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1 **Do the effects of Artificial Light at Night on behavioral rhythm and related gene expression are**
2 **wavelength dependent in the oyster *Crassostrea gigas*?**

3 Audrey BOTTÉ¹, Laura PAYTON¹, Damien TRAN^{1*}

4
5 ¹Univ. Bordeaux, CNRS, Bordeaux INP, EPOC, UMR 5805, F-33120 Arcachon, France

6
7 *Corresponding author: Damien Tran

8 E-mail: damien.tran@u-bordeaux.fr

9
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25 **Abstract**

26 Artificial Light At Night (ALAN) constitutes a growing threat to coastal ecosystems by altering natural light
27 cycles, which could impair organisms' biological rhythms, with resulting physiological and ecological
28 consequences. Coastal ecosystems are strongly exposed to ALAN but its effects on coastal organisms are poorly
29 studied. Besides ALAN's intensity, ALAN's quality exposure may change the impacts on organisms. This study
30 aims to characterize the effects of different ALAN's spectral compositions (monochromatic wavelength lights in
31 red (594–640 nm, peak at 626 nm), green (494–554 nm, peak at 515 nm), blue (447–511 nm, peak at 467 nm)
32 and white (410–680 nm, peak at 558 nm) light) at low and realistic intensity (1 lx) on the oyster *Crassostrea*
33 *gigas* daily rhythm. Results reveal that all ALAN's treatments affect the oysters' daily valve activity rhythm in
34 different manners and the overall expression of the 13 studied genes (clock, clock-associated, light perception
35 genes). The blue light has the most important effects on oysters' valve behavior and clock and clock-associated
36 gene expression. Interestingly, red and green lights also show significant impacts on the daily rhythm, while the
37 lowest impacts are shown with the green light. Finally, ALAN white light shows the same impact as the blue one
38 in terms of loss of rhythmic oysters' percentage, but the chronobiological parameters of the remaining rhythmic
39 oysters are less disrupted than when exposed to each of the monochromatic light's treatments alone. We
40 conclude that ALAN's spectral composition does influence its effect on oysters' daily rhythm, which could give
41 clues to limit physiological and ecological impacts on coastal environments.

42

43 **Keywords:** ALAN, *Crassostrea gigas*, circadian clock, daily rhythm, oyster behavior, valvometry

44 Introduction

45 ALAN is a worldwide phenomenon whose amplitude is still growing since its radiance increases each year
46 (1.8%) as well as lit areas (2.2%) (Falchi et al. 2016; Kyba et al. 2017). Despite some positive aspects for human
47 activities (security, social activities, advertising, or aesthetic), nocturnal lighting constitutes a threat to public
48 health and ecosystems by affecting organisms' physiology, behavior, communication, reproduction, or
49 communities structure (Brayley et al. 2022; Sanders et al. 2021). To limit these harmful effects, mitigation
50 strategies have been suggested by varying lighting intensity, spectrum, or duration (Gaston 2018). The growing
51 use of Light-Emitting Diodes (LEDs) can facilitate the application of these strategies since their lighting
52 characteristics (intensity, timing, spectrum) can be easily modulated compared to previous lighting technologies
53 (Gaston 2018). Narrow white LED spectrum to contain only the wavelengths least harmful to organisms in a
54 given environment could mitigate the increasing ALAN impacts caused by the widespread use of white LED,
55 which are rich in short wavelengths (Kyba 2018; Longcore and Rich 2016).

56 The increase in blue-rich sources of ALAN is shown to make brighter the anthropogenic nocturnal skyglow and
57 can be even more harmful to marine organisms since blue and green ones can penetrate deeper in seawater,
58 depending on its composition (Falcon et al. 2020). Finally, blue wavelengths are more susceptible to affect
59 organisms' physiology since they are used as signals in many biological processes, such as reproduction, daily
60 activity patterns, or orientation (Kaniewska et al. 2015; Leach and Reitzel 2020; Rivas et al. 2015).

61 Disruption of organisms' biological rhythms is an essential target of ALAN physiological and ecological
62 putative damages. Biological rhythms are ubiquitous and arise from an autonomous internal clock present in
63 each cell of organisms, coordinating physiological processes and behavior (Gaston et al. 2017). Endogenous
64 clocks are synchronized by the environment using reliable cues such as daily, annual, or lunar natural light
65 cycles, enabling organisms to anticipate predictable changes of their environment and to organize accordingly
66 their biological processes, improving their fitness and survival. They are also crucial for synchronizing intra-
67 specific interactions (reproduction) and inter-specific interactions (foraging and predation), and consequently
68 play a major role in ecosystems' functionality (Gaston et al. 2017). However, by masking natural light cycles,
69 ALAN impairs the synchronization of biological clocks. Considering the colossal diversity of light detection
70 systems in the realm of life, organisms' spectral sensitivity needs to be characterized to successfully apply the
71 alteration of ALAN spectral composition as a mitigation strategy (Alaasam et al. 2021; Falcon et al. 2020).

72 Coastal ecosystems are strongly exposed to ALAN with 1.6 million km² of the coastal seas affected by this
73 phenomenon at 10 m depth (Smyth et al. 2021). The importance of studying ALAN's effects on coastal

74 organisms has been addressed mainly recently and first studies show that ALAN affects a large diversity of
75 coastal organisms (Davies et al. 2014; Hussein et al. 2021; Marangoni et al. 2022). A keystone species in coastal
76 environments is the Pacific oyster *Crassostrea gigas*, which is today the most globalized marine invertebrate,
77 mainly due to its rapid growth and large tolerance to diverse environmental conditions (Ruesink et al. 2005).
78 These advantages for culture also confer to wild oysters the ability to colonize diversified environments, making
79 *C. gigas* an invasive species. Living in intertidal and subtidal areas and being a sessile mollusk, oysters are likely
80 exposed to ALAN, which could disrupt their daily, lunar, and annual rhythms synchronized with natural
81 variations of light (Payton et al. 2017b; Payton and Tran 2019; Tran et al. 2020). Moreover, it was sequenced
82 genes involved in the clock mechanism of *C. gigas* and its light perception (Payton et al. 2017a; Perrigault and
83 Tran 2017). Finally, it was recently shown that ALAN with a white spectrum disrupts the daily rhythm of
84 oyster's valve activity as well as its molecular clock, starting from a low intensity of 0.1 lx (Botté et al. 2023).
85 Therefore, characterizing ALAN's effects on oysters according to its spectral composition could give
86 supplementary clues to help preserve coastal ecosystems.

87 This study evaluates the effects of several monochromatic wavelengths of light (red, green, and blue
88 wavelengths) and white light spectrum as sources of ALAN on the daily valve behavioral rhythm and mRNA
89 level of genes involved in clockwork mechanism, light perception and melatonin synthesis of the oyster *C. gigas*.
90 A low and realistic ALAN intensity of 1 lx was chosen according to Botté et al. (2023) findings. The objective of
91 this study is to establish if ALAN's spectral composition modulates in a specific manner its effects on the
92 oysters' daily rhythm and to determine which ALAN wavelength is likely to cause the most and least damages.
93 We hypothesized that ALAN effect on oyster's daily rhythm is wavelength dependent. Thus, we also
94 hypothesize that ALAN blue and white lights have the most important effects, while the green and red lights
95 would have the least harmful effects.

