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1        **Is part-night lighting a suitable mitigation strategy to limit ALAN's effects on the**  
2 **biological rhythm at the behavioral and molecular scales of the oyster *Crassostrea gigas*?**

3

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21 **Abstract**

22 Artificial Light at Night (ALAN) is a fast-spreading threat to organisms, especially in coastal  
23 environments, where night lighting is increasing due to constant anthropization. Considering  
24 that ALAN affects a large diversity of coastal organisms, finding efficient solutions to limit  
25 these effects is of great importance but poorly investigated. The potential benefit of one  
26 strategy, in particular, should be studied since its use is growing: part-night lighting (PNL),  
27 which consists in switching off the lights for a few hours during nighttime. The aim of this  
28 study is to investigate the positive potential of the PNL strategy on the daily rhythm of the  
29 oyster *Crassostrea gigas*, a key species of coastal areas of ecological and commercial interest.  
30 Oysters were exposed to a control condition and three different ALAN modalities. A realistic  
31 PNL condition is applied, recreating a strategy of city policy in a coastal city boarding an  
32 urbanized bay (Lanton, Arcachon Bay, France). The PNL modality consists in switching off  
33 ALAN direct sources (5 lx) for four hours (23-3 h) during which oysters are in darkness.  
34 Then, a PNL + skyglow (PNL+S) modality reproduces the previous one mimicking a skyglow  
35 (0.1 lx), an indirect ALAN source, during the direct lighting switch off, to get as close as  
36 possible to realistic conditions. Finally, the third ALAN condition mimics full-night direct  
37 lighting (FNL). Results revealed that PNL reduces some adverse effects of FNL on the  
38 behavioral daily rhythm. But, counterintuitively, PNL+S appears more harmful than FNL for  
39 some parameters of the behavioral daily rhythm. PNL+S modality is also the only one that  
40 affect oysters' clock and melatonin synthesis gene expression, suggesting physiological  
41 consequences. Thus, in realistic conditions, the suggested PNL mitigation strategy, in the  
42 presence of skyglow, might be not beneficial, seeing worse for a coastal organism such as the  
43 oysters.

44 **Keywords (6/6):** ALAN; oysters' behavior; valvometry; daily rhythm; skyglow; part-night  
45 lighting

## 46        **1. Introduction**

47        Artificial Light at Night (ALAN) is a widespread pollution type, which can affect zones  
48        supposed to be preserved from human activities, such as marine or terrestrial protected areas  
49        (Davies et al., 2016; Davies and Smyth, 2018; Gaston et al., 2015). In these areas and more  
50        globally all over the world, ALAN is a threat to a wide diversity of organisms (from nocturnal  
51        to diurnal and from terrestrial to aquatic species) and in a large range of intensities (from  
52        below 1 lx to more than 100 lx) (Brayley et al., 2022; Sanders et al., 2021). A multitude of  
53        effects on organisms caused by a disruption of natural nocturnal lighting levels have been  
54        described, such as effects on sleep, circadian rhythm, endocrinology, orientation, migration,  
55        foraging, communication, or reproduction (Brayley et al., 2022; Burt et al., 2023; Sanders and  
56        Gaston, 2018; Sanders et al., 2021; Svechkina et al., 2020). Moreover, this threat to  
57        biodiversity is spreading fast, with a 2.2 % increase in surface area and a 1.8% increase in  
58        radiance per year (Kyba et al., 2017a). Thus, according to Davies and Smyth (2018), ALAN  
59        should be one of the main focuses of the global change research of this century. As a matter of  
60        fact, the number of studies focusing on ALAN's impacts increased in the last 20 years  
61        (Davies and Smyth, 2018). However, in addition to investigating ALAN's negative effects,  
62        finding solutions to limit them is also necessary. In this way, suggestions have been made  
63        including the protection and creation of dark areas, the reduction of the trespass of lighting in  
64        areas not supposed to be lit, the choice of dimmer intensities as well as narrower spectra, and  
65        the reduction of lighting duration (part-night lighting) (Gaston et al., 2012; Longcore and  
66        Rich, 2016). Part-night lighting (PNL) could be considered one of the best options since it  
67        involves turning off the lights during a part of the night, returning darkness to organisms  
68        along with an economic interest. To ensure that this strategy is helpful to ecosystems, it is  
69        necessary to test its efficiency and effects on organisms, which is currently poorly  
70        investigated. For example, Davies et al. (2017) showed that switching off ALAN (14 lx) for 4

71 hours from 00 h to 04 h was more beneficial to grassland invertebrate assemblages than a full-  
72 night lighting (FNL). However, in some cases, PNL can be more harmful than FNL. This is  
73 the case for the aphid *Sitobion avenae*, for which PNL induces the suppression of the colony  
74 growth whereas FNL allows a recovery of aphid number over time (Heinen et al., 2023). In  
75 addition, in the environment, during the supposed dark part of the night, organisms will  
76 certainly still be exposed to ALAN through its indirect source, the skyglow. This skyglow is  
77 created by artificial light scattered in the atmosphere leading to a brightening of the sky that  
78 can extend to hundreds of kilometers from its source at an estimated intensity ranging from  
79 0.007 to 0.55 lx (Gaston, 2018; Hänel et al., 2018; Luginbuhl et al., 2014). Therefore, a city  
80 applying PNL strategies could still be exposed to ALAN throughout nighttime through the  
81 skyglow emitted for instance by FNL of a distant large city.

82 ALAN is deeply present in coastal environments, with 1.9 million km<sup>2</sup> of the world's coastal  
83 seas exposed to biologically important ALAN at a depth of 1 m (Smyth et al., 2021). Even if  
84 the study of ALAN's impacts on marine environments increased these last few years, our  
85 knowledge on this topic remains poor compared to terrestrial environments, especially  
86 concerning the potential benefits of mitigation strategies. The oyster *Crassostrea gigas* is a  
87 key coastal species likely exposed to ALAN since it is a sessile mollusk living in intertidal or  
88 subtidal areas. This oyster of high commercial interest has been introduced in many coastal  
89 environments in which it is now present as cultured and wild oysters and is a keystone species  
90 (FAO, 2016; Ruesink et al., 2005). In oysters, ALAN at low and realistic intensities has been  
91 shown to disrupt their daily rhythm at the behavioral and molecular scales, suggesting  
92 consequences on physiological processes (Botté et al., 2023). The daily rhythm of oysters'  
93 valve activity has been well characterized as well as its endogenous clock (Mat et al., 2012;  
94 Payton et al., 2017a; Perrigault and Tran, 2017; Tran et al., 2020). Biological rhythms are  
95 ubiquitous and found their origin in each cell with an endogenous and autonomous clock that

96 coordinates physiological processes between them and with environmental timing using  
97 reliable cues such as natural light cycles (Bell-Pedersen et al., 2005; Partch et al., 2014).  
98 Being synchronized with natural light cycles enable physiological processes and behavior to  
99 be optimized, giving adaptive advantages to organisms (Emerson et al., 2008; Golombek and  
100 Rosenstein, 2010). Furthermore, biological rhythms contribute to maintain stable ecosystems  
101 by organizing temporally intra and interspecific interactions (Helm et al., 2017). However, by  
102 disrupting natural nocturnal light levels, ALAN can prevent clock synchronization with  
103 environmental light cycles and therefore have consequences on organisms' physiology and  
104 behavior (Botté et al., 2023; Botté et al., Unpublished results; Sanders et al., 2021). These  
105 findings highlight the importance of studying efficient mitigation strategies to limit ALAN's  
106 impacts (Gaston and Bennie, 2014; Gaston et al., 2017). In *C. gigas*, ALAN's effects on its  
107 daily rhythm can be reduced by decreasing ALAN intensities ( $< 0.1$  lx), which is largely too  
108 low for public lighting, and by changing its spectral composition to green wavelength (Botté  
109 et al., 2023; Botté et al., Unpublished results). However, these two strategies do not suppress  
110 ALAN's effects, which would be possibly further reduced by using PNL strategy.

