary both in populations and
428 throughout an organism's lifetime (i.e. shorter to long). Traits with inter- and intra-individual
429 variation are an important coping strategy, as they reduce the ability of predators to adapt to prey
430 behaviour (Briffa, 2013; Briffa et al., 2013). Although we found that hermit crabs adapt their
431 average day-time behaviour in response to constant light (i.e. decreasing boldness), there were no
432 differences in predictability (i.e. V_{WI}) between light treatments. Constant light did, however, lead
433 to an increase in energy consumption (when compared with standard light regimes). Therefore, it
434 is possible that under long-term constant light, hermit crabs need to increase their food
435 consumption to support their high energetic demand (Lima, 1998; Lima and Dill, 1990) which

436 increases foraging and, therefore, the predation risk (Lima, 1998; Lima and Dill, 1990). Animals
437 in captivity tend to have a more homogeneous behaviour due to reduced environmental
438 heterogeneity (Bell et al., 2009; Dammhahn and Almeling, 2012; Desy et al., 1990). However,
439 captive animals still have some activity pattern with higher and lower activity phases, usually
440 reinforced by light (e.g. circadian rhythms: (Palmer, 1973). Thus, under constant light
441 conditions, such patterns are expected to be minimised (Ringelberg, 2007; Wyse et al., 2011),
442 producing a more homogeneous response (e.g. lower variance or higher repeatability). However,
443 we found no evidence for this, with constant light exposed individuals similar to those under the
444 standard light and dark regime. The repeatability within groups (i.e., consistent behavioural
445 differences among individuals the day versus night) was also similar across treatment groups.

446 Individuals in both treatment groups and at both periods exhibit significant between- and
447 within-individual variance in boldness (V_{BI} and V_{WI} , respectively). There was also no significant
448 variation in V_{BI} between treatment groups and time. However, under standard light and dark
449 regime treatment, there was a substantial difference in the amount of within-individual variation
450 (V_{WI}) between day and night; crabs had more within-individual variation in behaviour (less
451 consistent) at night than during the day (Table 3). One possible explanation is that *P. bernhardus*
452 is a diurnal species, maintaining its activity pattern even under constant light (Mitchell, 1973).
453 Therefore, it is possible that in the absence of constant light exposure, other rhythms than
454 circadian (tidal or lunar) are more pronounced, resulting in a higher variation in behaviour
455 within-individuals. Alternatively, hermit crabs may be subjected to different pressures
456 throughout a 24-hour period. For instance, the predation risk increases during the night-time and
457 thus, decreasing predictability (increases V_{WI}) may increase survival in natural conditions.
458 Therefore low predictability (i.e. low behavioural consistency or high V_{WI}), alongside low
459 boldness, is potentially a strategy to cope with risk, as less predictable individuals might reduce
460 the chance of being captured (Briffa, 2013; Briffa et al., 2013).

461 How organisms respond to ALAN is not homogeneous and varies across species, seasons and
462 even daily activity patterns. For instance, some animals showed a significant decrease in energy
463 expenditure (Duarte et al., 2019; Manríquez et al., 2019; Pulgar et al., 2019; Touzot et al., 2019).
464 The usage of the crossover design allowed us to test the effect of the treatment while avoiding
465 confounding factors, such as time and habituation (Briffa, 2013; Briffa et al., 2008; Briffa and
466 Bibost, 2009; Briffa and Twyman, 2011; Mowles et al., 2012).

467 Light pollution is a modern, globally widespread (Cinzano et al., 2001) and fast-
468 expanding (Hölker et al., 2010) issue. Nevertheless, the effect on marine life is not well
469 documented, especially for invertebrates. To our knowledge, no prior studies have explored the
470 effects of constant light as a potential driver (or disruptor) of variation in repeatable personality
471 traits. Previous work examining ALAN induced personality changes in guppies did not appear to
472 influence variation (Kurvers et al., 2018). Therefore, our study shows how light pollution may
473 affect *P. bernhardus* physiologically, increasing metabolic rate and behaviourally by reducing
474 within-individual variation in behaviour and decreasing boldness overall. Further experiments are
475 required to explore the potential downstream effects of these light pollution-induced changes.

476 For instance, it has been shown that hermit crabs exposed to predator chemical cues (e.g. effluent
477 form containers with a predator) or visual (e.g. predator model) cues tend to adjust their
478 behaviour (Briffa, 2013; Briffa et al., 2008) and could be used to simulate risk under varying
479 light treatments. Our results (increased metabolic rate and decreased day-time boldness), indicate
480 that artificial light at night can cause systemic changes to hermit crab's physiology and risk
481 coping mechanisms. Furthermore, such changes in response to light conditions could interact
482 potentially in a non-linear way. If elevated metabolism requires greater foraging, under elevated
483 predation risk, then increased foraging behaviour could place crabs under even greater risk. On
484 the other hand, if crabs try to avoid elevated predation risk by foraging less, their energy balance
485 will be negatively impacted under conditions of increased metabolic rate. We did not measure
486 foraging in this experiment, but since boldness is linked to the exploration in hermit crabs
487 (Mowles and Briffa, 2012), the second of these two scenarios seem the most likely. Such
488 changes could influence individual fitness and population stability in affected areas.