96

97 **Material and methods**

98 *Experimental procedure*

99 160 oysters (85.2 ± 0.8 mm shell length; 45.6 ± 0.5 mm shell width, mean \pm SE) from an oyster's farm in Arcachon
100 Bay (France) were placed in group of 32 individuals into a tank (L x W x H: 74.8 x 54.8 x 40.8 mm) continuously
101 supplied with natural oxygenized and filtered ($< 1 \mu\text{m}$) seawater from the Arcachon Bay with a monitored
102 temperature ($T = 19.9 \pm 0.1$ °C). The tank was placed in an isolated room and equipped with an antivibration

103 system to avoid external disturbances for the oysters. The experiments were performed in the Marine Station of
104 Arcachon from June to October 2021. During the experiments, oysters were not fed and were kept under a 12L:12D
105 light cycle performed by programmable white LED light bars (MH3SP3 DSunY, 413–685 nm, peak at 553 nm;
106 Fig. S1A). Daytime was from 06:00 to 18:00 h (all times noted in local time, UTC +2) (Fig. S2) during which the
107 light intensity varied to mimic the natural light cycle with maximum intensity from 11:30 to 12:30 h of $1514.0 \pm$
108 140.6 lx (mean \pm SE) (Tab. S1). After 7 days of acclimation, oysters were exposed for 7 days from 18:30 to 05:30
109 h to four different ALAN's spectral compositions (Fig. S2B) using LED strips (MiBoxer Mi-Light WL5): white
110 light (410–680 nm, peak at 558 nm, 6000 K; Fig. S1B), red light (594–640 nm, peak at 626 nm; Fig. S1C), green
111 light (494–554 nm, peak at 515 nm, Fig. S1D), and blue light (447–511 nm, peak at 467 nm; Fig. S1E). The ALAN
112 intensity applied was 1 lx, which was chosen according to previous results (Botté et al. 2023). Illuminances were
113 measured underwater at the oysters' level, at five positions in the tank (Fig. S3 and Tab. S1), using a handheld
114 spectroradiometer (Blue-Wave UVN-100, StellarNet Inc.). A control condition was also realized without nocturnal
115 lighting and with a measured light intensity below 0.05 lx, which is the spectroradiometer detection limit (Fig.
116 S2A).

117 *Behavioral monitoring*

118 For each experiment, the valve activity of 16 oysters was continuously recorded using a High Frequency–Non
119 Invasive (HFNI) valvometer technology (Tran et al. 2020). It consists of lightweight electromagnets glued on
120 each valve of each oyster between which an electromagnetic current is formed and allows to record each
121 individual's valve activity every 4.8 s (Tran et al. 2023). Data were processed using Labview 8.0 (National
122 Instrument, Austin, TX, USA). The studied valve activity parameter is the hourly Valve Opening Duration
123 (VOD), meaning the percentage of time that each oyster spends with its valves open per hour. For instance, a
124 VOD of 100% means that the individual spent an entire hour with its valves open.

125 *Chronobiological analyses*

126 From hourly VOD data, chronobiological analyses were conducted at the individual and group levels using the
127 software Time Series Analysis Serie Cosinor 8.0 (Expert Soft Technologies) (for details, see Payton et al.
128 (2017b)). The spectral method of the Lomb and Scargle periodogram was performed to search for a periodicity
129 in the dataset, considering a periodicity as significant for $p > 0.95$. When a period was statistically validated, the
130 dataset rhythmicity was modeled using the Cosinor model, which is characterized by its amplitude (difference
131 between the rhythm highest value and average level), period (interval between two identical events), acrophase
132 (the rhythm highest value), and mesor (the rhythm average level). This model gives another chronobiological

133 parameter, the Percent Rhythm (PR), representing the percentage of cyclic behavior explained by the model,
134 meaning the strength of the rhythm.

135 *Molecular analyses*

136 On the 7th day of the experiment, the gill tissue of 8 oysters was sampled during daytime at 12:00 h and during
137 nighttime at 20:00 h. They were stored in Tri Reagent (Invitrogen) at -80°C for further analysis. From gills
138 tissues, total RNA was extracted using a SV Total RNA Isolation System kit (Promega). The quantity and
139 quality of the total RNA were evaluated by spectrophotometry (OD230, OD260, OD280). The RNA was reverse
140 transcribed using GoscriptTM Reverse Transcription System kits (Promega). The next step was to perform Real-
141 Time qPCR using the GoTaq[®] qPCR Master Mix kit (Promega). Primers sets of clock, clock-associated
142 (*CgClock*, *CgBmal*, *CgCry*, *CgPer*, *CgTim1*, *CgCry1*, *CgRev-erb*, *CgRor*, *CgHiomt-like*, *CgOctβ2*,
143 *CgRhodopsin-like 1*, *CgRhodopsin-like 2*, *CgRhodopsin-like 3*), and housekeeping genes (*CgEfl*, *Cg28S*,
144 *CgGadph*) used are described in the Tab. S2. The comparative Ct method $2^{-\Delta\text{Ct}}$ (Livak and Schmittgen 2001) was
145 used to determine the relative mRNA expression level of studied genes, where $\Delta\text{Ct} = \text{Ct}_{(\text{target gene})} - \text{Ct}_{(\text{housekeeping}$
146 $\text{gene})}$. Genes' expression levels were normalized using the geometric mean of the housekeeping genes *CgGadph*
147 and *CgEfl*, based on stability values.

148 *Statistical analyses*

149 Statistical analyses were performed using the software SigmaPlot (version 13.0; Systat Software, USA). T-tests
150 were used for two groups' comparisons and One-Way ANOVA for multiple groups' comparisons, after checking
151 prerequisites (homogeneity of variances, equal data). If the prerequisites were not validated, non-parametric tests
152 were performed instead: Mann Whitney rank sum for two groups' comparisons and Kruskal-Wallis One-Way
153 ANOVA on ranks for multiple groups' comparisons. A Three-Way ANOVA was performed on molecular
154 analyses results after checking assumptions (homogeneity of variances, equal data). For One-Way and Three-
155 Way ANOVA tests, if significant differences were found, the Fisher LSD test was performed for all pairwise
156 multiple comparisons. In the case of a non-parametric Kruskal-Wallis One-Way ANOVA on ranks test, the
157 Student-Newman-Keuls test was used for all pairwise multiple comparisons. For all test results, a difference was
158 considered significant when $p < 0.05$.

