



HAL
open science

Artificial light at night at environmental intensities disrupts daily rhythm of the oyster *Crassostrea gigas*

Audrey Botté, Laura Payton, Damien Tran

► To cite this version:

Audrey Botté, Laura Payton, Damien Tran. Artificial light at night at environmental intensities disrupts daily rhythm of the oyster *Crassostrea gigas*. *Marine Pollution Bulletin*, 2023, 191, pp.114850. 10.1016/j.marpolbul.2023.114850 . hal-04262331

HAL Id: hal-04262331

<https://hal.science/hal-04262331v1>

Submitted on 27 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1

1

2

3

2 **Artificial light at night at environmental intensities disrupts daily rhythm of the oyster**

4

5

3 *Crassostrea gigas*

6

7

8

4

9

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

10

11

5

12

13

14

6

15

16

17

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

18

19

8

20

21

22

9 *Corresponding author: Damien Tran

23

24

25

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

26

27

28

11

29

30

31

12

32

33

34

13

35

36

37

14

38

39

40

15

41

42

43

16

44

45

46

17

47

48

18

49

50

51

19

52

53

54

55

20

56

57

58

59

60

61

62

63

64

65

21 **Abstract**

1
2
3 22 Artificial Light At Night (ALAN) masks the natural light cycles and thus can disturb the
4
5 23 synchronization of organisms' biological rhythms with their environment. Although
6
7
8 24 coastlines are highly exposed to this growing threat, studies concerning the impacts of ALAN
9
10 25 on coastal organisms remain scarce. In this study, we investigated the ALAN exposure effects
11
12
13 26 at environmentally realistic intensities (0.1, 1, 10, 25 lux) on the oyster *Crassostrea gigas*, a
14
15 27 sessile bivalve subject to light pollution on shores. We focused on the effects on oyster's daily
16
17 28 rhythm at behavioral and molecular levels. Our results showed that ALAN disrupts the
18
19
20 29 oyster's daily rhythm by increasing valve activity and annihilating day / night differences of
21
22 30 expression of circadian clock and clock-associated genes. ALAN effects occur starting from
23
24
25 31 0.1 lux, in the range of artificial skyglow illuminances. We concluded that realistic ALAN
26
27 32 exposure affects oysters' biological rhythm, which could lead to severe physiological and
28
29
30 33 ecological consequences.

31
32
33 34 Keywords: ALAN, biological rhythm, circadian clock, *Crassostrea gigas*, behavior
34
35
36 35
37
38
39 36
40
41
42 37
43
44
45 38
46
47
48 39
49
50 40
51
52
53 41
54
55
56 42
57
58
59 43
60
61
62
63
64
65

44 **Introduction**

1
2
3 45 Natural light cycles play an important role in biological timings (Gaston et al., 2017). Indeed,
4
5 46 predictable variations of light intensity over a day, a month, or a year are used as signals by
6
7 47 organisms to synchronize their biological rhythms with their environment (Bradshaw and
8
9 48 Holzapfel, 2010; Gaston et al., 2017). These biological rhythms are ubiquitous and find their
10
11 49 origin in each cell with an endogenous clock, which uses environmental cues, such as natural
12
13 50 light cycles, to synchronize organisms' physiological processes and behavior with their
14
15 51 environment. This synchronization enables organisms to be fully adapted to their environment
16
17 52 and to anticipate its changes (Cermakian and Sassone-Corsi, 2000; Partch et al., 2014).
18
19 53 However, Artificial Light At Night (ALAN) affects the natural nocturnal lighting levels,
20
21 54 which can disrupt the organisms' perception of natural variations of light, and affect
22
23 55 organisms' biological rhythms (daily, lunar, seasonal) and their physiology (Falchi et al.,
24
25 56 2011; Gaston and Bennie, 2014). For example, it can disrupt migration, reproduction,
26
27 57 foraging, or prey/predator interactions (Davies et al., 2014; Gaston et al., 2017; Longcore and
28
29 58 Rich, 2004; Navara and Nelson, 2007). These ALAN's effects on organisms' physiology and
30
31 59 behavior occur in a large range of species, including diurnal ones, and at low intensities
32
33 60 (below 1 lux) (Sanders et al., 2021). Thus ALAN can have consequences at the individual,
34
35 61 population, and ecosystem scale. Nocturnal artificial lighting affects the whole world (Falchi
36
37 62 et al., 2016) and spreads fast with a 6% increase in the sky luminosity per year (Hölker et al.,
38
39 63 2010). The negative effects of ALAN on organism behavior or physiology become
40
41 64 increasingly studied over the years (Davies and Smyth, 2018) but they remain widely
42
43 65 investigated for terrestrial organisms. However, ALAN effects on marine organisms, and
44
45 66 especially benthic organisms, recently received more attention. Studying the impacts of
46
47 67 ALAN on species living in coastal ecosystems is of great importance considering that already
48
49 68 22.2 % of the world's coasts are exposed to ALAN as well as 35% of marine protected areas
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