111 This study investigates the potential of PNL mitigation strategy to reduce ALAN's impact on  
112 the oysters' daily rhythm. Thus, we evaluate the effects of several ALAN modalities on the  
113 valve behavior at the group and individual scales as well as on the expression of clock, light  
114 perception, and clock-associated genes of oysters. The first ALAN modality mimics PNL  
115 with total darkness when the nocturnal lighting is switched off. To approximate real  
116 environmental conditions, lighting off times reproduce PNL strategy applied in Lanton  
117 (Arcachon Bay, France), a city boarding a very anthropized bay, with an important oyster  
118 farm activity. Moreover, the intensity used to imitate ALAN direct sources (5 lx) was  
119 previously measured underwater, directly on the field, in the same bay. The second ALAN  
120 modality also reproduces PNL strategy with in addition the presence of a skyglow light

121 intensity (0.1 lx) throughout nighttime, to be as close as possible to realistic conditions.  
122 Finally, the third ALAN condition mimics FNL that is still largely applied in the cities policy  
123 strategy. We hypothesized that ALAN would affect oysters' daily rhythm at the behavioral  
124 and molecular levels no matter its modality. A priori, we suggested that the use of PNL  
125 strategy would reduce these ALAN's negative effects on the oysters' daily rhythm, although  
126 we expected that the dispensed advantages by PNL strategies would be reduced in presence of  
127 skyglow throughout nighttime.

128

## 129 **2. Materials and methods**

### 130 *2.1. Experimental protocol*

131 The experiments were conducted in the Marine Station of Arcachon from May to August  
132 2022 on 130 oysters ( $83.3 \pm 1.3$  mm shell length;  $38.7 \pm 0.6$  mm shell width; mean  $\pm$  SE)  
133 coming from an oyster's farm source of the Arcachon bay (France). The experiments were  
134 realized using four tanks (L x W x H: 74.8 x 54.8 x 40.8 mm) placed in an isolated room and  
135 equipped with an antivibration bench to reduce disturbances to oysters' behavior. Each tank  
136 was continuously supplied with natural, oxygenized, and filtered ( $< 1\mu\text{m}$ ) seawater of stable  
137 composition (temperature =  $20.3 \pm 0.1$  °C, pH =  $8.0 \pm 0.1$ , salinity =  $34.9 \pm 0.1$  ‰) pumped  
138 from the Arcachon bay. For each experimental condition, 32 oysters were used. The valve  
139 behavior of 16 of them was continuously recorded and 24 oysters (including 8 of them studied  
140 for valve behavior) were used for molecular analyses. Oysters were not fed during the  
141 experiments and were acclimated for 7 – 10 days before the experiment to an L:D 14:10 cycle  
142 with daytime from 5 to 19 h (all times noted in Universal Time Coordinated, UTC) (Fig. S1).  
143 Daylight intensity varied gradually with a maximum intensity of  $1388.47 \pm 260.89$  lx (mean  
144  $\pm$  SE) (Fig. S2) between 11:30 to 12:30 h to mimic the daily cycle of light using  
145 programmable white (391–720 nm, peak at 553 nm; Fig. S3A) LED light bars (MH3SP3

146 DSunY). Following acclimation, 4 conditions were tested, including a control condition and  
147 three ALAN exposure modalities (Fig. S1) during 10 days, using white (411–687 nm, peak at  
148 563 nm; Fig. S3B-C) LED strips (MiBoxer Mi-Light WL5): part-night lighting (PNL), part-  
149 night lighting + skyglow (PNL+S), and full-night lighting (FNL). For each experimental  
150 condition, underwater illuminances were measured at five positions in the tank, at oysters'  
151 level (Fig. S2A) using a handheld spectroradiometer (Blue-Wave UVN-100, StellarNet Inc.).  
152 The control group was in complete darkness at night, with a light intensity below the  
153 detection limit of the spectroradiometer ( $< 0.05$  lx). PNL condition involves an ALAN  
154 exposure from 19 to 23 h, and from 3 to 5 h at an intensity of 5 lx. The choice of the ALAN  
155 intensity of 5 lx (Fig. S2B) was motivated by measures made at 19:15 h in May (civil  
156 twilight) underwater at the lighting Eyrac Pier in Arcachon Bay, France (44.64902, -  
157 1.163639) at a depth of 1.5 m. Thus, this intensity best mimics ALAN direct sources. During  
158 the 4 h interruption of ALAN, the oysters were in complete darkness ( $< 0.05$  lx) (Fig. S1).  
159 PNL+S group was subject to the same ALAN exposure modality as the previous condition,  
160 except during the 4 h interruption of ALAN direct source, where oysters were exposed to the  
161 indirect source of ALAN from 23 to 3 h with an intensity of 0.1 lx (Fig. S1; Fig. S2B) to  
162 mimic the presence of the skyglow. Finally, FNL condition is characterized by an exposure to  
163 direct ALAN sources (5 lx) throughout nighttime from 19 to 5 h (Fig. S1). For each  
164 experimental condition, the valve behavior of 16 oysters was continuously recorded using the  
165 High Frequency–Non Invasive (HFNI) valvometer technology (Andrade et al., 2016). On the  
166 10<sup>th</sup> day of each experiment, gill tissues of 8 oysters were sampled at three sampling times.  
167 The first sampling time occurs at 12 h when the daylight intensity is at its maximum. The two  
168 remaining sampling times occur during nighttime at 22 h when oysters are exposed to ALAN  
169 direct sources in all conditions, and at 1 h when ALAN's intensity varies across experimental



170 conditions. Gills tissues were stored in RNAlater (Invitrogen) at  $-80^{\circ}\text{C}$  for further molecular  
171 analysis.

## 172 2.2. *Crassostrea gigas* behavior measurements and chronobiological analyses

173 Measurement of oyster's valve activity is performed using a HFNI valvometer technology  
174 (see details in Andrade et al. (2016)), for which lightweight's electromagnets are glued on the  
175 oysters' valve. An electromagnetic current is generated between these two electromagnets,  
176 linked to a valvometer device by flexible wires, and allows to continuously measure oyster  
177 valve activity. For each oyster, the signal is recorded every 4.8 s and data were processed  
178 using Labview 8.0 (National Instrument, Austin, TX, USA). Here we focused on the oysters'  
179 hourly Valve Opening Duration (VOD) meaning that we established for each individual and  
180 each hour the percentage of time spent with valves open. For example, an individual having  
181 its valves closed during a whole hour corresponds to a VOD of 0 % while an oyster with its  
182 valves open during one hour corresponds to a VOD of 100 %. From the hourly VOD data,  
183 chronobiological analyses were performed at the group and individual scales using the  
184 software Time Series Analysis Serie Cosinor 8.0 (Expert Soft Technologies). The first step is  
185 to evaluate the data quality using the autocorrelation diagram to control the absence of  
186 random repartition of data and to determine the absence of stationary character using the  
187 Partial Autocorrelation Function (PACF) calculation (Gouthière and Mauvieux, 2003). The  
188 second step involves the Lomb and Scargle periodogram used to search for periodicities in the  
189 dataset, which are considered significant for  $p > 0.95$  (Scargle, 1982). Then, the two main  
190 rhythmicities of the dataset were modeled using the Cosinor model. This model uses a cosine  
191 function calculated by regression (Bingham et al., 1982), and for a given period, the model  
192 is written as:  $Y(t) = A\cos\left(\frac{2\pi t}{\tau} + \phi\right) + M + \varepsilon(t)$  where A is the amplitude (difference  
193 between the average level and the highest value of the rhythm),  $\tau$  the given period (interval  
194 between two identical events),  $\phi$  the acrophase (the highest value of the rhythm), M the mesor

195 (average level of the rhythm), and  $\varepsilon(t)$  the relative error. This model gives a chronobiometric  
196 parameter, the percent rhythm (PR), which is the percentage of cyclic behavior explained by  
197 the model, that represents the strength of the rhythm. Two tests validated the calculated model  
198 and the existence of rhythmicity: the ellipse test must be rejected, and the probability for the  
199 null amplitude hypothesis must be lower than 0.05.

### 200 *2.3.Total RNA extraction and cDNA synthesis*

201 From gill tissues, the total RNA was extracted using an SV Total RNA Isolation System kit  
202 (Promega). The quantity and quality of the total RNA were evaluated by spectrophotometry  
203 (OD230, OD260, OD280). Then a RNA reverse transcription was realized using Goscript™  
204 Reverse Transcription System kits (Promega).