489 **Acknowledgements**

490 The work leading to this publication was part of a PhD scholarship awarded to MV funded by
491 Coordination for the Improvement of Higher Education- Brazil (CAPES), Science Without
492 Borders Program, CsF. We thank Marie Palmer, Richard Ticehurst and Ann Torr for technical
493 assistance during fieldwork and animal husbandry.

494 **Declaration of interests**

495 The authors declare that they have no known competing financial interests or personal
496 relationships that could have appeared to influence the work reported in this paper.

497

498 **References**

- 499 Alaasam, V.J., Liu, X., Niu, Y., Habibian, J.S., Pieraut, S., Ferguson, B.S., Zhang, Y., Ouyang,
500 J.Q., 2021. Effects of dim artificial light at night on locomotor activity, cardiovascular
501 physiology, and circadian clock genes in a diurnal songbird. *Environ. Pollut.* 282,
502 117036. <https://doi.org/10.1016/j.envpol.2021.117036>
- 503 Amadi, N., Luiselli, L., Belema, R., Nyiwale, G.A., Wala, C., Urubia, N., Meek, R., 2021. From
504 diurnal to nocturnal activity: a case study of night-light niche expansion in *Agama agama*
505 lizards. *Ethology Ecology & Evolution*. <https://doi.org/10.1080/03949370.2021.1883120>
- 506 Ayalon, I., Rosenberg, Y., Benichou, J.I.C., Campos, C.L.D., Sayco, S.L.G., Nada, M.A.L.,
507 Baquiran, J.I.P., Ligson, C.A., Avisar, D., Conaco, C., Kuechly, H.U., Kyba, C.C.M.,
508 Cabaitan, P.C., Levy, O., 2021. Coral Gametogenesis Collapse under Artificial Light
509 Pollution. *Curr. Biol.* 31, 413-419.e3. <https://doi.org/10.1016/j.cub.2020.10.039>
- 510 Baskir, E.A., Hazen, R.E., Powell, D.M., 2021. Documenting nocturnal activity of dragon-
511 headed katydids (*Lesina blanchardi*) under artificial light. *Zoo Biology*.
512 <https://doi.org/10.1002/zoo.21608>
- 513 Belgrad, B.A., Karan, J., Griffen, B.D., 2017. Individual personality associated with interactions
514 between physiological condition and the environment. *Anim. Behav.* 123, 277–284.