69 (Davies et al., 2014, 2016), percentages that certainly will increase since the human
70 population living in coastal areas is predicted to grow (Kummu et al., 2016). Furthermore,
71 ALAN's negative effects could be worsened by the increasing use of LED (Light-Emitting
72 Diode) in public lighting (Gaston, 2018; Zisis and Bertoldi, 2018). Indeed, LED lights
73 spectrum contains more short-wavelength than spectra of other lighting types such as HPS
74 (High-Pressure Sodium) lighting (Bierman, 2012; Falchi et al., 2011; Longcore et al., 2018;
75 Luginbuhl et al., 2014). Considering that short-wavelength, such as blue wavelength, go
76 further into seawater (Davies et al., 2014; Grubisic et al., 2019) and that marine organisms
77 would be highly sensitive to blue wavelength (Grubisic, 2018), the negative effects of ALAN
78 could be amplified on the coastal fauna. Therefore, ALAN becomes over the years a growing
79 threat to marine life.

80 The oyster *Crassostrea gigas* is a marine bivalve of commercial importance with a large
81 distribution. Oysters are also sessile organisms living in benthic areas, thus inevitably exposed
82 to ALAN through direct or indirect sources of artificial light. The direct sources of lighting on
83 the coast are for example streetlights on a pier, harbors, etc. The indirect source of ALAN is
84 called skyglow, with a lower light intensity but much more spatially extensive than direct
85 lighting. It takes its source mostly in big cities from which artificial lights are diffused into the
86 atmosphere, resulting in a global lightening of the sky at night (Gaston, 2018). Both of these
87 ALAN sources could disrupt oysters' biological rhythms. These oysters' biological rhythms
88 related to daily (Mat et al., 2012), tidal (Tran et al., 2011), lunar (Payton and Tran, 2019), and
89 seasonal (Payton et al., 2017b) natural cycles have already been described. Concerning their
90 daily rhythm, oysters have a plastic endogenous circadian rhythm generated by a molecular
91 clock (Mat et al., 2012; Mat et al., 2014; Payton et al., 2017a; Perrigault and Tran, 2017; Tran
92 et al., 2020). This plasticity enables oysters to easily adapt to new environments but it also

93 could make them vulnerable to disruption by ALAN, which could have consequences on their
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65
94 physiology and ecology.

95 The objective of this study is to evaluate the effects of several ALAN intensities encountered
96 in the environment on the daily rhythm of the oyster *C. gigas*. To achieve that, valve behavior
97 rhythm and circadian clock genes expression were studied. We exposed oysters during 7 days
98 to ALAN intensities ranging from 0.1 to 25 lux with the lowest intensity representing a
99 skyglow intensity (Gaston, 2018) and the highest intensity being in the range of direct ALAN
100 due to a lighted parking lot (Rich and Longcore, 2006). We hypothesized that ALAN would
101 disrupt oysters' daily rhythm at the behavioral and molecular levels. We assumed that ALAN
102 exposure disrupts the oysters' circadian clock machinery, impairing the clock's outputs such
103 as clock-associated genes' expression and rhythmic behavior, and may also lead to a direct
104 behavioral response during nighttime. Finally, we hypothesized that some of these effects
105 would occur in an intensity-dependent manner.

107 **Materials and methods**

108 *General conditions*

109 The experiment was conducted from January to April 2021 in the Marine Station of Arcachon
110 on 160 oysters (85.8 ± 0.7 mm shell length; 48.4 ± 0.5 mm shell width; mean \pm SE) coming
111 from an oyster's farm source of the Arcachon bay (France). During the acclimation and
112 experimental duration, oysters were placed into tanks (L x W x H: 74.8 x 54.8 x 40.8 mm)
113 continuously supplied with natural seawater from the Arcachon bay, which was filtered (<
114 $1\mu\text{m}$), oxygenized, and the temperature was monitored ($T = 15.0 \pm 0.1$ °C). Tanks were
115 placed in an isolated room and equipped with an antivibration bench to minimize external
116 disturbances to animal behavior. The oysters were not fed during the experiment.