### 205 *2.4.Real-Time PCR analyses*

206 Real-Time qPCR was performed using GoTaq® qPCR Master Mix kit (Promega) and primers  
207 sets of clock (*CgClock*, *CgBmal*, *CgCry*, *CgPer*, *CgTim1*, *CgCry1*, *CgRev-erb*, *CgRor*), light  
208 perception (*CgRhodopsin-like 1*), clock-associated genes (*CgHiomt-like*, *CgOctβ2*), and  
209 housekeeping genes (*CgEfl*, *Cg28S*, *CgGadph*) (Tab. S1). The expression level of clock and  
210 clock-associated genes were evaluated using the comparative Ct method  $2^{-\Delta Ct}$  (Livak and  
211 Schmittgen, 2001), where  $\Delta Ct = Ct_{(\text{target gene})} - Ct_{(\text{housekeeping gene})}$ . Based on stability values,  
212 gene's expression was normalized with the geometric mean of the two housekeeping genes  
213 *CgGadph* and *CgEfl* (Xie et al., 2012).

### 214 *2.5.Statistical analyses*

215 The SigmaPlot software (version 13.0; Systat Software, USA) was used to perform the  
216 statistical analyses. T-tests were used to realize two groups multiple comparisons after  
217 checking assumptions (normality of data and equal variance). When these assumptions  
218 weren't validated, the non-parametric Mann Whitney rank sum was performed. For multiple

219 comparisons, one-way ANOVA were performed after checking assumptions (normality of  
220 data and equal variance) followed by the Student-Newman-Keuls test for all pairwise multiple  
221 comparisons in case of significant differences. If the assumptions weren't checked, the  
222 Kruskal-Wallis One-Way ANOVA on ranks test was performed. For all test results, a  
223 difference was considered significant when  $p < 0.05$ .

224

### 225 3. Results

226 Figure 1 shows the overall oysters' behavioral mean daily pattern for the control condition  
227 and the three ALAN conditions (PNL, PNL+S, FNL) with a black arrow indicating the  
228 maximal activity during the day. The oysters in the control condition show a strong diurnal  
229 pattern with a daily VOD peak at 7–8 h. When oysters are exposed to the three tested ALAN  
230 modalities, their diurnal activity pattern is disrupted with a 13 h shift of the oysters' activity  
231 peak delayed (peak at 20–21 h) from daytime to nighttime in FNL. The daily VOD peak  
232 during PNL and PNL+S is still present during daytime although the mean hourly VOD  
233 increases during nighttime and decreases during daytime. Then, statistical analyses were  
234 performed first at the group level (Fig. 2 and 3). Figure 2A shows significant daytime /  
235 nighttime differences of the mean VOD for control oysters with an increased VOD during  
236 daytime, revealing a diurnal behavior ( $p < 0.001$ ). This significant difference tends to  
237 decrease for PNL and PNL+S conditions, caused by a decrease in the mean VOD during  
238 daytime as well as an increase during nighttime, which is significantly higher for the PNL+S  
239 condition. Finally, this daytime / nighttime difference disappears during FNL, characterized  
240 by a significantly more important decrease of the mean VOD during daytime compared to  
241 PNL and PNL+S conditions. Figure 2B focus on the mean VOD difference between  
242 conditions during different parts of the night. From 19 to 23 h, in all of the tested ALAN  
243 modalities oysters are exposed to a direct source of ALAN (5 lx) and for all of the conditions

244 their mean VOD increases significantly compared to control oysters ( $p < 0.001$ ). From 23 to 3  
245 h ALAN intensity varies between condition with a null intensity ( $< 0.05$  lx) for PNL, a  
246 skyglow intensity (0.1 lx) for PNL+S and an ALAN direct source intensity (5 lx) in FNL.  
247 During this period, oysters mean VOD remains significantly higher for all ALAN modalities  
248 compared to the control ( $p < 0.001$ ) but with weaker effects with PNL, intermediate with FNL  
249 and the most important increase for PNL+S. Finally, from 3 to 5 h, the oysters are once more  
250 exposed to ALAN direct source (5 lx) in all ALAN conditions, inducing a significant increase  
251 of their mean VOD in all ALAN modalities compared to control oysters with the least  
252 important increase for PNL and FNL and the most important for PNL+S.

253 Figure 3A shows Lomb and Scargle periodograms at the group level revealing that in each  
254 experimental condition oysters' mean VOD express two significant and distinct periodicities  
255 ( $\tau_1$ ,  $\tau_2$ ) of 12 h and 24 h, revealing two rhythms, validated by the Cosinor model ( $p < 0.001$ ),  
256 more or less pronounced according to the condition. In the control condition, oysters'  
257 behavior expresses a first significant period ( $\tau_1$ ) of  $24.00 \pm 0.02$  h (mean  $\pm$  SD) and a less  
258 pronounced period ( $\tau_2$ ) of  $12.00 \pm 0.08$  h. In the PNL+S condition, VOD data also express a  
259 first significant period ( $\tau_1$ ) of  $23.80 \pm 0.05$  h and a less pronounced period ( $\tau_2$ ) of  $12.00 \pm 0.16$   
260 h, but with a reduced difference between the 24 h and 12 h period compared to the control  
261 condition. On the contrary, the PNL and FNL conditions show a more pronounced 12 h period  
262 ( $\tau_1$ ) ( $12.00 \pm 0.12$  h and  $12.00 \pm 0.05$  h respectively) compared to the 24 h one ( $\tau_2$ ) ( $23.80 \pm$   
263  $0.03$  h and  $23.80 \pm 0.08$  h respectively), with a difference more pronounced between the two  
264 significant periods for FNL. The figure 3B shows the results of the analyses of  
265 chronobiological parameters (percent rhythm (PR), mesor, amplitude, acrophase) for each of  
266 the two main significant periodicities (12 h and 24 h) to reveal ALAN effects according to its  
267 modality on these two rhythms at the group level (results are detailed in Table S2).  
268 Concerning the 12h-rhythm, the tested ALAN modalities have no significant effect on the

269 strength of the rhythm (PR) ( $p = 0.102$ ), while the mesor significantly increases for PNL and  
270 PNL+S but not for FNL compared to the control ( $p = 0.004$ ). The 12h-rhythm amplitude is  
271 significantly lower for PNL+S and FNL only compared to the control ( $p < 0.001$ ). Finally, the  
272 12h-rhythm acrophase is significantly delayed ( $p = 0.046$ ) for PNL and PNL+S but not for  
273 FNL even if an increasing trend is noticeable. ALAN conditions also disrupt the 24h-rhythm  
274 parameters. All ALAN modalities induce a significant decrease of the strength of the rhythm  
275 with a significantly more important decrease in the FNL condition. The mesor of the 24h-  
276 rhythm significantly increases during PNL and PNL+S only ( $p = 0.003$ ) and its amplitude  
277 significantly decreases for all of the ALAN conditions compared to the control, with a  
278 significantly more important decrease for FNL ( $p < 0.001$ ). Finally, the 24h-rhythm shows a  
279 significantly delayed acrophase ( $p < 0.001$ ) for PNL and FNL but not for PNL+S.

280 Then, statistical analyses were conducted at the individual level on oysters keeping a  
281 significant daily rhythm (Fig. 4 and 5). The analyses on the daytime / nighttime difference of  
282 their mean daily VOD reveals that in all conditions a diurnal pattern is expressed ( $p < 0.001$ ;  
283 Fig. 4A). However, this difference is reduced for all ALAN modalities, due on one hand to a  
284 decrease of the mean VOD during daytime with a more important decrease for FNL. On the  
285 other hand, oysters' activity shows a significant increase for all ALAN modalities during  
286 nighttime compared to the control, but significantly higher for PNL+S and FNL. Then, Fig.  
287 4B shows mean VOD of rhythmic oysters during different parts of the night. From 19 h to 23  
288 h, when oysters in all ALAN condition are exposed to a direct ALAN source (5 lx), their  
289 mean VOD significantly increases compared to the control condition ( $p < 0.001$ ). From 23 to  
290 3 h ALAN intensity varies between conditions with a null intensity ( $< 0.05$  lx) for PNL, a  
291 skyglow intensity (0.1 lx) for PNL+S and an ALAN direct source intensity (5 lx) for FNL.  
292 Results show that the oysters mean VOD significantly increases in presence of ALAN no  
293 matter its intensity, but with a significantly more important increase for PNL+S and FNL

294 compared to PNL ( $p < 0.001$ ). Finally, for the last part of the nighttime (from 3 h to 5 h),  
295 oysters were once again exposed to a direct ALAN source (5 lx) in each ALAN condition. On  
296 the contrary to the first part of the night, there were no significant difference of the mean  
297 VOD between the control and PNL conditions. However, the mean VOD significantly  
298 increases during PNL+S and FNL, with a significantly more important increase for PNL+S ( $p$   
299  $< 0.001$ ).