159

160 **Results**

161 Figure 1 shows the overall oysters' behavioral mean daily pattern in the control condition and the four ALAN
162 conditions, with a black arrow indicating the activity peak during the day. In the control condition, oysters
163 exhibit a diurnal behavioral pattern with a daily VOD peak at 10–11 h. When exposed to ALAN, oysters show a
164 disruption of their diurnal behavioral pattern for all ALAN's treatments. Mainly, the principal daily VOD peak
165 shifts by 9 hours, being delayed from daytime to nighttime, for the red, green, and blue light treatments. In the
166 white light treatment, the main VOD peak remains slightly dominant during daytime. The mean daily VOD (Fig.
167 2A) shows a significant increase when oysters are exposed to ALAN, except for the green wavelength, with an
168 increase significantly higher for the blue wavelength, and intermediate for the white light and the red wavelength
169 ($p < 0.001$). Figure 2B shows significant daytime / nighttime differences of the mean VOD in the control
170 condition with an increase during daytime, expressing a diurnal pattern. When oysters are exposed to ALAN,
171 this diurnal trend tends to decrease in the white light condition but disappears in the green and red wavelength
172 treatments. Finally, when exposed to ALAN with the blue wavelength treatment, oysters change their behavior
173 from a diurnal to a nocturnal pattern, characterized by a mean VOD significantly higher during nighttime than
174 during daytime. During daytime, the figure 2C shows a significant decrease in oysters' mean VOD for the red,
175 green, and blue wavelength treatments ($p < 0.001$), but not for the white light treatment. On the contrary, during
176 nighttime, the figure 2D reveals a significant increase in oysters' mean VOD for all ALAN treatments ($p <$
177 0.001), with the smallest increase in the white and green light conditions, intermediate for the red wavelength
178 and the highest for the blue wavelength.

179 Figure 3 shows the results of the chronobiological parameters analyses (period, amplitude, acrophase, and
180 percent rhythm (PR)), characterizing ALAN effects according to its spectral composition on the oysters' daily
181 behavioral rhythm at the group level (detailed results in Table S3). Oysters show a significant daily rhythm
182 (rhythm with a period in the circadian range of 20-28 h) that persists for all conditions (Fig. 3A). However, all of
183 the ALAN treatments affect the chronobiological parameters of this daily behavioral rhythm. The strength of the
184 rhythm (PR) decreases significantly for all wavelengths tested, and also for the white light treatment with a PR
185 intermediate between the control and the monochromatic lights ($p < 0.001$; Fig. 3B). ALAN also induces a
186 significant decrease of the daily rhythm's amplitude. This decrease of amplitude is maximal in red and blue
187 wavelength and is less intense in green and white lights ($p < 0.001$; Fig. 3C). Finally, the results show a
188 significantly delayed acrophase for all ALAN treatments, with a minimum shift in white light, intermediate in
189 red and green wavelength conditions and maximal in the blue wavelength treatment ($p < 0.001$; Fig. 3D). Then,
190 chronobiological analyses were performed at the individual level (Fig. 4; detailed results in Table S4). Results

191 show a decrease in the percentage of oysters having a significant daily behavioral rhythm according to the
192 ALAN treatments (Fig. 4A). In the control condition, the percentage of oysters having a significant daily rhythm
193 is 87.5 %. This percentage decreases to 75.0 %, 43.7 %, 35.7 %, and 33.3 % for the green, red, white, and blue
194 light treatments, respectively, which corresponds to a loss of 14.3 %, 50.1 %, 59.2 %, and 61.9 % of individuals
195 having a daily rhythm compared to the control. Therefore, ALAN with the blue and white light treatments cause
196 the most important loss of oysters with a daily rhythm. Concerning oysters maintaining a daily behavioral
197 rhythm, the chronobiological parameters are also disrupted by ALAN. The PR decreases significantly for the
198 red, green, and blue wavelength conditions but not for the white light ($p = 0.004$; Fig. 4B). The rhythm
199 amplitude decreases significantly only for the green wavelength treatment ($p = 0.022$; Fig. 4C). Finally, the
200 acrophase significantly shifts to a later hour during nighttime for the red, green and blue wavelength treatments,
201 but not for the white light condition ($p = 0.038$; Fig. 4D).

202 Figures 5 and S4 show the relative expression of thirteen clock (*CgClock*, *CgBmal*, *CgCry2*, *CgPer*, *CgTim1*,
203 *CgCry1*, *CgRev-erb*, *CgRor*), clock-associated (*CgHiomt-like*, *CgOctβ2*), and putative light signalization
204 (*CgRhodopsin-like 1*, *CgRhodopsin-like 2*, *CgRhodopsin-like 3*) genes for all conditions. For each gene, the left
205 graph shows the daytime / nighttime expression difference while the middle and right graphs show the
206 expression difference between the experimental conditions during daytime and nighttime respectively. ALAN
207 affects the expression of three clock genes (*CgBmal*, *CgTim1*, *CgCry1*) and one clock-associated gene
208 (*CgHiomt*) (Fig. 5). ALAN affects *CgBmal* expression during nighttime by causing a significant decrease only
209 for the white light condition ($p = 0.037$). Concerning *CgTim1*, its expression is significantly different between
210 daytime and nighttime in the control condition, as well as in the red and blue wavelength treatments. This
211 daytime / nighttime difference is lost for the green and white light treatments, consequently to a decrease of the
212 relative mRNA level during nighttime ($p = 0.016$). The expression of *CgCry1* shows a significant daytime /
213 nighttime difference for the blue wavelength treatment, which is not significant for the control condition and the
214 other ALAN treatments. Finally, the clock-associated gene *CgHiomt* shows a gene expression significantly
215 higher during nighttime compared to daytime in the control condition, and this daytime / nighttime difference
216 disappears for all ALAN's treatments. Furthermore, there is a significant decrease, in comparison to the control
217 condition, of *CgHiomt* expression during nighttime for all ALAN treatments ($p = 0.006$). To evaluate an overall
218 effect on all genes expression of ALAN treatments, table 1 shows the results of a Three-Way ANOVA, testing
219 the effects of ALAN treatments, the moment of the day (Daytime / Nighttime), and different gene expressions.
220 This analysis reveals a significant ALAN effect on the overall mRNA level ($p = 0.001$). More precisely, a

221 significant difference in expression is observed between the control condition and all ALAN treatments, as well
222 as between the red wavelength and the white light treatments. This three-way ANOVA shows as well a
223 significant difference in the overall gene expression between nighttime and daytime ($p = 0.005$). Moreover, the
224 interactions tested show a significant difference ($p = 0.002$) in the overall mRNA level between daytime and
225 nighttime in the control condition, as well as the red and green wavelength treatments. On the contrary, this
226 daytime / nighttime difference in gene expression is abolished for the white ($p = 0.213$) and blue light treatments
227 ($p = 0.081$).

228

229 **Discussion**

230 This study aims to evaluate ALAN effects according to its spectral composition (red, green, blue monochromatic
231 wavelengths and also full visible spectrum, i.e. white light), using LED technology lighting, on the daily rhythm
232 of the oyster *C. gigas*. Results reveal that at 1 lx, all tested ALAN monochromatic wavelengths and white light
233 deeply disrupt the oyster's behavioral daily rhythm as well as gene expression involved in the molecular
234 circadian clock mechanism, and melatonin synthesis, and this with different effects according to ALAN' light
235 quality.