515 <https://doi.org/10.1016/j.anbehav.2016.11.008>
516 Bell, A.M., Hankison, S.J., Laskowski, K.L., 2009. The repeatability of behaviour: a meta-
517 analysis. *Animal Behaviour*. <https://doi.org/10.1016/j.anbehav.2008.12.022>
518 Benedict, J.E., 1901. The hermit crabs of the *Pagurus bernhardus* type. *Proceedings of the United*
519 *States National Museum*. <https://doi.org/10.5479/si.00963801.23-1216.451>
520 Bennie, J., Davies, T.W., Cruse, D., Inger, R., Gaston, K.J., 2018. Artificial light at night causes
521 top-down and bottom-up trophic effects on invertebrate populations. *J. Appl. Ecol.* 55,
522 2698–2706. <https://doi.org/10.1111/1365-2664.13240>
523 Bergmüller, R., 2010. Animal personality and behavioural syndromes, in: *Animal Behaviour:*
524 *Evolution and Mechanisms*. pp. 587–621. https://doi.org/10.1007/978-3-642-02624-9_19
525 Bevan, P.A., Gosetto, I., Jenkins, E.R., Barnes, I., Ioannou, C.C., 2018. Regulation between
526 personality traits: individual social tendencies modulate whether boldness and leadership
527 are correlated. *Proc. Biol. Sci.* 285. <https://doi.org/10.1098/rspb.2018.0829>
528 Biro, P.A., Abrahams, M.V., Post, J.R., Parkinson, E.A., 2006. Behavioural trade-offs between
529 growth and mortality explain evolution of submaximal growth rates. *J. Anim. Ecol.* 75,
530 1165–1171. <https://doi.org/10.1111/j.1365-2656.2006.01137.x>
531 Botha, L.M., Michael Botha, L., Jones, T.M., Hopkins, G.R., 2017. Effects of lifetime exposure
532 to artificial light at night on cricket (*Teleogryllus commodus*) courtship and mating
533 behaviour. *Animal Behaviour*. <https://doi.org/10.1016/j.anbehav.2017.05.020>
534 Bridger, D., Bonner, S.J., Briffa, M., 2015. Individual quality and personality: bolder males are
535 less fecund in the hermit crab *Pagurus bernhardus*. *Proc. Biol. Sci.* 282, 20142492.
536 <https://doi.org/10.1098/rspb.2014.2492>
537 Briffa, M., 2013. Plastic proteans: reduced predictability in the face of predation risk in hermit
538 crabs. *Biol. Lett.* 9, 20130592. <https://doi.org/10.1098/rsbl.2013.0592>
539 Briffa, M., Bibost, A.-L., 2009. Effects of shell size on behavioural consistency and flexibility in
540 hermit crabs. *Can. J. Zool.* 87, 597–603. <https://doi.org/10.1139/Z09-047>
541 Briffa, M., Bridger, D., Biro, P.A., 2013. How does temperature affect behaviour? Multilevel
542 analysis of plasticity, personality and predictability in hermit crabs. *Anim. Behav.* 86,
543 47–54. <https://doi.org/10.1016/j.anbehav.2013.04.009>
544 Briffa, M., Elwood, R.W., 2007. Monoamines and decision making during contests in the hermit
545 crab *Pagurus bernhardus*. *Anim. Behav.* 73, 605–612.
546 Briffa, M., Rundle, S.D., Fryer, A., 2008. Comparing the strength of behavioural plasticity and
547 consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*.
548 *Proc. Biol. Sci.* 275, 1305–1311. <https://doi.org/10.1098/rspb.2008.0025>
549 Briffa, M., Twyman, C., 2011. Do I stand out or blend in? Conspicuousness awareness and
550 consistent behavioural differences in hermit crabs. *Biol. Lett.* 7, 330–332.
551 <https://doi.org/10.1098/rsbl.2010.0761>
552 Briffa, M., Weiss, A., 2010. Animal personality. *Current Biology*.
553 <https://doi.org/10.1016/j.cub.2010.09.019>
554 Brisbane, J.L.K., van den Burg, M.P., 2020. No need for artificial light: nocturnal activity by a
555 diurnal reptile under lunar light. *Neotropical Biodiversity*.
556 <https://doi.org/10.1080/23766808.2020.1844993>
557 Bubac, C.M., Coltman, D.W., Don Bowen, W., Lidgard, D.C., Lang, S.L.C., den Heyer, C.E.,
558 2018. Repeatability and reproductive consequences of boldness in female gray seals.
559 *Behav. Ecol. Sociobiol.* 72, 527. <https://doi.org/10.1007/s00265-018-2515-5>
560 Burgess, S.C., Marshall, D.J., 2014. Adaptive parental effects: the importance of estimating

561 environmental predictability and offspring fitness appropriately. *Oikos*.
562 <https://doi.org/10.1111/oik.01235>

563 Calosi, P., Turner, L.M., Hawkins, M., Bertolini, C., Nightingale, G., Truebano, M., Spicer, J.I.,
564 2013. Multiple physiological responses to multiple environmental challenges: an
565 individual approach. *Integr. Comp. Biol.* 53, 660–670. <https://doi.org/10.1093/icb/ict041>

566 Careau, V., Thomas, D., Humphries, M.M., Réale, D., 2008. Energy metabolism and animal
567 personality. *Oikos*. <https://doi.org/10.1111/j.0030-1299.2008.16513.x>

568 Carter, A.J., Feeney, W.E., Marshall, H.H., Cowlishaw, G., Heinsohn, R., 2013. Animal
569 personality: what are behavioural ecologists measuring? *Biological Reviews* 88, 465–
570 475. <https://doi.org/10.1111/brv.12007>

571 Cinzano, P., Falchi, F., Elvidge, C.D., 2001. The first World Atlas of the artificial night sky
572 brightness. *Monthly Notices of the Royal Astronomical Society*.
573 <https://doi.org/10.1046/j.1365-8711.2001.04882.x>

574 Dammhahn, M., Almeling, L., 2012. Is risk taking during foraging a personality trait? A field
575 test for cross-context consistency in boldness. *Anim. Behav.* 84, 1131–1139.
576 <https://doi.org/10.1016/j.anbehav.2012.08.014>

577 Dammhahn, M., Dingemanse, N.J., Niemelä, P.T., Réale, D., 2018. Pace-of-life syndromes: a
578 framework for the adaptive integration of behaviour, physiology and life history. *Behav.*
579 *Ecol. Sociobiol.* 72, 62. <https://doi.org/10.1007/s00265-018-2473-y>

580 Davies, T.W., Bennie, J., Inger, R., de Ibarra, N.H., Gaston, K.J., 2013. Artificial light pollution:
581 Are shifting spectral signatures changing the balance of species interactions? *Glob.*
582 *Chang. Biol.* 19, 1417–1423. <https://doi.org/10.1111/gcb.12166>

583 Davies, T.W., Duffy, J.P., Bennie, J., Gaston, K.J., 2014. The nature, extent, and ecological
584 implications of marine light pollution. *Front. Ecol. Environ.* 12, 347–355.
585 <https://doi.org/10.1890/130281>

586 Degen, T., 2015. Female Mating Success Under Altered Sex-specific Mortality and Various
587 Male and Female Encounter Rates: Does Artificial Light at Night Force Evolution?