300 Figure 5A reveals that the percentage of oysters maintaining a daily rhythm is reduced in all  
301 ALAN conditions. This percentage decreases from 87.5% in the control condition to 56.3%  
302 for PNL and FNL and to 31.3% for PNL+S, which corresponds respectively to a loss of 35.7  
303 % and 64.2 % of individuals having a daily rhythm compared to the control. Therefore,  
304 PNL+S is the ALAN modality causing the most important loss of oysters having a daily  
305 rhythm. Then, chronobiological analyses were performed on these oysters that kept a  
306 significant daily behavioral rhythm (results are detailed in Table S3). First, ALAN modalities  
307 do not affect the mesor of their behavioral rhythm ( $p = 0.817$ ; Fig. 5B). However, the other  
308 parameters are affected by ALAN such as the strength of the rhythms (PR), which  
309 significantly decreases for FNL but not for PNL and PNL+S even if a decreasing trend is  
310 observed ( $p = 0.012$ ; Fig. 5C). Moreover, the rhythm's amplitude is significantly lower in  
311 PNL and FNL compared to the control, with a decreasing trend for PNL+S ( $p = 0.008$ ; Fig.  
312 5D). Finally, ALAN conditions do not significantly affect the acrophase ( $p = 0.427$ ; Fig. 5E).

313 Finally, Figure 6 shows the effects of ALAN modalities on the expression of clock, light  
314 perception and clock-associated genes at three sampling times: one during daytime (12 h, left  
315 column), and two during nighttime (22 h and 1 h, middle and right column respectively).  
316 During daytime, ALAN shows a significant effect only on the expression of the clock gene  
317 *CgTim1* with a significant decrease for PNL+S only ( $p = 0.015$ ). Furthermore, at 12 h the  
318 expression of the light perception gene *CgRhodopsin-like1* tends to decrease along the ALAN

319 modalities tested ( $p = 0.077$ ). The first sampling time during nighttime (22 h) corresponds to  
320 an intensity of 5 lx for all of the ALAN modalities. At this time, the results show a significant  
321 decrease in the expression of the clock-associated gene *CgHiomt-like* for PNL+S only ( $p =$   
322  $0.026$ ). The expression of the other studied genes is not significantly affected at this sampling  
323 time. The last sampling time occurs at 1 h, when the ALAN intensity varies among the  
324 experimental conditions: no ALAN ( $< 0.05$  lx) for the control and PNL, a skyglow intensity  
325 (0.1 lx) for PNL+S, and a direct source of ALAN intensity (5 lx) for FNL. At this time, the  
326 expression of three clock genes (*CgBmal*, *CgCry2*, *CgRev-erb*) and one clock-associated gene  
327 (*CgHiomt-like*) significantly decreases during PNL+S only. Finally, the table 1 shows the  
328 results of a Three-Way ANOVA, testing the effects of the experimental conditions (control,  
329 PNL, PNL+S, and FNL), sampling times (12, 22 and 1 h), and different gene expression on  
330 the overall relative mRNA levels of the eleven genes studied. This analyses reveals a  
331 significant effect of ALAN conditions on the overall gene expression ( $p < 0.001$ ). More  
332 precisely, the expression is significantly different between PNL+S and all of the other  
333 conditions, including the control. The Three-Way ANOVA also show a significant difference  
334 between the sampling times for PNL+S only ( $p = 0.001$ ).

335

#### 336 4. Discussion

337 The objective of this study is to investigate the potential benefits of using PNL as an ALAN  
338 mitigation strategy on the daily rhythm of the oyster *C. gigas*. Results show that all of the  
339 tested ALAN modalities (PNL, PNL+S and FNL) affect oysters' behavioral daily rhythm but  
340 with reduced effects with PNL, intermediate effects with FNL, and the most important effects  
341 with PNL+S. PNL+S is also the only ALAN modality to affect the expression of genes  
342 involved in the circadian clock machinery and in the melatonin synthesis.

343 First, our study show that ALAN causes harmful effects on oysters' behavioral daily rhythm  
344 at the group and individual levels no matter its modality. The valve behavior of control  
345 oysters follows a daily rhythm, characterized by a main period of 24 h, and also show a weak  
346 but significant period of 12 h. When exposed to ALAN, this 12h-rhythm is more pronounced  
347 for PNL + S and even becomes the dominant rhythm for PNL and FNL, while the 24h-rhythm  
348 is still present but less pronounced. The presence of these behavioral 12h-rhythm could  
349 suggest that the oysters' behavior follows a more or less marked bimodal pattern  
350 characterized by one peak activity during daytime and another during nighttime spaced about  
351 12 h apart. This bimodal pattern has been already shown and discussed in *C. gigas* (Mat et al.,  
352 2014; Mat et al., 2016; Tran et al., 2011; Tran et al., 2020) suggesting a real circadian bimodal  
353 rhythm or a unimodal circadian rhythm coupled to a circatidal rhythm in free-running. Then,  
354 whether we analyze the oysters' 12h- or 24h-rhythms, ALAN affects chronobiological  
355 parameters of both, whatever its modality, as it has been observed on their 24h-rhythm at  
356 several ALAN intensities (from 0.1 to 25 lx) and wavelengths (white, blue, green, and red  
357 lights) (Botté et al., 2023; Botté et al., Unpublished results).

358 Biological rhythms such as daily rhythm enable the synchronization of multiple physiological  
359 processes with the environment, such as foraging or reproduction, etc, giving adaptive  
360 advantages to organisms (Bradshaw and Holzapfel, 2010; Golombek and Rosenstein, 2010;  
361 Kaniewska et al., 2015). The diurnal behavior of *C. gigas* during spring and summer, as in  
362 this study, enables oysters to feed during daytime when the phytoplankton is the most  
363 abundant, answering the high energy needs especially due to growth and gametogenesis  
364 (Enríquez-Díaz et al., 2008; Mat et al., 2012; Payton et al., 2017b). The disruption during  
365 PNL and PNL+S or loss during FNL of this diurnal behavior can induce a less efficient  
366 feeding, which might not answer the oysters' energy needs, and therefore induce potential  
367 physiological consequences, reducing its fitness. For instance, a reduced growth has been



368 observed in the juvenile toad *Anaxyrus americanus* at an intensity of 15 lx as well as in the  
369 gammarids *Gammarus jazdzewskii* and *Dikerogammarus villosus* at an intensity of 2 lx  
370 (Czarnecka et al., 2021; Dananay and Benard, 2018). Other oysters' biological rhythms might  
371 be disrupted by ALAN. ALAN intensity, ranging from 0.007 (skyglow) to more than 100 lx  
372 (direct ALAN source), very often exceeds that of moonlight, which has a maximum of 0.1 –  
373 0.3 lx in full moon (Gaston, 2018; Hänel et al., 2018; Kyba et al., 2017b). Masking the  
374 moonlight cycle could alter its perception by oysters and thus disrupt their behavioral lunar  
375 rhythm (Payton and Tran, 2019). ALAN masking effects on the natural lunar cycle have been  
376 investigated in the corals *Acropora millepora* and *Acropora digitifera* for which ALAN  
377 induces a delay in corals' gametogenesis, having for consequence the desynchronization of  
378 their spawning (Ayalon et al., 2021). ALAN is also susceptible to alter oysters' reproduction  
379 success since their spawning synchronization mainly depends on daily, lunar, and annual  
380 cycles, as many parameters that ALAN can disrupt by altering their perception by oysters  
381 (Bernard et al., 2016; Payton et al., 2017b). Thus, the disruption of oysters' behavioral  
382 rhythms by ALAN is likely to have harmful consequences on their physiology and fitness as  
383 well as the ecosystems functioning since *C. gigas* provide important ecosystems services (van  
384 der Schatte Olivier et al., 2018).