236 First, a question that could be raised is if ALAN affects directly valve behavior or through a disruption of the
237 clock mechanism. In this study, all ALAN's treatments affect oysters' behavioral daily rhythm and the overall
238 expression of the thirteen studied genes (clock, clock-associated, and light perception genes). Therefore,
239 ALAN's effects on oyster's behavior might not be due, or at least not only, to a direct reaction of oysters to
240 nocturnal lighting, but to a disruption of their internal clock by ALAN. This finding has been already suggested
241 by Botté et al. (2023), which has evaluated ALAN's effects on oyster's daily rhythm according to its intensity.
242 Previous results also revealed that the majority of ALAN's effects on oysters occurred starting from 0.1 lx.
243 However, some observed ALAN's effects at the behavioral level occurred starting from the 1 lx intensity (Botté
244 et al. 2023). Here, we confirm that ALAN affects oysters' daily rhythm at 1 lx whatever the light quality.

245 Daily rhythms play a fundamental role in organisms to anticipate predictable changes in their environment and
246 optimize their physiological and behavioral processes according to the moment of the day, improving then their
247 fitness (Hafker and Tessmar-Raible 2020). A clue supporting the idea that loss of fitness can result from the
248 disruption of oysters' daily rhythm by ALAN is the impairment of diurnal behavior observed in our study.

249 Indeed, oysters have been shown in the field to be nocturnal during autumn and winter, and diurnal during spring

250 and summer. This annual alternation of daily behavior is suspected to have an internal origin and to be an
251 adaptive advantage related to seasonal variations of phytoplankton availability and energy needs (Mat et al.
252 2012). Indeed, as the plankton abundance increases during spring and summer, as well as the oyster's energy
253 needs, mainly due to gametogenesis and growth, being diurnal would enable them to consume more food
254 (Enríquez-Díaz et al. 2008; Payton et al. 2017b). Thus, losing diurnal behavior or switching from a diurnal to a
255 nocturnal activity pattern could impair food intake and thus induce an imbalance between energy generation and
256 metabolic demand. A second clue suggesting that physiological consequences can result from the disruption of
257 oysters' daily rhythm is the significant decrease of the clock-associated gene *CgHiomt-like* expression during
258 nighttime, whatever the ALAN spectral composition tested. Previous results have shown that ALAN in white
259 spectrum also disrupts oysters *CgHiomt-like* gene expression starting from 0.1 lx (Botté et al. 2023). This gene is
260 a homolog of the mammalian gene *Hiomt*, involved in the melatonin synthesis directly under the control of the
261 circadian clock. This hormone is involved in many physiological processes (homeostasis, reproduction,
262 immunology ...). Its daily rhythmic synthesis is believed to give organisms information about day and night
263 length (Tan et al. 2010). Similar results have been found in aquatic species such as the fish *Rutilus rutilus* and
264 *Perca fluviatilis*. ALAN in white, blue, red, and green lights at comparable intensities disrupted their melatonin
265 production rhythm by decreasing its production (Bruning et al. 2016; Bruning et al. 2018). Given the multiple
266 physiological functions that fulfill melatonin, impairment of its expression can induce negative consequences on
267 oyster physiology even though mitigation strategies focusing on ALAN's spectral composition or intensities are
268 implemented.

269 Among the ALAN treatments, the blue and white light conditions have the most important effects on oysters'
270 daily rhythm, while red wavelength induces intermediate effects and the green light treatment the least important
271 effects. Indeed, for the blue and white lights only, results reveal an overall abolition of the daytime / nighttime
272 gene expression difference as well as a stronger loss of rhythmic individuals. The blue wavelength causes the
273 most impacting effects on the oysters' diurnal behavior by making it nocturnal and on most of the
274 chronobiological parameters of the daily rhythm at the group (mesor, acrophase, PR), and individual levels (%
275 rhythmic oysters, PR, acrophase in oysters that keep a daily rhythm). The second most harmful ALAN treatment
276 is the white light since it causes a strong loss of rhythmic oysters in the same order as the blue wavelength
277 treatment. But, unlike in the blue light condition, most of the chronobiological parameters at the group level
278 (mesor, PR, acrophase) are not significantly affected by the white light condition. This could be due to the few
279 rhythmic individuals (35.7 %) that maintain a daily rhythm without significant impacts on chronobiological

280 parameters. The red wavelength treatment shows intermediate effects on the oysters' daily rhythm at the group
281 (mesor, acrophase) and individual levels (% rhythmic oysters). Finally, the green monochromatic light has the
282 least important effects on the oysters' daily rhythm (mesor, amplitude). Especially, the green light condition
283 causes a loss of only 14 % of oysters having a daily rhythm, while other monochromatic lights induce a loss of
284 more than 50 %. The wavelengths in the green light range from 494 to 554 nm and thus contain some
285 wavelengths belonging to the blue light (447–511 nm). Blue wavelengths having the most harmful effects on
286 oysters, green light impacts might have been even lower if its spectrum had strictly contained wavelengths
287 corresponding to a green light.

288 Although studies about the effects of different ALAN wavelengths on marine organisms remain scarce, oysters
289 are not the only ones to show differential responses according to wavelengths. Christoforou et al. (2023) showed
290 that ALAN (19.86 lx) affects the pattern of the valve activity (expressed as the gaping frequency) of the mussel
291 *Mytilus edulis*. Mussels were less active in white and red ALAN with a decrease of gaping frequency by 32 %
292 and 41 % respectively. However, when exposed to ALAN in green monochromatic wavelength, mussels were
293 more active with a 10 % increase in the gaping frequency (Christoforou et al. 2023). Thus, these results show
294 that changing the ALAN monochromatic wavelength can induce opposite effects on mussels' valve behavior,
295 contrary to oysters, highlighting the diversity of responses to ALAN among ecosystems. However, the devices
296 and methodology used by Christoforou et al. (2023) to measure mussels' valve activity were more invasive and
297 less accurate than the ones used in this study. Another marine organism able to discriminate wavelengths and to
298 have a greater sensitivity to short wavelengths like oysters is the sea anemone *Nematostella vectensis* (Leach and
299 Reitzel 2020). Indeed, the blue light is able to entrain a circadian activity profile in *N. vectensis*, contrary to the
300 red one. However, *N. vectensis* expresses under red light an activity profile following the one expressed under
301 constant darkness, suggesting that the sea anemone's circadian clock is not sensitive to the red light, while red
302 monochromatic wavelength has intermediate but significant effects on the oysters behavioral daily rhythm and
303 impacts the overall gene expression (Leach and Reitzel 2020). In contrast to the red light, the green wavelength
304 is efficient to entrain a circadian activity in the sea anemone, while oysters were the least sensitive to it. Contrary
305 to our study, Li et al. (2022) demonstrated that ALAN's effects on the reproduction, development, and defenses
306 in response to predation risk of the freshwater crustacean *Daphnia magna* were the least important when using a
307 blue light compared to white, red, or green lights. Bruning et al. (2016) made the same analysis in the European
308 perch *Perca fluviatilis* by studying ALAN effects in blue, green, and red wavelengths at comparable intensities
309 on melatonin production. All ALAN's wavelengths tested induced a disruption of the European perch daily

310 melatonin synthesis rhythm. Also, a loss of daily rhythmicity of melatonin concentration for the green and red
311 wavelength treatments are shown, whereas under the blue wavelength, the melatonin production was reduced but
312 kept its daily rhythmicity (Bruning et al. 2016).