117 *Experimental protocol*

1
2 118 Oysters were maintained under a L:D 10:14 cycle with daytime from 7:00 to 17:00 h (all
3
4
5 119 times noted in local time, UTC +1) (Fig. S1A). The light intensity during daytime varied
6
7 120 gradually to mimic the natural cycle of light using programmable white (413–688 nm, peak at
8
9 121 551 nm; Fig. S2A) LED light bars (MH3SP3 DSunY). The maximum intensity during
10
11 122 daytime was 1473.42 ± 106.21 lux (mean \pm SE) (Tab. S.1) between 11:30 to 12:30 h. After 7
12
13 123 to 9 days of acclimation to the experimental setup, the proper experiment started for a
14
15 124 duration of 7 days. There were 5 conditions: a control condition and 4 conditions where
16
17 125 oysters were exposed to several ALAN intensities (Tab. S1): 0.1, 1, 10, and 25 lux. In the 4
18
19 126 ALAN conditions, oysters were exposed to ALAN from 17:30 to 6:30 h (Fig. S1B) using
20
21 127 white (411–687 nm, peak at 563 nm; Fig. S2B–S2E) LED strips (MiBoxer Mi-Light WL5).
22
23 128 Illuminances underwater were measured at five positions in the tank (Fig. S3) using a
24
25 129 handheld spectroradiometer (Blue-Wave UVN-100, StellarNet Inc.). The control group was in
26
27 130 the complete dark at night, with a light intensity inferior to the detection limit of the
28
29 131 spectroradiometer (0.05 lux), mentioned in the study as 0 lux for convenience. For each of the
30
31 132 5 experimental conditions, 32 oysters were placed in the same tank, and the valve activity of
32
33 133 16 of them was continuously measured using a High Frequency–Non Invasive (HFNI)
34
35 134 valvometer technology (Andrade et al., 2016). For each experimental conditions, 32 different
36
37 135 oysters were used. On the 7th day of the experiment, the gill tissue of 8 oysters were sampled
38
39 136 during daytime at 12:00 h and during nighttime at 19:00 h and stored in Tri Reagent
40
41 137 (Invitrogen) at -80 °C for further molecular analysis.
42
43
44
45
46
47
48
49
50
51

52 138 *Behavioral measurements*

53
54 139 For valve activity measurement using HFNI valvometer technology, lightweight's
55
56 140 electromagnets were glued on each valve of oysters and linked to a valvometer device using
57
58 141 flexible wires (Andrade et al., 2016). The electromagnetic current generated between the two
59
60
61
62
63
64
65

142 electromagnets allows to measure oyster valve activity. For each individual, the signal was
1 recorded every 4.8s. Data were processed using Labview 8.0 (National Instrument, Austin,
2 143 TX, USA). The study focused on the hourly Valve Opening Duration (VOD) meaning that we
3
4 144 determined the percentage of time each individual spent with its valve open for each hour. For
5
6 145 example, an individual with its valves open during a whole hour corresponds to a VOD of 100
7
8 146 %, while an individual with its valves closed during one hour corresponds to a VOD of 0 %.
9
10
11
12 147

148 *Chronobiological analyses*

149 Chronobiological analysis of the hourly VOD data were performed at groups and individual
150 levels using the software Time Series Analysis Serie Cosinor 8.0 (Expert Soft Technologies).
151 First, data quality was evaluated using the autocorrelation diagram to control the absence of
152 random repartition of data, and the Partial Autocorrelation Function (PACF) calculation to
153 assess the absence of stationary character (Gouthière and Mauvieux, 2003). Then the Lomb
154 and Scargle periodogram was used to search for periodicity in the data (Scargle, 1982). A
155 significant period was accepted for $p > 0.95$. Data rhythmicity was then modeled using the
156 Cosinor model, which uses a cosine function calculated by regression (Bingham et al., 1982).
157 For a given period, the model is written as: $Y(t) = A \cos\left(\frac{2\pi t}{\tau} + \phi\right) + M + \varepsilon(t)$ where A is
158 the amplitude (difference between the average level and the highest value of the rhythm), ϕ
159 the acrophase (the highest value of the rhythm), τ the given period (interval between two
160 identical events), M the mesor (average level of the rhythm), and $\varepsilon(t)$ the relative error. Then
161 the calculated model and the existence of rhythmicity were validated by two tests: the ellipse
162 test had to be rejected, and the probability for the null amplitude hypothesis had to be lower
163 than 0.05. The percent rhythm (PR), a chronobiometric parameter, had been calculated and
164 represents the percentage of cyclic behavior explained by the model, meaning the strength of
165 the rhythm.