385 Given the potential physiological and ecological consequences of ALAN exposure, limiting  
386 its effects is necessary to preserve ecosystems and can be done by testing and applying  
387 suggested mitigation strategies (dark protected areas, dim lighting, narrower spectrum, limit  
388 lighting duration...) (Gaston et al., 2012). Previous studies showed that dimming ALAN  
389 intensity up to 0.1 lx or restricting its spectrum to green wavelengths could reduce, but not  
390 suppress, ALAN's effects on oysters daily rhythm (Botté et al., 2023; Botté et al.,  
391 Unpublished results). This study brings new knowledge on the potential benefits of another  
392 mitigation strategy on oysters, which consist in changing ALAN timing and duration.

393 Potential ecological advantages of PNL have been rarely studied and mainly on bats, which  
394 make this study one of the first to investigate PNL effects on coastal organisms and especially  
395 the first to investigate them on a bivalve to our knowledge. Our results reveal that ALAN  
396 effects on oysters' daily rhythm vary according to its modality. PNL induces the least  
397 important effects on the oysters' daily rhythm, while FNL shows intermediate effects and  
398 surprisingly, PNL+S induces the most important effects. Thus, PNL mitigation strategy  
399 applied here limits ALAN effects on the oysters' daily rhythm (nighttime VOD increase,  
400 daytime VOD decrease, the strength and amplitude of the rhythm at the group level) but only  
401 when skyglow is not present throughout nighttime. PNL strategy advantages have already  
402 been showed in some of the few studies investigating the ecological interest of this mitigation  
403 strategy. For instance, Bauer et al. (2022) showed that the shift of feeding activity from  
404 daytime to nighttime in the sea urchins *Paracentrotus lividus* was reduced when a PNL  
405 strategy (ALAN switched off from 0 to 5 h) was applied.

406 However, Bauer et al. (2022), as in our PNL condition, does not take into account the  
407 skyglow that could be present throughout the nighttime. In PNL, oysters were in total  
408 darkness between 23 h and 3 h, when the direct ALAN source was switched off, which rarely  
409 occurs in anthropized environments. Indeed, the skyglow has a weaker intensity (estimated  
410 inferior to 0.55 lx) than direct ALAN sources but spreads further, until hundreds of km from  
411 its source (Gaston et al., 2017; Gaston, 2018; Hänel et al., 2018). Despite its weak intensity,  
412 the skyglow is not negligible since it can affect several biological processes, such as the  
413 diurnal locomotor activity of the fish *Lepomis macrochirus* (skyglow intensity of 0.5 lx)  
414 (Latchem et al., 2021). The skyglow (intensity of 0.2 lx) can also disrupt in situ the nocturnal  
415 migration of the amphipod *Talitrus saltator* guided by the moon by reducing the probability  
416 that the amphipod undertook them and by altering its orientation (Torres et al., 2020). In  
417 oysters, it is established that a 0.1 lx skyglow intensity affects their behavioral daily rhythm as

418 well as the expression of clock, clock-associated, and light perception genes (Botté et al.,  
419 2023). The town of Lanton (Arcachon bay), used here as a model to mimic PNL strategies, is  
420 only about 40 km from Bordeaux city, a community of nearly 820 000 inhabitants, which  
421 owns a multitude of direct ALAN sources generating skyglow. Furthermore, Lanton is  
422 surrounded by other towns in Arcachon bay that do not all apply PNL strategies. Therefore, to  
423 best recreate in the laboratory realistic conditions of the PNL strategy applied in Lanton,  
424 simulating skyglow when direct ALAN sources are switched off is necessary. This was the  
425 aim of PNL+S condition in which oysters were exposed to an intensity of 0.1 lx when direct  
426 ALAN sources (5 lx) were switched off. Results show that adding skyglow to PNL condition  
427 annihilates the benefits acquired by PNL for some studied parameters (mean nighttime VOD  
428 and 12h-rhythm's amplitude at the group level, mean VOD from 23 to 3 h at the individual  
429 scale). Furthermore, contrary to our a priori hypothesis, PNL+S also worsens some behavioral  
430 effects caused by FNL. The most striking result is a loss of 44.4 % of PNL+S oysters that kept  
431 a daily rhythm compared to FNL and PNL. Furthermore, at the group level, the mean VOD  
432 from 23 to 3 h is significantly higher for PNL+S compared to FNL, although ALAN intensity  
433 during this period is higher for FNL. Moreover, the mean VOD of oysters in PNL+S is still  
434 significantly the highest from 3 to 5 h, while the intensity is the same in the three ALAN  
435 conditions. Even more surprising, PNL+S is the only tested ALAN modality to show  
436 molecular effects. In PNL+S condition, the overall expression of the eleven genes studied is  
437 significantly different from all of the other conditions (control, PNL, and FNL). Furthermore,  
438 the overall expression is significantly different according to the sampling time only in this  
439 condition. In particular, PNL+S significantly decreases the expression of clock genes during  
440 daytime (*CgTim1*) or nighttime at 1 h (*CgBmal*, *CgCry2*, *CgRev-erb*). Thus, the stronger  
441 behavioral effects caused by PNL+S might be explained by the fact that this ALAN modality  
442 could induce a clearer disruption of oysters' molecular clockwork contrary to the others.

443 PNL+S also caused a significant decrease of the expression of the clock-associated gene  
444 *CgHiomt-like*, a homolog of the mammalian gene *Hiomt*, coding for an enzyme involved in  
445 the synthesis of melatonin (Pevet et al., 1980). Melatonin is a hormone believed to be  
446 produced exclusively during nighttime and known to be involved in diverse physiological  
447 functions (homeostasis, reproduction, immunity, antioxidant system ...) and especially to be a  
448 signal of darkness (Jones et al., 2015; Tan et al., 2010). In oysters, results suggest that PNL+S  
449 decreases melatonin synthesis during nighttime (22 h and 1 h) whatever ALAN intensity (0.1  
450 or 5 lx), suggesting potential physiological consequences. The decrease of melatonin  
451 production by ALAN has been already observed in the fish *Rutilus rutilus* at 1 lx, but also at  
452 the lower intensities of 0.1–0.3 lx for *Salvelinus alpinus* and of 0.01 lx for *Perca fluviatilis*  
453 (Bruning et al., 2018; Kupprat et al., 2020; Liu et al., 2019). Two previous studies showed  
454 that ALAN affects the oyster's molecular clock, as well as light perception and clock-  
455 associated genes (especially the gene *CgHiomt-like*) no matter its intensity (from 0.1 lx to 25  
456 lx) or its wavelength (blue, green, red or white lights) (Botté et al., 2023; Botté et al.,  
457 Unpublished results). Here, ALAN molecular effects have not been observed for the studied  
458 light perception genes. Moreover, ALAN affects clock and clock-associated genes expression  
459 in PNL+S only and not in FNL, which is the modality approaching most the ALAN  
460 conditions used in previous studies. One explanation can be the sampling times, which might  
461 not occur at the most appropriate hours to detect significant differences between conditions.  
462 Nevertheless, behavioral and molecular results, in particular the loss of oysters having a daily  
463 rhythm, might suggest that PNL mitigation strategies would be harmful to oysters in the  
464 current environmental context, partly because of the skyglow. However, studies are needed to  
465 confirm or disconfirm this hypothesis in focusing on specific physiological consequences  
466 such as the growth rate, the microbiote, etc.

467 A few in situ studies have investigated the effects of PNL mostly on bats. Hooker et al. (2022)  
468 tested the impacts of FNL and two PNL strategies, where ALAN, starting from the sunset,  
469 was switched off after 2 or 4 h, on the feeding activity of bats in situ. Results show that FNL  
470 induced a significant decrease of the feeding activity of the bats *Pipistrellus pygmaeus* and  
471 *Myotis* spp.. Both PNL strategies abolished FNL effects on the feeding activity of *Pipistrellus*  
472 *pygmaeus*, while it is the case only for the 2h-PNL strategy for *Myotis* spp.. In the third  
473 studied bat species *Pipistrellus pipistrellus*, the 2h-PNL strategy is the only condition to affect  
474 significantly its feeding activity (Hooker et al., 2022). Thus, these results highlight the  
475 species-dependent positive or negative effects of PNL strategy. Azam et al. (2015) also shows  
476 the species-dependent effects of PNL strategies on the activity of bats in situ. In *Plecotus* sp.,  
477 PNL strategy had a more harmful effect on its activity than FNL, while in *Pipistrellus*  
478 *pipistrellus* PNL annihilated FNL effects. Finally, in most of the studied species (*Pipistrellus*  
479 *kuhlii*, *Pipistrellus nathusius*, *Nyctalus leislerii*, *Myotis* spp) PNL affected their activity as did  
480 FNL. Bats are nocturnal organisms but with species specific activity ranges, coinciding or not  
481 with the dark part of PNL, which might explain the difference in responses to PNL between  
482 species (Azam et al., 2015). Day et al. (2015) also highlighted the importance of timing in the  
483 implementation of PNL strategies. They tested the effect of 8 scenarios of PNL on the activity  
484 of the bat *Rhinolophus ferrumequinum* by shifting the switch-off hours by one hour from 22 to  
485 5 h. They found that the sooner the lights are switched off, the less ALAN disrupts bat  
486 activity. Although PNL strategies usually implemented allow hours of darkness at night, the  
487 lighting switches off after midnight, which limits their benefits for this bat species (Day et al.,  
488 2015). More globally, current PNL schemes do not usually coincide with periods of high  
489 nocturnal biological activity, which are mostly around dusk and dawn (Gaston et al., 2012). In  
490 oysters, the masking of dusk and dawn by ALAN could disrupt their perception of the day  
491 length as well as the photoperiod increase or decrease, which can be used by oysters to