313 Therefore, our study shows that the oyster *C. gigas* perceives light even though it's an eyeless organism,
314 contrary to other bivalve mollusks such as hammer oysters or scallops for instance, which possess more or less
315 primitive eyes (Audino et al. 2020). Photoreceptors along with photopigments can be associated with an eye
316 structure or not and therefore be visual or not visual (Falcon et al. 2020). Each cell is able of non-visual
317 photodetection by using photoreceptors such as the cryptochrome, which is usually involved in the internal clock
318 molecular machinery (Cronin and Johnsen 2016; Falcon et al. 2020). Another example is the opsins, which are
319 the most widespread and diverse photopigments family that can be involved in visual or non-visual functions
320 (Cronin and Johnsen 2016). This ability to detect light without an eye is of great importance since it enables
321 oysters to synchronize their internal clock with daily, lunar, or seasonal natural light cycles (Mat et al. 2012;
322 Payton et al. 2017b; Payton and Tran 2019). Light detection in organisms without visual structures is also found
323 for example in corals, which are able to detect light intensity as low as moonlight (Kaniewska et al. 2015). Here,
324 we learn a bit more about the ability of the eyeless *C. gigas* to perceive light since our study shows that they
325 react differently according to the wavelength. Oysters seem to have a higher sensitivity to the blue wavelength, a
326 moderate sensitivity to the red wavelength, and the lowest sensitivity to the green wavelength. These
327 wavelength-dependent effects do not result from the penetration difference between wavelengths occurring in
328 seawater since the 1 lux intensity was measured at the oysters' level for all tested wavelengths. Therefore, the
329 wavelength-dependent effects result from real biological reactions due to the spectral sensitivity of oysters'
330 photoreceptors. One of them is the cryptochrome 1, which is known to be involved in the entrainment of oysters'
331 daily rhythm (Mat et al. 2016; Perrigault and Tran 2017). Even if its spectral sensitivity in oysters isn't
332 established, cryptochromes are believed to be receptors of blue and ultraviolet lights (Lin and Todo 2005). Here,
333 ALAN affects the cryptochrome 1 expression only in the blue wavelength condition and therefore might explain
334 that oysters' daily rhythm is more sensitive to short wavelengths. In addition to cryptochrome, other known
335 oysters' photoreceptors might be involved in the light-entrainment of *C. gigas* daily rhythm and therefore in its
336 spectral sensitivity, such as rhodopsin (Wu et al. 2018). For instance, in *Drosophila melanogaster* rhodopsins
337 seem to play a role in the light-entrainment of its internal clock in addition to cryptochromes (Senthilan et al.
338 2019). Another example is the marine bristle worm *Platynereis dumerilii*, which reproduces by synchronized
339 swarming followed by nocturnal mass spawning during specific nights of the month and especially specific

340 hours during these nights (Zurl et al. 2022). To synchronize this process, two distinct photoreceptors interact: a
341 cryptochrome (L-Cry) is used to discriminate sunlight versus moonlight and an opsin (r-Opsin1) is used to sense
342 moonrise (Zurl et al. 2022). Here, the expression of the three studied rhodopsin-like genes in oyster gills was not
343 affected by ALAN's treatments and thus does not seem to be involved in the disruption mechanism of the
344 oysters' daily rhythm. However, cryptochrome and rhodopsins might not be the only non-visual photoreceptors
345 in oysters to be involved in the daily rhythm light-entrainment, since a large diversity of them exists in the opsin
346 family for instance (Falcon et al. 2020). Furthermore, we cannot exclude that a molecular response of the tested
347 genes has been missed due to the fact that oysters have been sampled at only two-time points over the daily
348 cycle. Finally, a stronger transcriptional response might be observed in other tissues, such as the mantle edge,
349 which is, as the gills, at the interface between the environment and more inner tissue.

350

351 **Conclusion**

352 This study contributes to fill a gap by characterizing the effects of different ALAN monochromatic wavelengths
353 on a marine bivalve, the oyster *Crassostrea gigas*, a key species of coastal ecosystems. Results reveal that
354 ALAN's effects on oysters' daily rhythm are influenced by its wavelength at the behavioral and molecular levels
355 with the blue monochromatic wavelength and the white light having the most deleterious effects, while the green
356 wavelength seems to have the least important impact. Thus, to limit ALAN's effects on oysters by altering its
357 spectral composition, green nocturnal lighting could be used, instead of the red one usually advocated.
358 Considering ALAN harmful effects on oysters' daily rhythm and its potential physiological and ecological
359 consequences, which need to be investigated, limiting ALAN effects on coastal organisms is of great importance
360 and this study gives a clue to reduce its impact. However, other studies need to complete our knowledge
361 concerning the diversity of spectral sensitivities among coastal organisms to find an ALAN spectral composition
362 affecting the least the coastal communities.

363

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468

469 **Statements and Declarations**

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473 **Competing interests**

474 The authors have no relevant financial or non-financial interests to disclose.

475 **Author contributions**

476 Study conception, design and methodology, D.T., A.B., L.P.; experimentation, A.B., D.T.; molecular analysis:
477 A.B., L.P.; data treatment, A.B.; interpretation, A.B., D.T., L.P.; manuscript writing, A.B.; review and editing,
478 all authors; funding, D.T. All authors contributed critically to the drafts, and gave final approval for publication.

479 **Ethics**

480 All experiments complied with the laws in effect in France and they conformed to international ethical standards.

481 **Data accessibility**

482 The data underlying this study are available on Supplementary data.

483 **Figures & legends**

484 **Figure 1.** Behavioral daily valve activity of oysters in the control condition (< 0.05 lx) and exposed to different
 485 ALAN spectral compositions (white light, and red, green, blue wavelengths) at an intensity of 1 lx at the oyster
 486 level (n = 14–16 oysters / condition). Mean hourly valve opening duration (VOD, %) data are expressed as mean
 487 ± SE (n = 7 days). White backgrounds indicate daytime and black, yellow, red, green, and blue backgrounds
 488 indicate nighttime. Arrows show the peak of maximal hourly VOD during the day.

489 **Figure 2.** Effect of ALAN spectral composition on (A) the mean daily valve opening duration (VOD, %), (B)
 490 the mean VOD difference between daytime and nighttime, (C) the mean VOD during daytime, and (D) the mean
 491 VOD during nighttime. Data are expressed in mean ± SE (n = 7 days). Different letters indicate significant
 492 differences between ALAN conditions, and asterisks indicate significant differences between daytime and
 493 nighttime ($p < 0.05$).