588 Desy, E. a., Batzli, G.O., Liu, J., 1990. Effects of food and predation on behaviour of prairie
589 voles : a field experiment. *Oikos* 58, 159–168. <https://doi.org/10.2307/3545423>

590 Dickerson, A.L., Hall, M.L., Jones, T.M., 2022. The effect of natural and artificial light at night
591 on nocturnal song in the diurnal willie wagtail. *Sci. Total Environ.* 808, 151986.
592 <https://doi.org/10.1016/j.scitotenv.2021.151986>

593 Dingemanse, N.J., Kazem, A.J.N., Réale, D., Wright, J., 2010. Behavioural reaction norms:
594 animal personality meets individual plasticity. *Trends Ecol. Evol.* 25, 81–89.
595 <https://doi.org/10.1016/j.tree.2009.07.013>

596 Duarte, C., Quintanilla-Ahumada, D., Anguita, C., Manríquez, P.H., Widdicombe, S., Pulgar, J.,
597 Silva-Rodríguez, E.A., Miranda, C., Manríquez, K., Quijón, P.A., 2019. Artificial light
598 pollution at night (ALAN) disrupts the distribution and circadian rhythm of a sandy
599 beach isopod. *Environ. Pollut.* 248, 565–573.
600 <https://doi.org/10.1016/j.envpol.2019.02.037>

601 Dupont-Prinet, A., Chatain, B., Grima, L., Vandeputte, M., Claireaux, G., McKenzie, D.J., 2010.
602 Physiological mechanisms underlying a trade-off between growth rate and tolerance of
603 feed deprivation in the European sea bass (*Dicentrarchus labrax*). *J. Exp. Biol.* 213,
604 1143–1152.

605 Elwood, R.W., n.d. Hermit Crabs – Information Gathering by the Hermit Crab, *Pagurus*
606 *bernhardus*. *Field and Laboratory Methods in Animal Cognition*.

607 <https://doi.org/10.1017/9781108333191.012>

608 Farnworth, B., Innes, J., Kelly, C., Littler, R., Waas, J.R., 2018. Photons and foraging: Artificial
609 light at night generates avoidance behaviour in male, but not female, New Zealand weta.
610 *Environmental Pollution*. <https://doi.org/10.1016/j.envpol.2018.01.039>

611 Finch, D., Smith, B.R., Marshall, C., Coomber, F.G., Kubasiewicz, L.M., Anderson, M., Wright,
612 P.G.R., Mathews, F., 2020. Effects of Artificial Light at Night (ALAN) on European
613 Hedgehog Activity at Supplementary Feeding Stations. *Animals (Basel)* 10.
614 <https://doi.org/10.3390/ani10050768>

615 Finstad, A.G., Forseth, T., Ugedal, O., Næsje, T.F., 2007. Metabolic rate, behaviour and winter
616 performance in juvenile Atlantic salmon. *Functional Ecology*.
617 <https://doi.org/10.1111/j.1365-2435.2007.01291.x>

618 Forsburg, Z.R., Guzman, A., Gabor, C.R., 2021. Artificial light at night (ALAN) affects the
619 stress physiology but not the behavior or growth of *Rana berlandieri* and *Bufo valliceps*.
620 *Environ. Pollut.* 277, 116775. <https://doi.org/10.1016/j.envpol.2021.116775>

621 Galliard, J., Paquet, M., Cisel, M., Montes-Poloni, L., 2013. Personality and the pace-of-life
622 syndrome: variation and selection on exploration, metabolism and locomotor
623 performances. *Funct. Ecol.* 27, 136–144.

624 Galliard, J.-F.L., Le Galliard, J.-F., Paquet, M., Cisel, M., Montes-Poloni, L., 2013. Personality
625 and the pace-of-life syndrome: variation and selection on exploration, metabolism and
626 locomotor performances. *Functional Ecology*. <https://doi.org/10.1111/1365-2435.12017>

627 Gasparini, C., Speechley, E.M., Polverino, G., 2019. The bold and the sperm: positive
628 association between boldness and sperm number in the guppy. *Royal Society Open
629 Science* 6, 190474. <https://doi.org/10.1098/rsos.190474>

630 Gaston, K.J., Bennie, J., Davies, T.W., Hopkins, J., 2013. The ecological impacts of nighttime
631 light pollution: a mechanistic appraisal. *Biol. Rev. Camb. Philos. Soc.* 88, 912–927.
632 <https://doi.org/10.1111/brv.12036>