492 synchronize their valve behavior at an annual scale (Payton et al., 2017b). Therefore,  
493 implementing a PNL strategy should require studying the benefits of the chosen duration and  
494 timing of lighting shutdowns in local communities.

495

## 496 **5. Conclusion**

497 With the fast increase of ALAN extent in coastal environments, the studies on this thematic  
498 are increasing, mainly focusing on the characterization on its effects on coastal organisms, but  
499 mostly forgetting to investigate the potential benefits of mitigation strategies, such as PNL.

500 This study contributes to fill this gap by investigating the effects of PNL strategy on the  
501 oyster daily rhythm at the behavioral and molecular scales by recreating a realistic context in  
502 the laboratory. Results show that the use of PNL strategies could in theory reduce the oysters'  
503 daily rhythm disruption by ALAN. However, when adding the skyglow throughout nighttime  
504 mimicking real conditions in anthropized environments (PNL+S), many PNL strategy  
505 benefits are annihilating and can even be worsened compared to FNL effects on the oyster  
506 behavioral daily rhythm and on the molecular clock functioning. Molecular results also  
507 suggest a disruption of the melatonin synthesis in PNL+S, which might result in physiological  
508 consequences. Thus, in realistic conditions, the use of PNL as a mitigation strategy might be  
509 not suitable to limit ALAN's effects on oysters' daily rhythm and can even be more harmful  
510 than FNL. However, other studies are needed to confirm these effects in situ on oysters and to  
511 evaluate PNL effects at a community level.

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514

515 **Ethics:** All experiments complied with the laws in effect in France and they conformed to  
516 international ethical standards.

517 **Data accessibility:** The data underlying this study are available on Supplementary data.

#### 518 **CRedit authorship contribution statement**

519 **Audrey Botté:** Conceptualization, Methodology, Formal analysis, Investigation, Writing -  
520 Original Draft, Writing - Review & Editing, Visualization. **Laura Payton:** Conceptualization,  
521 Methodology, Investigation, Writing - Review & Editing, Visualization. **Elisa Lefeuvre:**  
522 Formal analysis, Investigation. **Damien Tran:** Conceptualization, Methodology,  
523 Investigation, Writing - Review & Editing, Visualization, Funding acquisition.  
524 All authors contributed critically to the drafts, and gave final approval for publication.

#### 525 **Competing interests**

526 We have no competing interests.

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731 **Figures & legends**

732 **Figure 1.** Behavioral daily valve activity of oysters in the control condition and exposed to  
733 different ALAN modalities (n = 16 oysters / condition): part-night lighting (PNL: ALAN  
734 direct source (5 lx) switched off from 19 h to 23 h), part-night lighting + skyglow (PNL+S:  
735 skyglow throughout nighttime added to PNL), and full-night lighting (FNL: ALAN direct  
736 source (5 lx) throughout nighttime). Mean hourly Valve Opening Duration (VOD, %) data are  
737 expressed as mean  $\pm$  SE (n = 10 days). White backgrounds indicate daytime while grey and  
738 yellow backgrounds indicate nighttime (grey: < 0.05 lx, dark yellow: 5 lx, pale yellow: 0.1  
739 lx). Arrows show the peak of maximal hourly VOD.

740 **Figure 2.** Effect of ALAN modalities (PNL, PNL+S, FNL) at the group level on (A) the mean  
741 VOD during daytime and nighttime, (B) the mean VOD during three period of nighttime:  
742 from 19 h to 21 h, when oysters are exposed to 5 lx in all ALAN conditions, from 21 h to 3 h,  
743 when oysters are exposed to darkness during PNL, to 0.1 lx during PNL+S, and to 5 lx during  
744 FNL, and from 3 h to 5 h, when oysters are exposed to 5 lx in all ALAN conditions. Data are  
745 expressed in mean  $\pm$  SE (n = 10 days). Different letters indicate significant differences  
746 between conditions ( $p < 0.05$ ).

747 **Figure 3.** Chronobiological analysis of the oysters' mean valve behavior at the group level.  
748 (A) Effects of ALAN modalities (PNL, PNL+S, FNL) on significant periodicities detected  
749 with the Lomb and Scargle periodograms (above the dotted line ( $p > 0.95$ )) and validated by  
750 the cosinor model ( $p < 0.05$ ). (B) Effect of ALAN modalities (PNL, PNL+S, FNL) on  
751 chronobiological parameters of oysters' 12h (left column) and 24h (right column) -rhythms:  
752 the percent rhythm (PR), the mesor, the amplitude, and the acrophase of the rhythm. Data are  
753 expressed in mean  $\pm$  SE with the SE showing the daily variability of the group's significant  
754 rhythm parameters (n = 10 days). Different letters show significant differences between  
755 conditions ( $p < 0.05$ ).

756 **Figure 4.** Effect of ALAN modalities (PNL, PNL+S, FNL) at the individual level on oysters  
757 that maintain a significant daily rhythm on (A) the mean VOD during daytime and nighttime,  
758 (B) the mean VOD during three period of nighttime: from 19 h to 21 h, when oysters are  
759 exposed to 5 lx in all ALAN conditions, from 21 h to 3 h, when oysters are exposed to < 0.05  
760 lx (PNL), 0.1 lx (PNL+S) or 5 lx (FNL), and from 3 h to 5 h, when oysters are exposed to 5 lx  
761 in all ALAN conditions. Data are expressed in mean  $\pm$  SE (n = 5 – 14 rhythmic oysters /  
762 condition). Different letters indicate significant differences ( $p < 0.05$ ).

763 **Figure 5.** Effect of ALAN modalities (PNL, PNL+S, FNL) on the chronobiological  
764 parameters of oysters' daily rhythm at the individual level: (A) the percentage of oysters  
765 having a daily rhythm (in the period range of 20–28 h), (B) the mesor, (C) the percent rhythm  
766 (PR), (D) the amplitude, and (E) the acrophase. The chronobiological parameters studied (B-  
767 E) are expressed only for oysters that maintain a significant daily rhythm. Data are expressed  
768 in mean  $\pm$  SE (n = 5 – 14 rhythmic oysters / condition). Different letters show significant  
769 differences ( $p < 0.05$ ).

770 **Figure 6.** Effect of ALAN modalities (PNL, PNL+S, FNL) on the difference in relative  
771 mRNA level of eleven clock, light perception and clock-associated genes in oyster gills  
772 tissues between conditions during daytime at 12 h (left column), when oysters are exposed to  
773 daylight (1400 lx), during nighttime at 22 h (middle column), when ALAN intensity is of 5  
774 lux for all conditions, and at 1 h (right column), when oysters are exposed to darkness during  
775 PNL and to ALAN at an intensity of 0.1 lx during PNL+S and of 5 lx during FNL. White bars  
776 indicate the gene level during daytime and, colored bars during nighttime. Data are expressed  
777 in mean  $\pm$  SE (n = 8 gills / condition). Different letters indicate differences between  
778 conditions ( $p < 0.05$ ).