494 **Figure 3.** Effect of ALAN spectral composition (white light, and red, green, blue wavelengths) on
 495 chronobiological parameters of oysters' daily rhythm at the group level: (A) the period, (B) the percent rhythm
 496 (PR), (C) amplitude of the rhythm, (D) the acrophase of the rhythm. Data are expressed in mean ± SE with the
 497 SE showing the daily variability of the group's significant rhythm parameters (n = 7 days). Different letters
 498 indicate significant differences ($p < 0.05$).

499 **Figure 4.** Effect of ALAN spectral composition on the chronobiological parameters of oysters' daily rhythm at
 500 the individual level: (A) the percentage of oysters having a daily rhythm (period range of 20–28 h), (B) the
 501 percent rhythm (PR) of the daily rhythm, (C) the amplitude of the daily rhythm, (D) the acrophase of the daily
 502 rhythm. The parameters of the daily rhythm (B–D) are shown only for oysters that maintain a significant daily
 503 rhythm. Data are expressed in mean ± SE with the SE showing the individual variability (n = 5–14 oysters /
 504 condition). Different letters indicate significant differences ($p < 0.05$).

505 **Figure 5.** Effect of ALAN spectral composition on the difference in relative mRNA level in oyster gills tissues,
 506 between daytime and nighttime (left column), between conditions during daytime (middle column), and between
 507 conditions during nighttime (right column) of four clock and clock-associated genes (mean ± SE, n = 8 oysters).
 508 White bars indicate the gene level during daytime and, colored bars during nighttime. Asterisks indicate
 509 significant differences between daytime and nighttime, and different letters indicate differences between
 510 conditions ($p < 0.05$).

511 **Table 1.** Three-Way Analysis of Variance testing the effect of the ALAN spectral composition (control
 512 condition, white light, and red, green, blue wavelengths), Daytime / Nighttime, and the genes, on the relative
 513 mRNA level on gill tissues of 13 clock and clock-associated genes. In bold, significant *p*-values ($p < 0.05$).

514	Source of variation	<i>p</i> -value
515	ALAN treatment	0.001
	Control x White	<0.001
516	Control x Red	0.042
517	Control x Green	0.011
	Control x Blue	0.005
518	Red x White	0.032
	Red x Green	0.984
	Red x Blue	0.933
	Green x White	0.499
	Green x Blue	0.999
	Blue x White	0.661
	Daytime / Nighttime	0.005
	Daytime / Nighttime x ALAN wavelength	0.002
	Daytime / Nighttime within Control	<0.001
	Daytime / Nighttime within White	0.213
	Daytime / Nighttime within Red	0.004
	Daytime / Nighttime within Green	<0.001
	Daytime / Nighttime within Blue	0.081
	Gene x Daytime / Nighttime x ALAN wavelength	0.008

Figure 1

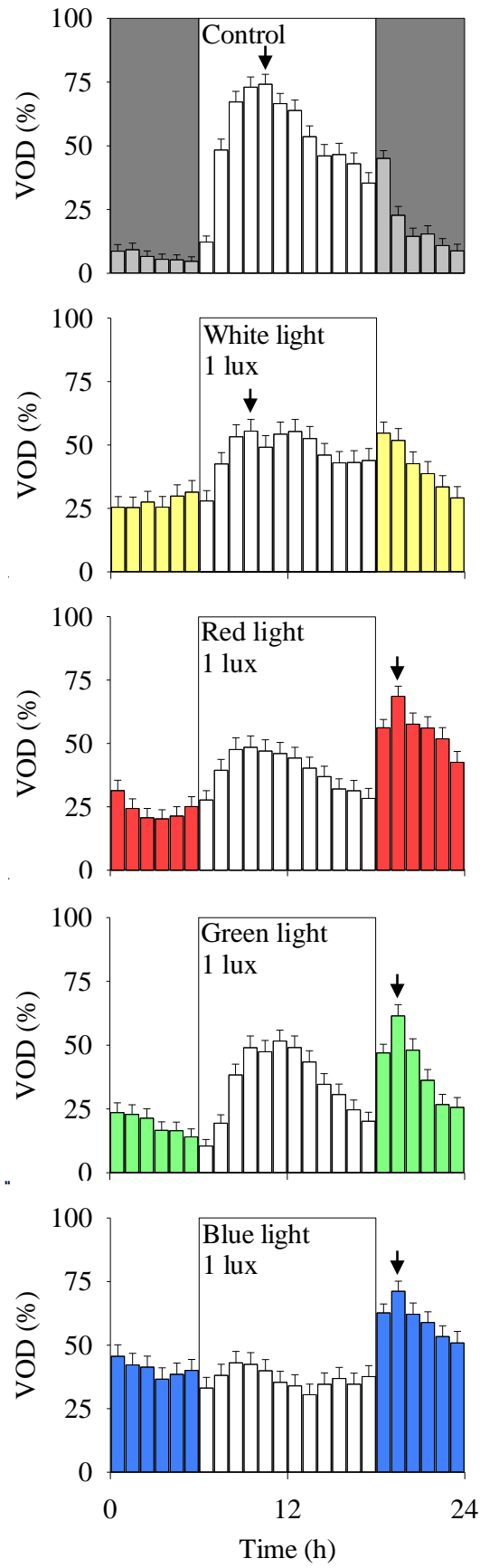


Figure 1. Botté et al.