166 *Total RNA extraction and cDNA synthesis*

1
2 167 Total RNA was extracted from gills using Tri reagent (Invitrogen) and an SV Total RNA
3
4
5 168 Isolation System kit (Promega). The total RNA quantity and quality were assessed by
6
7 169 spectrophotometry (OD230, OD260, OD280). RNA reverse transcription was realized using
8
9
10 170 Goscript™ Reverse Transcription System kits (Promega).

13 171 *mRNA expression analysis by Real-Time PCR*

15 172 Real-Time qPCR was realized using GoTaq® qPCR Master Mix kit (Promega). Primers sets
16
17
18 173 of clock and clock-associated genes (*CgClock*, *CgBmal*, *CgCry*, *CgPer*, *CgTim1*, *CgCry1*,
19
20 174 *CgRev-erb*, *CgRor*, *CgHiomt-like*, *CgOctβ2*, *CgRhodopsin-like 1*, *CgRhodopsin-like 2*,
21
22
23 175 *CgRhodopsin-like 3*), and housekeeping genes (*CgEfl*, *Cg28S*, *CgGadph*) are listed in the
24
25 176 table S2. qPCR reactions were realized as follows: 95 °C for 2 min to activate the GoTaq
26
27
28 177 polymerase, 40 cycles of 95 °C for 15 seconds (denaturation), and 60 °C for 1 minute
29
30 178 (annealing and extension) for amplification of target cDNA, followed by 2 minutes at 60 °C
31
32
33 179 for the final elongation. In the end, melting curves were generated by gradually decreasing the
34
35 180 temperature from 95 °C to 60 °C to control the primer specificity. The comparative Ct method
36
37 181 $2^{-\Delta Ct}$ (Livak and Schmittgen, 2001) was used to determine the relative transcript level of clock
38
39
40 182 and clock-associated genes, where $\Delta Ct = Ct(\text{target gene}) - Ct(\text{housekeeping gene})$. Gene's
41
42 183 expression was normalized with the geometric mean of the housekeeping genes *CgEfl* and
43
44
45 184 *Cg28S*, based on stability values (Xie et al., 2012).

48 185 *Statistical analysis*

50
51 186 All statistical analysis were performed using SigmaPlot software (version 13.0; Systat
52
53 187 Software, USA). T-tests were performed for two groups' comparisons after checking
54
55
56 188 assumptions (normality of data and equal variance) and if they were not validated the non-
57
58 189 parametric Mann-Whitney rank sum test was performed. Two groups' comparisons were used
59
60 190 for molecular analysis results. For multiple comparisons, one-way ANOVA were used after
61
62
63
64
65

191 checking assumptions (normality of data and equal variance). When assumptions were not
192 validated, the non-parametric Kruskal-Wallis One-Way ANOVA on ranks test was
193 performed. In case of significant differences, a Tukey test was realized for all pairwise
194 multiple comparisons unless the groups did not have equal size, in which case the Dunn's test
195 was realized. Multiple comparisons were performed for behavioral analyses. On the whole
196 molecular data, a two-way ANOVA was also performed after checking assumptions
197 (normality of data and equal variance), and in case of significant differences, a Tuckey test
198 was used for all pairwise multiple comparisons. For all test results, a difference was
199 considered significant when $p < 0.05$.