779

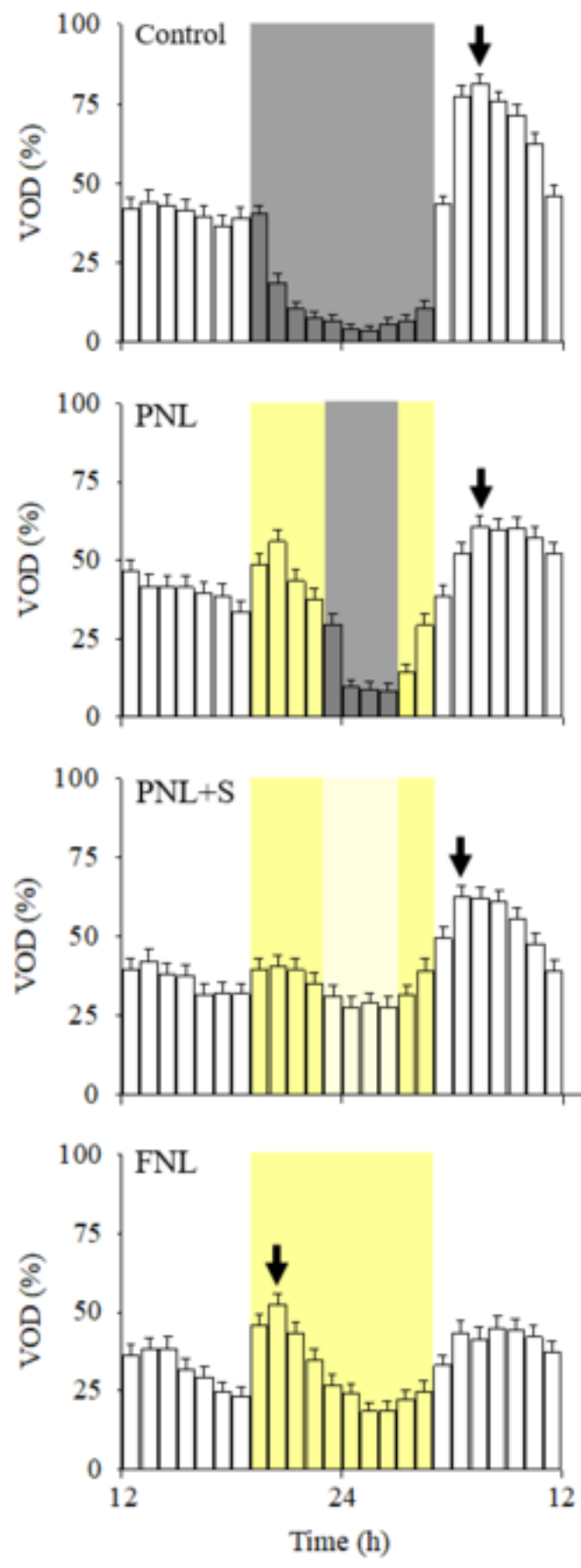
780 **Table 1.** Three-Way Analysis of Variance testing the effect of the ALAN condition (control,  
 781 PNL, PNL+S, FNL), sampling time, and the genes, on the relative mRNA level on gill tissues  
 782 of eleven clock, light perception and clock-associated genes. In bold, significant  $p$ -values ( $p <$   
 783 0.05).

<b>Sources of variation</b>	<b><math>p</math>-value</b>
Genes	<b>&lt; 0.001</b>
Sampling time	0.237
Conditions	<b>&lt; 0.001</b>
Control vs PNL	0.174
Control vs PNL+S	<b>&lt; 0.001</b>
Control vs FNL	0.333
PNL vs PNL+S	<b>&lt; 0.001</b>
PNL vs FNL	0.424
PNL+S vs FNL	<b>&lt; 0.001</b>
Sampling time x Conditions	<b>0.024</b>
Sampling time within Control	0.449
Sampling time within PNL	0.781
Sampling time within PNL+S	<b>0.001</b>
Sampling time within FNL	0.419

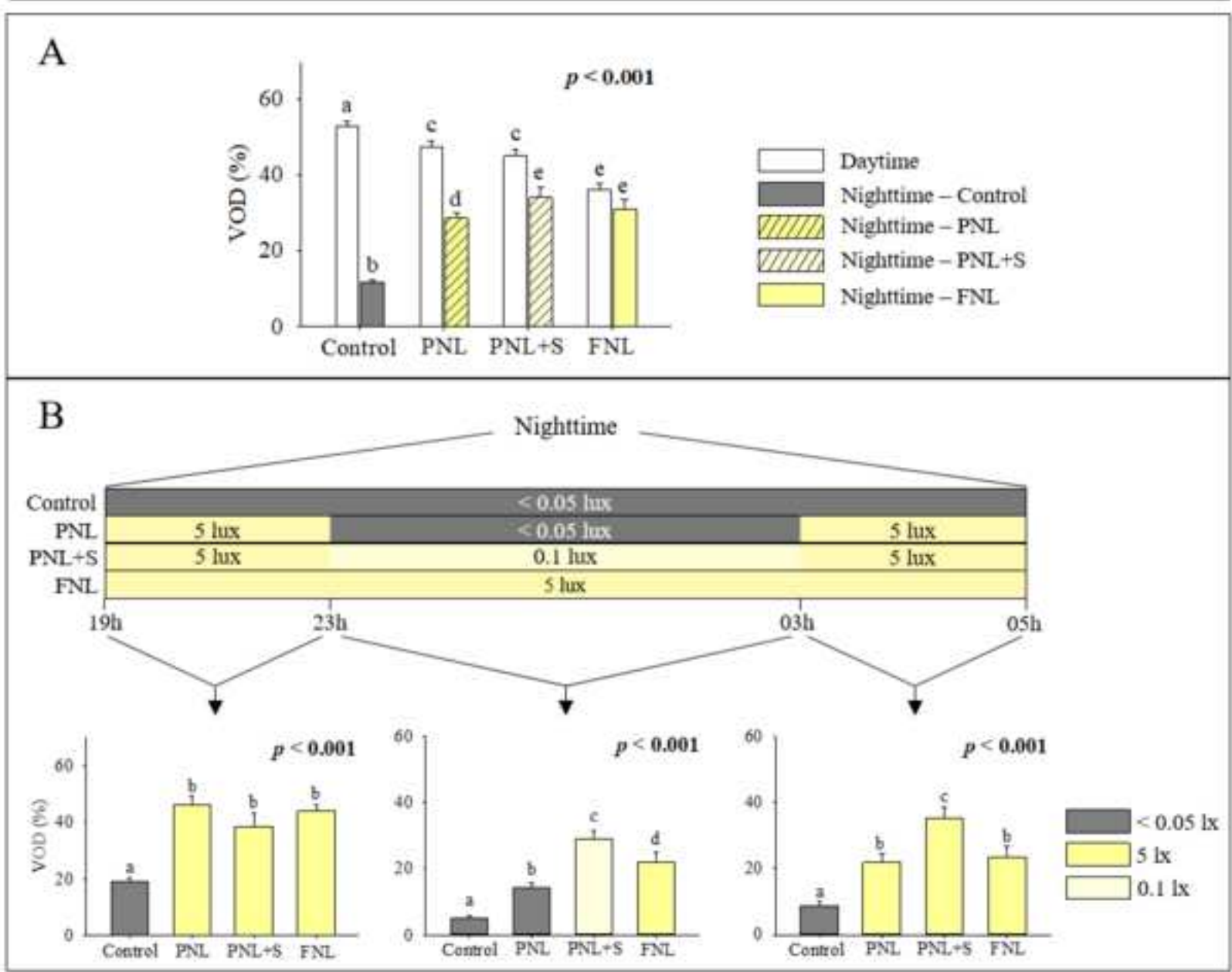
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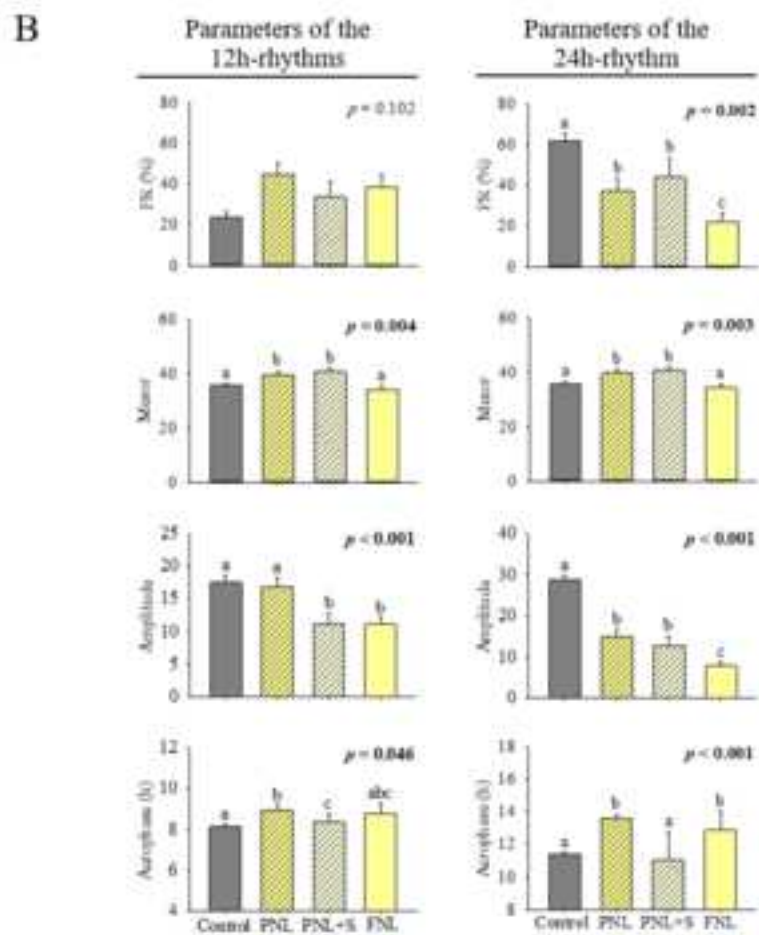
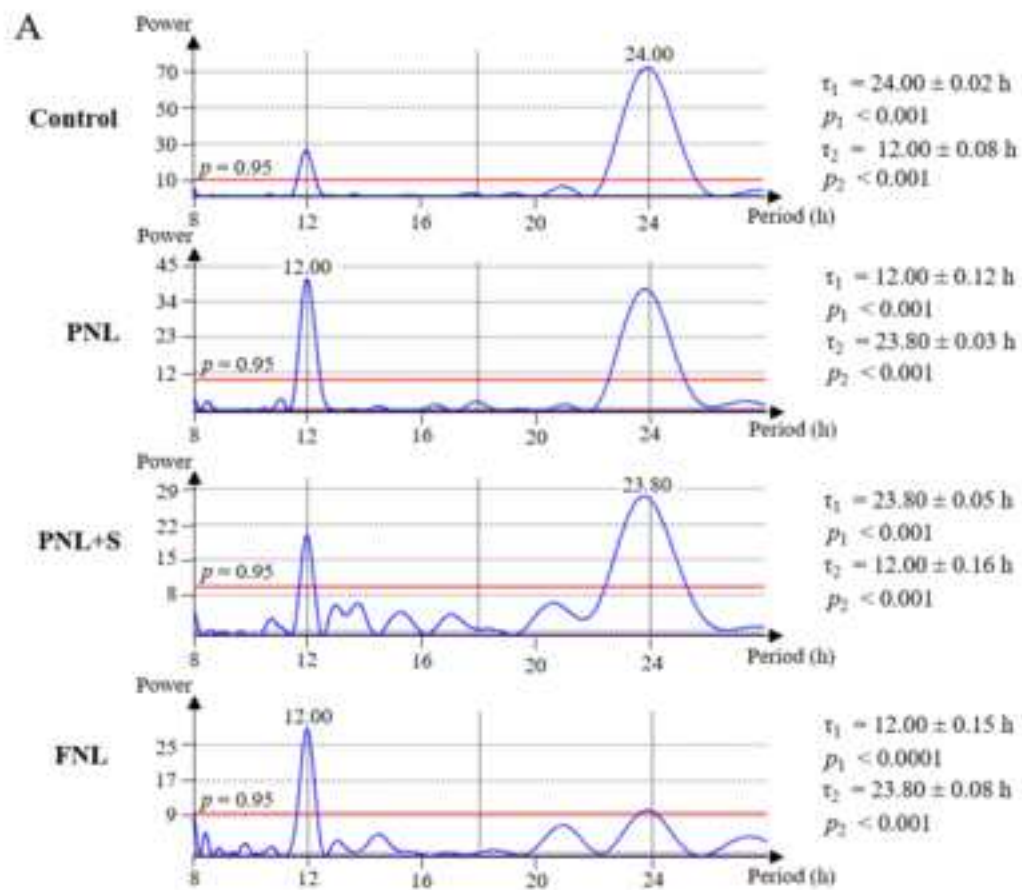