Figure 2

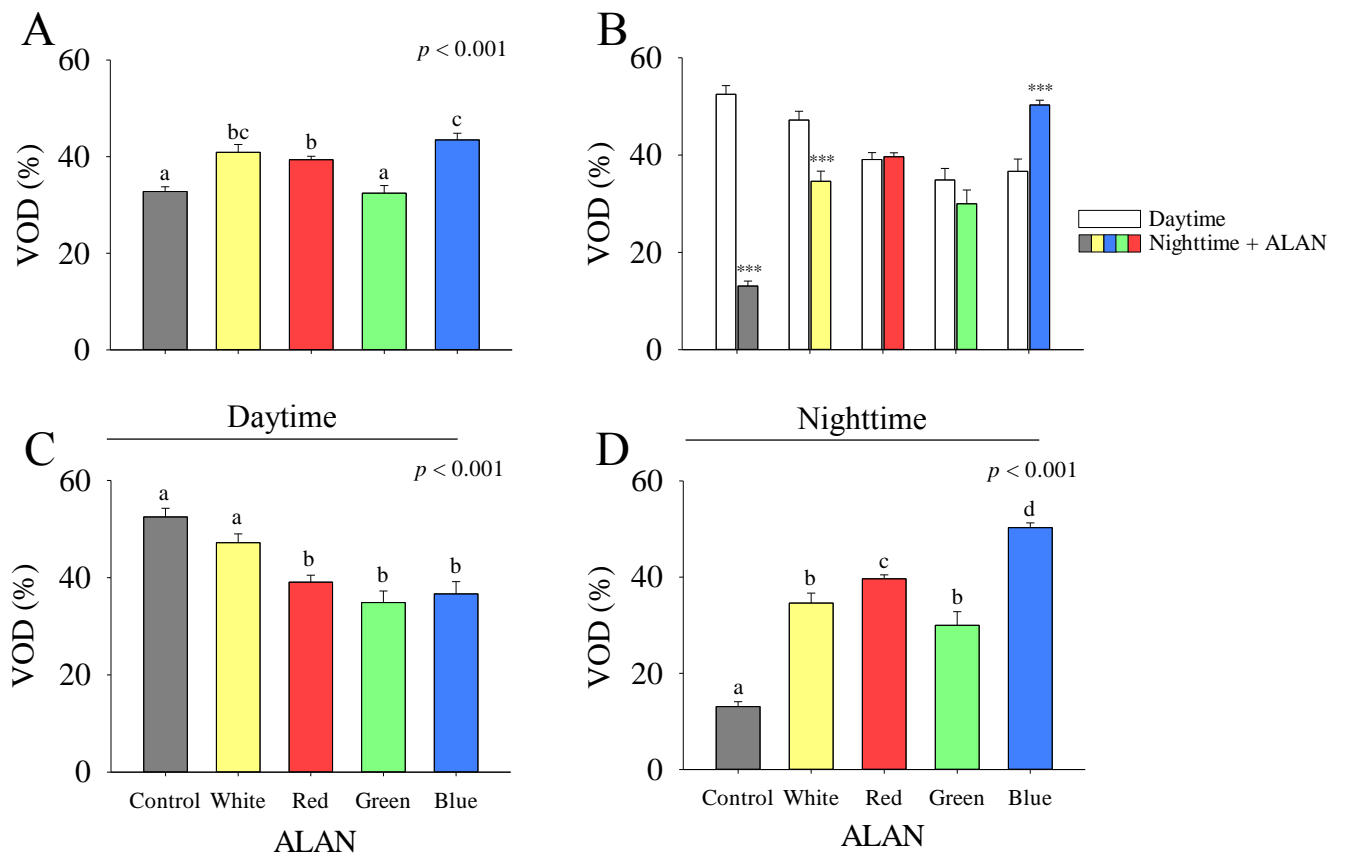


Figure 2. Botté et al.

Group level

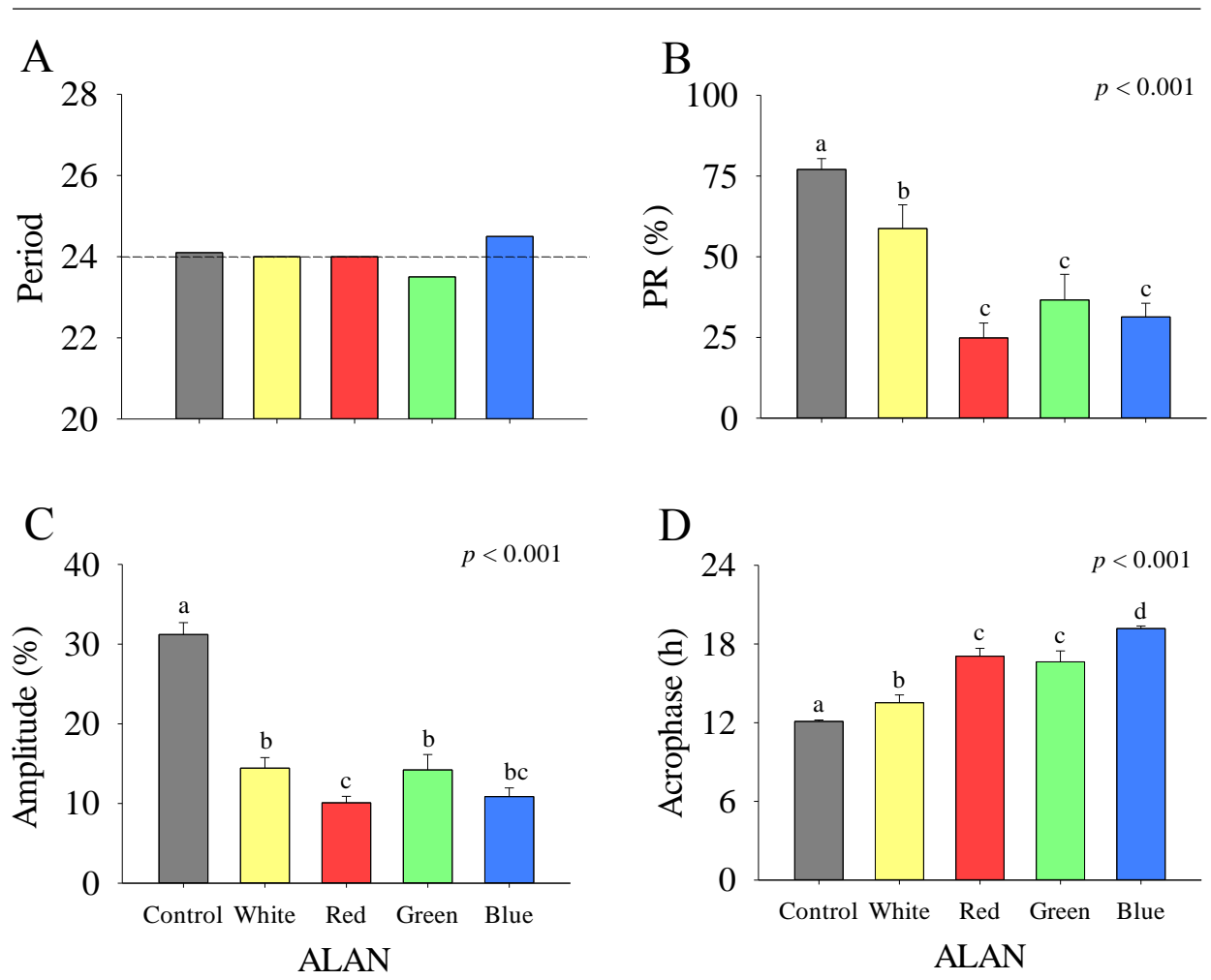


Figure 3. Botté et al.