633 Gaston, K.J., Spicer, J.I., 2013. *Biodiversity: An Introduction*. John Wiley & Sons.

634 Gerlach, S.A., Ekstrøm, D.K., Eckardt, P.B., 1976. Filter feeding in the hermit crab : *Pagurus
635 bernhardus*. *Oecologia* 24, 257–264. <https://doi.org/10.1007/BF00345477>

636 Hadfield, J.D., 2010. MCMC methods for multi-response generalized linear mixed models: the
637 MCMCglmm R package. *J. Stat. Softw.* 33, 1–22. <https://doi.org/10.1002/ana.22635>

638 Hazlett, B.A., 1966. Social behavior of the Paguridae and Diogenidae of Curacao. *Studies on the
639 Fauna of Curaçao and other Caribbean Islands* 23, 1–143.

640 Hillyer, K.E., Beale, D.J., Shima, J.S., 2021. Artificial light at night interacts with predatory
641 threat to alter reef fish metabolite profiles. *Sci. Total Environ.* 769, 144482.
642 <https://doi.org/10.1016/j.scitotenv.2020.144482>

643 Hölker, F., Wolter, C., Perkin, E.K., Tockner, K., 2010. Light pollution as a biodiversity threat.
644 *Trends Ecol. Evol.* 25, 681–682.

645 Holtmann, B., Lagisz, M., Nakagawa, S., 2017. Metabolic rates, and not hormone levels, are a
646 likely mediator of between-individual differences in behaviour: a meta-analysis. *Funct.
647 Ecol.* 31, 685–696. <https://doi.org/10.1111/1365-2435.12779>

648 Hut, R.A., Beersma, D.G.M., 2011. Evolution of time-keeping mechanisms: early emergence
649 and adaptation to photoperiod. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 366, 2141–2154.
650 <https://doi.org/10.1098/rstb.2010.0409>

651 Jiang, J., He, Y., Kou, H., Ju, Z., Gao, X., Zhao, H., 2020. The effects of artificial light at night
652 on Eurasian tree sparrow (*Passer montanus*): Behavioral rhythm disruption, melatonin

653 suppression and intestinal microbiota alterations. *Ecol. Indic.* 108, 105702.
654 <https://doi.org/10.1016/j.ecolind.2019.105702>

655 Kurvers, R.H.J.M., Drägestein, J., Hölker, F., Jechow, A., Krause, J., Bierbach, D., 2018.
656 Artificial Light at Night Affects Emergence from a Refuge and Space Use in Guppies.
657 *Sci. Rep.* 8, 14131. <https://doi.org/10.1038/s41598-018-32466-3>

658 Lancaster, I.L.B.-. 2763, 1990. Reproduction and life history strategy of the hermit crab *Pagurus*
659 *bernhardus*. *Journal of Marine Biology Assessment* 70, 129–142.
660 <https://doi.org/10.1017/S0025315400034251>

661 Lantová, P., Zub, K., Koskela, E., Šichová, K., Borowski, Z., 2011. Is there a linkage between
662 metabolism and personality in small mammals? The root vole (*Microtus oeconomus*)
663 example. *Physiology & Behavior* 104, 378–383.
664 <https://doi.org/10.1016/j.physbeh.2011.04.017>

665 Le Tallec, T., Perret, M., Théry, M., 2013. Light pollution modifies the expression of daily
666 rhythms and behavior patterns in a nocturnal primate. *PLoS One* 8, e79250.
667 <https://doi.org/10.1371/journal.pone.0079250>

668 Lima, S.L., 1998. Nonlethal Effects in the Ecology of Predator-Prey Interactions. *Bioscience* 48,
669 25–34. <https://doi.org/10.2307/1313225>

670 Lima, S.L., Dill, L.M., 1990. Behavioral decisions made under the risk of predation: a review
671 and prospectus. *Can. J. Zool.* 68, 619–640. <https://doi.org/10.1139/z90-092>

672 Lines, E.R., Zavala, M.A., Purves, D.W., Coomes, D.A., 2012. Predictable changes in
673 aboveground allometry of trees along gradients of temperature, aridity and competition.
674 *Global Ecology and Biogeography*. <https://doi.org/10.1111/j.1466-8238.2011.00746.x>

675 Lu, Z.L., Yuan, K.-H., 2010. Welch's t test. <https://doi.org/10.13140/RG.2.1.3057.9607>

676 Duarte, T., Bonta, C.C., Silva-Rodríguez, E.A., Quijón, P.A., Miranda, C., Farias, A.A., Duarte,
677 C., 2016. Light pollution reduces activity, food consumption and growth rates in a sandy
678 beach invertebrate. *Environ. Pollut.* 218, 1147–1153.
679 <https://doi.org/10.1016/j.envpol.2016.08.068>