Figure 1

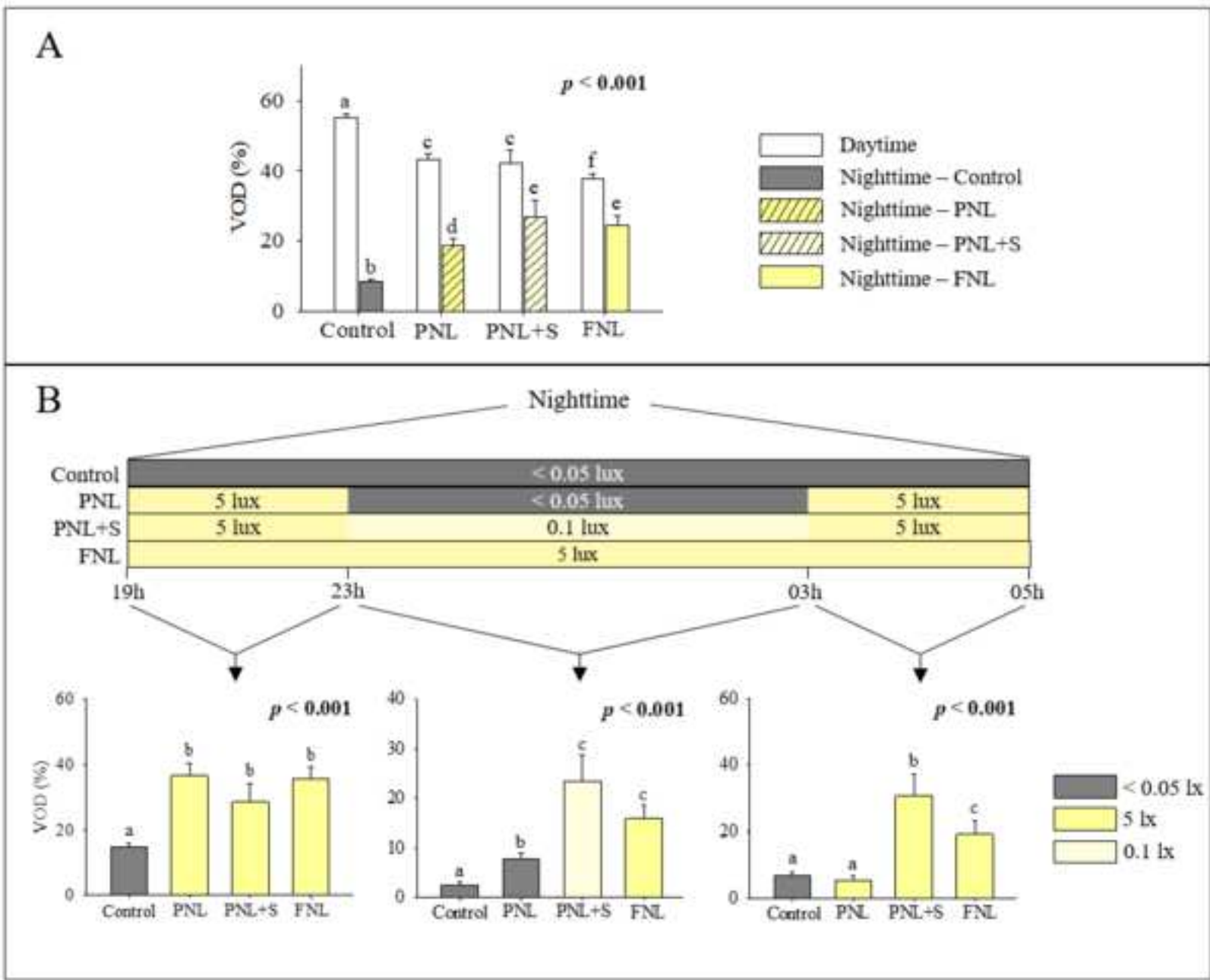


### Group level





### Individual level



## Individual level