Individual level

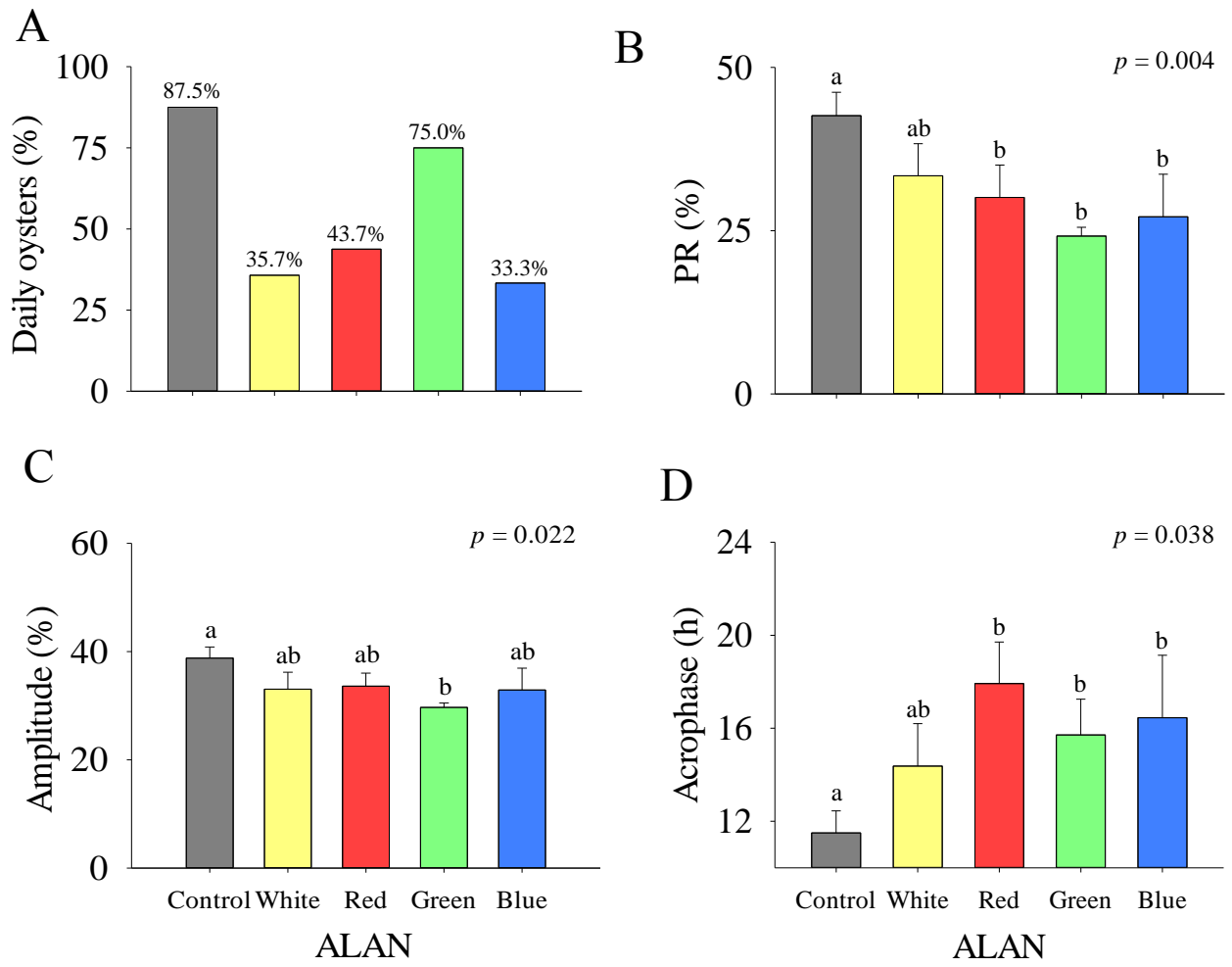


Figure 4. Botté et al.

Figure 5

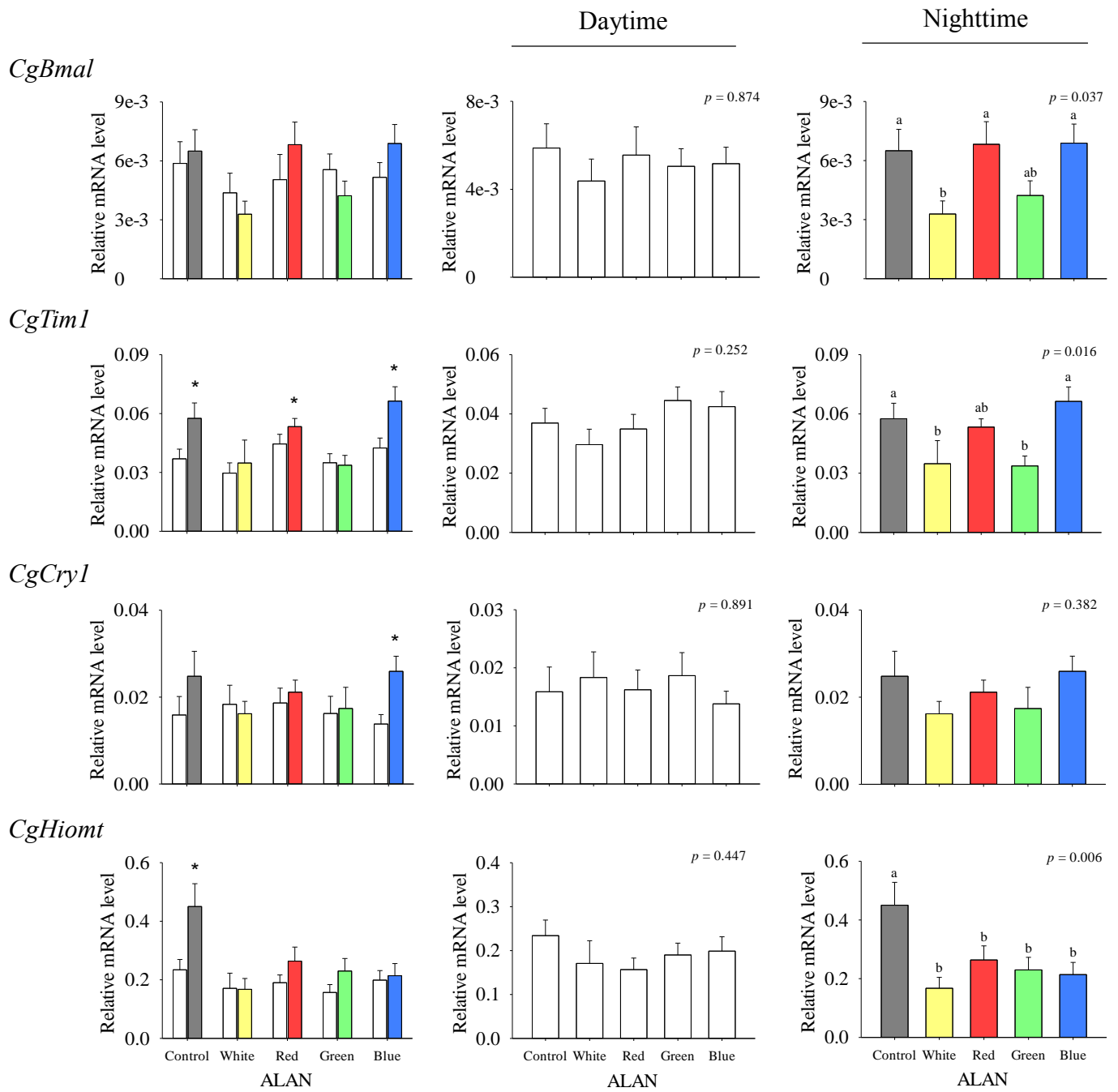


Figure 5. Botté et al.