200

201 **Results**

202 Figure 1 shows the behavioral mean daily pattern of oysters in the control condition, and
203 exposed to ALAN ranging from 0.1 to 25 lux. In the control condition, we show a diurnal
204 pattern with a maximal VOD at 11–12 h (peak showed by the black arrow). When exposed to
205 ALAN, the overall daily pattern of oysters' behavior is modified, with an increase of the
206 hourly VOD during nighttime and a 7-hours shift of the daily VOD peak, delayed from
207 daytime to nighttime, for all ALAN intensities starting from 0.1 lux. Statistical analysis of the
208 mean daily VOD shows a significant increase from 0.1 lux, of the mean VOD when oysters
209 are exposed to ALAN for all of the tested intensities ($p = 0.002$; Fig. 2A), without an intensity
210 effect. The figure 2B shows no significant differences in VOD during daytime between all
211 conditions. However, ALAN causes a significant increase of the mean VOD during nighttime
212 starting from 0.1 lux. The significant difference of VOD between daytime and nighttime
213 observed in the control condition decreases at 0.1 lux and significantly disappears at 1, 10,
214 and 25 lux. Figure 3 shows chronobiological analysis, characterizing the effects of ALAN
215 exposure on the oysters' daily behavioral rhythm at the group and individual levels (detailed

216 results in Table S3 and Table S4). At the group level, a significant daily rhythm persists in all
1
2 217 conditions with no significant differences of period length between the conditions (Fig. 3A).
3
4 218 However, ALAN affected several parameters of this rhythm. Indeed, there is a significant
5
6
7 219 decrease ($p < 0.001$) of the percent rhythm (PR, %) of the daily rhythm when oysters are
8
9
10 220 exposed to ALAN starting from 1 lux (Fig. 3B). Furthermore, the amplitude of the daily
11
12 221 rhythm decreases significantly ($p < 0.001$) in all ALAN conditions, starting from 0.1 lux and
13
14 222 without significant ALAN intensity-effect (Fig. 3C). At the individual level, results show an
15
16 223 ALAN intensity-dependent decrease of the percentage of oysters having a significant daily
17
18
19 224 rhythm (Fig. 3D). Figure 3E shows a decrease ($p < 0.001$) of the individual rhythmic period
20
21
22 225 lengths, getting out the daily range for many individuals exposed to ALAN (Table S3). The
23
24 226 mean period of oysters exposed to 1 lux and 25 lux are 17.9 ± 2.1 and 15.2 ± 1.7 h
25
26 227 respectively, under the circadian range (20-28 h), explaining the global loss of rhythmicity at
27
28
29 228 the daily scale shown in figure 3D. Furthermore, the PR of oysters having a daily rhythm
30
31 229 tends to decrease starting from 0.1 lux, and it decreases significantly ($p = 0.015$) when they
32
33
34 230 are exposed to 10 lux (Fig. 3F). Finally, the amplitude of oysters having a daily rhythm show
35
36 231 no significant differences ($p = 0.169$) but a trend to decrease when oysters are exposed to
37
38
39 232 ALAN (Fig 3G). Figures 4 and S4 shows the mRNA level during daytime and nighttime of
40
41 233 thirteen circadian clock (*CgClock*, *CgBmal*, *CgCry*, *CgPer*, *CgTim1*, *CgCry1*, *CgRev-erb*,
42
43 234 *CgRor*) and clock-associated (*CgHiomt-like*, *CgOctβ2*, *CgRhodopsin-like 1*, *CgRhodopsin-*
44
45 235 *like 2*, *CgRhodopsin-like 3*) genes for all of the tested ALAN intensities. No significant
46
47
48 236 differences of gene expression are observed between the control and the ALAN conditions
49
50
51 237 neither at daytime nor at nighttime. However, results show significant differences of mRNA
52
53 238 expression levels between daytime and nighttime in the control condition for five genes (two
54
55 239 circadian clock genes: *CgTim1* and *CgRev-erb*; and three clock-associated genes: *CgHiomt-*
56
57 240 *like*, *CgOctβ2* and *CgRhodopsin-like 1*), with an increase of expression during nighttime (Fig.

241 4). This daytime / nighttime difference of gene expression significantly disappears in all of the
1
2 242 five genes for all ALAN intensities tested. Finally, the table 1 shows the results of a two-way
3
4 243 ANOVA, testing the effects of the period of the day (daytime and nighttime), ALAN
5
6
7 244 intensities, and their interaction on the overall relative mRNA levels of the thirteen genes
8
9 245 studied. These results reveal a significant difference ($p = 0.046$) of the overall mRNA level
10
11 246 between daytime and nighttime only for the control condition ($p = 0.014$). In all ALAN
12
13 247 conditions, the daytime / nighttime difference of gene expression is abolished.
14
15
16
17 248