680 Manríquez, P.H., Jara, M.E., Diaz, M.I., Quijón, P.A., Widdicombe, S., Pulgar, J., Manríquez,
681 K., Quintanilla-Ahumada, D., Duarte, C., 2019. Artificial light pollution influences
682 behavioral and physiological traits in a keystone predator species, *Concholepas*
683 *concholepas*. *Sci. Total Environ.* 661, 543–552.
684 <https://doi.org/10.1016/j.scitotenv.2019.01.157>

685 Marangoni, L.F.B., Davies, T., Smyth, T., Rodríguez, A., Hamann, M., Duarte, C., Pendoley, K.,
686 Berge, J., Maggi, E., Levy, O., 2022. Impacts of artificial light at night in marine
687 ecosystems-A review. *Glob. Chang. Biol.* 28, 5346–5367.
688 <https://doi.org/10.1111/gcb.16264>

689 Mitchell, K.A., 1973. Activities of two British species of *Pagurus* (Crustacea, Decapoda,
690 *Paguroidea*). *Mar. Freshw. Behav. Physiol.* 2, 229–236.

691 Mowles, S.L., Briffa, M., 2012. Forewarned is forearmed: early signals of RHP predict opponent
692 fatigue in hermit crab shell fights. *Behavioral Ecology* 23, 1324–1329.
693 <https://doi.org/10.1093/beheco/ars124>

694 Mowles, S.L., Cotton, P.A., Briffa, M., 2012. Consistent crustaceans: The identification of stable
695 behavioural syndromes in hermit crabs. *Behav. Ecol. Sociobiol.* 66, 1087–1094.
696 <https://doi.org/10.1007/s00265-012-1359-7>

697 Naylor, E., 2010. *Chronobiology of Marine Organisms*. Cambridge University Press.
698 <https://doi.org/10.1017/cbo9780511803567>

699 Nelson, R.J., 2019. Light at night influences neuroinflammation and metabolism.
700 Psychoneuroendocrinology. <https://doi.org/10.1016/j.psyneuen.2018.12.198>

701 Nickerson, K.W., Van Holde, K.E., 1971. A comparison of molluscan and arthropod
702 hemocyanin—I. Circular dichroism and absorption spectra. *Comparative Biochemistry*
703 *and Physiology Part B: Comparative Biochemistry* 39, 855–872.
704 [https://doi.org/10.1016/0305-0491\(71\)90109-X](https://doi.org/10.1016/0305-0491(71)90109-X)

705 O'Connor, J.J., Fobert, E.K., Besson, M., Jacob, H., Lecchini, D., 2019. Live fast, die young:
706 Behavioural and physiological impacts of light pollution on a marine fish during larval
707 recruitment. *Mar. Pollut. Bull.* 146, 908–914.
708 <https://doi.org/10.1016/j.marpolbul.2019.05.038>

709 Oosten, J.E., Magnhagen, C., Hemelrijk, C.K., 2010. Boldness by habituation and social
710 interactions: a model. *Behav. Ecol. Sociobiol.* 64, 793–802.
711 <https://doi.org/10.1007/s00265-009-0896-1>

712 Osborn, A., Briffa, M., 2017. Does repeatable behaviour in the laboratory represent behaviour
713 under natural conditions? A formal comparison in sea anemones. *Anim. Behav.* 123,
714 197–206. <https://doi.org/10.1016/j.anbehav.2016.10.036>

715 Palmer, J.D., 1973. Tidal rhythms: the clock control of the rhythmic physiology of marine
716 organisms. *Biological Reviews* 48, 377–418. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-185x.1973.tb01008.x)
717 [185x.1973.tb01008.x](https://doi.org/10.1111/j.1469-185x.1973.tb01008.x)

718 Pamela Delarue, E.M., Kerr, S.E., Lee Rymer, T., 2015. Habitat complexity, environmental
719 change and personality: A tropical perspective. *Behav. Processes* 120, 101–110.
720 <https://doi.org/10.1016/j.beproc.2015.09.006>

721 Polak, T., Korine, C., Yair, S., Holderied, M.W., 2011. Differential effects of artificial lighting
722 on flight and foraging behaviour of two sympatric bat species in a desert. *Journal of*
723 *Zoology*. <https://doi.org/10.1111/j.1469-7998.2011.00808.x>

724 Porter, R.K., Brand, M.D., 1995. Cellular oxygen consumption depends on body mass. *American*
725 *Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 269, R226–
726 R228. <https://doi.org/10.1152/ajpregu.1995.269.1.r226>

727 Prugh, L.R., Golden, C.D., 2014. Does moonlight increase predation risk? Meta-analysis reveals
728 divergent responses of nocturnal mammals to lunar cycles. *J. Anim. Ecol.* 83, 504–514.
729 <https://doi.org/10.1111/1365-2656.12148>