20 249 **Discussion**

23 250 Our study shows that ALAN affects the daily behavioral rhythm and the molecular clock of
24
25 251 oysters starting from the lowest ALAN intensity tested, 0.1 lux, in the range of artificial
26
27 252 skyglow. Multiples ALAN's effects on oyster's behavioral rhythm are observed: a decrease of
28
29 253 the percentage of oysters having a daily rhythm, a decrease of the rhythm's robustness, a
30
31 254 decrease of the rhythmic amplitude, an increase of the mesor (mean VOD), and a shift from a
32
33 255 diurnal to a nocturnal peak activity. At the molecular level, the overall difference of clock and
34
35 256 clock-associated gene expression observed in control condition between daytime and
36
37 257 nighttime is abolished when oysters are exposed to ALAN. While a clear ALAN intensity-
38
39 258 effect is observed at the individual level regarding the percentage of oysters having a daily
40
41 259 behavioral rhythm, most of ALAN's effects at both behavioral and molecular levels occur
42
43 260 from 0.1 to 25 lux without significant intensity-effect.
44
45
46
47
48

49 261 We showed that ALAN exposure leads to an increase of oysters' VOD, explained by an
50
51 262 increase of activity during nighttime leading to a decrease (at 0.1 lux) or a loss (at 1, 10, and
52
53 263 25 lux) of daytime / nighttime differences of valve behavior and a shift of peak activity from
54
55 264 daytime to nighttime. Activity increase during nighttime as an effect of ALAN has been also
56
57 265 shown in other marine organisms, such as the diurnal fish *Girella laevis* exposed to 70 lux
58
59
60
61
62
63
64
65

266 at night (Pulgar et al., 2019). On the contrary, the nocturnal crayfish *Pacifastacus leniusculus*
267 showed a decrease of activity during nighttime when exposed to ALAN at 12 lux using a HPS
268 streetlight bulb (Thomas et al., 2016). ALAN effects on organisms' behavior have also been
269 investigated in juvenile gastropods *Concholepas concholepas*, in which ALAN at an intensity
270 of 329.9 lux (LED technology) induces a less efficient search for prey during nighttime
271 (Manriquez et al., 2019). Moreover, some previous results of bivalve behavior disruption by
272 ALAN was shown by Christoforou (2022), which investigated the effects of several ALAN
273 wavelengths at an intensity of 19.86 lux using LED lightings on the behavior of the mussel
274 *Mytilus edulis*. The exposure of mussels to white ALAN induces a decrease during both
275 nighttime and daytime of the open/close frequency valve events. However, in this study, no
276 chronobiological analysis were performed and the relatively invasive experimental device
277 used to record mussels' behavior could have mask ALAN effects on mussels valve activity. In
278 our study, we went further in the investigation of ALAN's impacts on daily behavioral rhythm
279 using chronobiological analysis and lower ALAN intensities, using a non-invasive biosensor
280 (Andrade et al., 2016).

281 Daily rhythms and more generally biological rhythms are of great importance since they
282 enable organisms to anticipate cyclic changes in their environment and regulate accordingly
283 the temporal organization of their physiological processes such as feeding, respiration,
284 immunity, or growth (Helm et al., 2017; Yerushalmi and Green, 2009). Thus, the clear
285 disruption of oysters' daily rhythm by ALAN observed in this study, starting from 0.1 lux,
286 could have deep consequences on organism's physiology and fitness. The oysters were not
287 fed during the experiments to avoid an effect of food supply that may fluctuate, and thus acts
288 as a zeitgeber of daily rhythm. However, during our experimentation duration in the control
289 condition, we did not observe a modification of behavior, showing that food absence did not
290 affect ALAN's effects on oysters' daily rhythm. Furthermore, the experiments were