730 Pulgar, J., Zeballos, D., Vargas, J., Aldana, M., Manriquez, P.H., Manriquez, K., Quijón, P.A.,
731 Widdicombe, S., Anguita, C., Quintanilla, D., Duarte, C., 2019. Endogenous cycles,
732 activity patterns and energy expenditure of an intertidal fish is modified by artificial light
733 pollution at night (ALAN). *Environ. Pollut.* 244, 361–366.
734 <https://doi.org/10.1016/j.envpol.2018.10.063>

735 Raap, T., Casasole, G., Costantini, D., AbdElgawad, H., Asard, H., Pinxten, R., Eens, M., 2016a.
736 Artificial light at night affects body mass but not oxidative status in free-living nestling
737 songbirds: an experimental study. *Sci. Rep.* 6, 35626. <https://doi.org/10.1038/srep35626>

738 Raap, T., Pinxten, R., Eens, M., 2018a. Artificial light at night causes an unexpected increase in
739 oxalate in developing male songbirds. *Conservation Physiology*.
740 <https://doi.org/10.1093/conphys/coy005>

741 Raap, T., Pinxten, R., Eens, M., 2016b. Artificial light at night disrupts sleep in female great tits
742 (*Parus major*) during the nestling period, and is followed by a sleep rebound. *Environ.*
743 *Pollut.* 215, 125–134. <https://doi.org/10.1016/j.envpol.2016.04.100>

744 Raap, T., Pinxten, R., Eens, M., 2015. Light pollution disrupts sleep in free-living animals. *Sci.*

745 Rep. 5, 13557. <https://doi.org/10.1038/srep13557>

746 Raap, T., Thys, B., Grunst, A.S., Grunst, M.L., Pinxten, R., Eens, M., 2018b. Personality and
747 artificial light at night in a semi-urban songbird population: No evidence for personality-
748 dependent sampling bias, avoidance or disruptive effects on sleep behaviour. *Environ.*
749 *Pollut.* 243, 1317–1324. <https://doi.org/10.1016/j.envpol.2018.09.037>

750 Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V., Montiglio, P.-O., 2010.
751 Personality and the emergence of the pace-of-life syndrome concept at the population
752 level. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 365, 4051–4063.

753 Reddon, A.R., 2012. Parental effects on animal personality. *Behavioral Ecology* 23, 242–245.
754 <https://doi.org/10.1093/beheco/arr210>

755 Ringelberg, J., 2007. The photobehaviour of *Daphnia* spp. as a model to explain diel vertical
756 migration in zooplankton. *Biol. Rev. Camb. Philos. Soc.* 74, 397–423.
757 <https://doi.org/10.1111/j.1469-185X.1999.tb00036.x>

758 Royauté, R., Greenlee, K., Baldwin, M., Dochtermann, N.A., 2015. Behaviour, metabolism and
759 size: phenotypic modularity or integration in *Acheta domesticus*? *Animal Behaviour* 110,
760 163–169. <https://doi.org/10.1016/j.anbehav.2015.09.027>

761 Sanders, D., Kehoe, R., Cruse, D., van Veen, F.J.F., Gaston, K.J., 2018. Low Levels of Artificial
762 Light at Night Strengthen Top-Down Control in Insect Food Web. *Curr. Biol.* 28, 2474-
763 2478.e3. <https://doi.org/10.1016/j.cub.2018.05.078>

764 Sanders, D., Kehoe, R., Tiley, K., Bennie, J., Cruse, D., Davies, T.W., Frank van Veen, F.J.,
765 Gaston, K.J., 2015. Artificial nighttime light changes aphid-parasitoid population
766 dynamics. *Sci. Rep.* 5, 15232. <https://doi.org/10.1038/srep15232>

767 Sinn, D.L., Moltschanivskyj, N. a., 2005. Personality traits in dumpling squid (*Euprymna*
768 *tasmanica*): context-specific traits and their correlation with biological characteristics. *J.*
769 *Comp. Psychol.* 119, 99–110. <https://doi.org/10.1037/0735-7036.119.1.99>

770 Smith, B.R., Blumstein, D.T., 2008. Fitness consequences of personality: a meta-analysis.
771 *Behavioral Ecology* 19, 448–455. <https://doi.org/10.1093/beheco/arm144>

772 Sneddon, L.U., 2003. The bold and the shy: individual differences in rainbow trout. *Journal of*
773 *Fish Biology* 62, 971–975. <https://doi.org/10.1046/j.1095-8649.2003.00084.x>

774 Speakman, J.R., 2013. Measuring energy metabolism in the mouse - theoretical, practical, and
775 analytical considerations. *Front. Physiol.* 4, 34. <https://doi.org/10.3389/fphys.2013.00034>

776 Spicer, J.I., Baden, S.P., 2000. Natural variation in the concentrations of haemocyanin from three
777 decapod crustaceans, *Nephrops norvegicus*, *Liocarcinus depurator* and *Hyas araneus*.
778 *Mar. Biol.* 136, 55–61.

779 Spoelstra, K., Verhagen, I., Meijer, D., Visser, M.E., 2018. Artificial light at night shifts daily
780 activity patterns but not the internal clock in the great tit (*Parus major*). *Proc. Biol. Sci.*
781 285. <https://doi.org/10.1098/rspb.2017.2751>

782 Stamps, J.A., 2007. Growth-mortality tradeoffs and ?personality traits? in animals. *Ecology*
783 *Letters* 10, 355–363. <https://doi.org/10.1111/j.1461-0248.2007.01034.x>

784 Stamps, J.A., Briffa, M., Biro, P.A., 2012. Unpredictable animals: Individual differences in
785 intraindividual variability (IIV). *Anim. Behav.* 83, 1325–1334.
786 <https://doi.org/10.1016/j.anbehav.2012.02.017>

787 Touzot, M., Teulier, L., Lengagne, T., Secondi, J., Théry, M., Libourel, P.-A., Guillard, L.,
788 Mondy, N., 2019. Artificial light at night disturbs the activity and energy allocation of the
789 common toad during the breeding period. *Conserv Physiol* 7, cozo02.
790 <https://doi.org/10.1093/conphys/cozo02>

791 Troscianko, T., Benton, C.P., Lovell, P.G., Tolhurst, D.J., Pizlo, Z., 2009. Camouflage and visual
792 perception. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 449–461.

793 Truscott, Z., Booth, D.T., Limpus, C.J., 2017. The effect of on-shore light pollution on sea-turtle
794 hatchlings commencing their off-shore swim. *Wildlife Research*.
795 <https://doi.org/10.1071/wr16143>

796 Tuomainen, U., Candolin, U., 2011. Behavioural responses to human-induced environmental
797 change. *Biological Reviews*. <https://doi.org/10.1111/j.1469-185x.2010.00164.x>

798 Welbers, A.A.M.H., Welbers, A.A.M., van Dis, N.E., Kolvoort, A.M., Ouyang, J., Visser, M.E.,
799 Spoelstra, K., Dominoni, D.M., 2017. Artificial Light at Night Reduces Daily Energy
800 Expenditure in Breeding Great Tits (*Parus major*). *Frontiers in Ecology and Evolution*.
801 <https://doi.org/10.3389/fevo.2017.00055>

802 Westneat, D.F., Schofield, M., Wright, J., 2013. Parental behavior exhibits among-individual
803 variance, plasticity, and heterogeneous residual variance. *Behavioral Ecology* 24, 598–
804 604. <https://doi.org/10.1093/beheco/ars207>

805 Westneat, D.F., Wright, J., Dingemanse, N.J., 2015. The biology hidden inside residual within-
806 individual phenotypic variation. *Biol. Rev. Camb. Philos. Soc.* 90, 729–743.

807 White, J.R., Meekan, M.G., McCormick, M.I., Ferrari, M.C.O., 2013. A Comparison of
808 Measures of Boldness and Their Relationships to Survival in Young Fish. *PLoS ONE* 8,
809 e68900. <https://doi.org/10.1371/journal.pone.0068900>

810 White, S.J., Briffa, M., 2017. How do anthropogenic contaminants (ACs) affect behaviour?
811 Multi-level analysis of the effects of copper on boldness in hermit crabs. *Oecologia* 183,
812 391–400. <https://doi.org/10.1007/s00442-016-3777-0>

813 Widdows, J., Donkin, P., F. J Staff, Matthiessen, P., Law, R.J., Allen, Y.T., Thain, J.E., Allchin,
814 C.R., Jones, B.R., 2002. Measurement of stress effects (scope for growth) and
815 contaminant levels in mussels (*Mytilus edulis*) collected from the Irish Sea. *Marine*
816 *Environmental Research* 53, 327–356. [https://doi.org/10.1016/s0141-1136\(01\)00120-9](https://doi.org/10.1016/s0141-1136(01)00120-9)

817 Wyse, C.A., Selman, C., Page, M.M., Coogan, A.N., Hazlerigg, D.G., 2011. Circadian
818 desynchrony and metabolic dysfunction; did light pollution make us fat? *Med.*
819 *Hypotheses* 77, 1139–1144. <https://doi.org/10.1016/j.mehy.2011.09.023>

820 Zubidat, A.E., Fares, B., Fares, F., Haim, A., 2018. Artificial Light at Night of Different Spectral
821 Compositions Differentially Affects Tumor Growth in Mice: Interaction With Melatonin
822 and Epigenetic Pathways. *Cancer Control* 25, 1073274818812908.
823 <https://doi.org/10.1177/1073274818812908>
824