291 conducted in winter, when the food supply is very low in the environment. Moreover, the
1
2 292 rhythm at the daily scale could not be the only rhythm that can be disrupted by ALAN. It has
3
4
5 293 been shown that oysters have other biological rhythms. For example, oysters showed a lunar
6
7 294 rhythm i.e. their valve behavior is synchronized with lunar cycles by detecting moonlight
8
9
10 295 illumination cycle (Payton and Tran, 2019). This lunar rhythm could be affected by ALAN
11
12 296 since the maximal intensity of the moonlight ranges from 0.1 to 0.3 lux (Rich and Longcore,
13
14 297 2006), thus the natural variations of moonlight intensity can be easily masked by ALAN
15
16
17 298 intensities, such as the skyglow. The masking effect of lunar cycles by ALAN using LED has
18
19 299 been investigated in the corals *Acropora millepora* and *Acropora digitifera*, in which
20
21
22 300 exposition to ALAN delayed or masked their gametogenesis leading to a spawning
23
24 301 desynchronization (Ayalon et al., 2021). Another example of ALAN effect on lunar rhythm is
25
26 302 the disruption of the monthly nocturnal foraging pattern of the gastropod *Nucella lapillus*,
27
28
29 303 based on the lunar cycle, by ALAN produced by LED lighting at an intensity of 10 lux (Tidau
30
31 304 et al., 2022). Moreover, oysters' seasonal rhythms could be disrupted by ALAN. Oysters use
32
33
34 305 the variation of photoperiod through the year to anticipate seasonal changes and synchronize
35
36 306 physiological processes such as behavior, growth, and spawning (Bernard et al., 2016; Payton
37
38
39 307 et al., 2017b). However, ALAN being often present during dusk and dawn, it can mask these
40
41 308 two natural phenomena, preventing oysters to detect the variation of photoperiod along the
42
43
44 309 year, which could affect several seasonal physiological processes and decrease the fitness of
45
46 310 organisms (Gaston et al., 2017). ALAN's effects on organisms' physiological processes and
47
48
49 311 fitness have been shown for example in the amphipod *Orchestroidea tuberculata*, in which
50
51 312 ALAN (halogen light) induces a decrease of growth (Luarte et al., 2016), and in barnacles, in
52
53 313 which ALAN (LED lighting) affects the settlement process (Lynn et al., 2021; Manriquez et
54
55
56 314 al., 2021). Moreover, ALAN's effects on organisms' fitness have also been observed in the
57
58 315 juvenile crabs *Neohelice granulata* since it increases mortality, mainly by increasing
59
60
61
62
63
64
65

316 cannibalistic interactions with adults at an intensity of approximately 40 lux (LED lighting)
1
2 317 (Nunez et al., 2021).
3
4
5 318 In addition to the effects on the oyster's behavioral daily rhythm, this study also showed that
6
7 319 ALAN disturbs the circadian clockwork of *C. gigas* by annihilating differences between
8
9 320 daytime and nighttime in clock and clock-associated genes expression starting from the
10
11 321 intensity 0.1 lux. Genes for which their expression was mainly affected by ALAN are
12
13 322 *CgTim1*, *CgRev-erb*, *CgHiomt-like*, *CgOctβ2*, and *CgRhodopsin-like 1*. *CgTim1* and *CgRev-*
14
15 323 *erb* are both involved in the regulation's feedback loops of the oysters' molecular clock and
16
17 324 thus are core clock genes (Perrigault and Tran, 2017). On the other hand, *CgHiomt-like*,
18
19 325 *CgOctβ2*, and *CgRhodopsin-like 1* are clock-associated genes. The first one is a homologue of
20
21 326 the mammal gene *Hiomt* involved in the final step of the synthesis of melatonin, known to be
22
23 327 a key molecule of the day / night rhythm and a potent antioxidant with a proposed role in
24
25 328 immune function (Jones et al., 2015; Pevet et al., 1980). *CgOctβ2* is the gene of the
26
27 329 octopamine receptor β-2R. Octopamine is a biogenic amine well studied in arthropods and
28
29 330 gastropods where it functions as a neurotransmitter and hormone, fulfilling the roles played
30
31 331 by adrenalin in vertebrates. It is an extremely pleiotropic substance, participating in
32
33 332 invertebrate development such as growth, maturation, and reproduction by activating their
34
35 333 corresponding G protein-coupled receptors (GPCRs), and may interact with the circadian
36
37 334 clock (Roeder, 2020). Finally, the last gene to show a disrupted expression in presence of
38
39 335 ALAN is the gene of the rhodopsin (*CgRhodopsin-like 1*), a photoreceptor involved in
40
41 336 oysters' light-sensitivity (Wu et al., 2018). Thus, ALAN affects five of the thirteen genes
42
43 337 studied here. This relative low transcriptional response observed might be due to the fact that
44
45 338 oysters have been sampled at only two sampling times over the daily cycle. Indeed, a stronger
46
47 339 transcriptional disruption might possibly have been revealed by sampling at another time of
48
49 340 the day. However, our results still show that ALAN can affect genes of the molecular clock of
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

341 *C. gigas* (*CgTim1*, *CgRev-erb*), suggesting that the observed disruption of oyster's daily
342 behavioral rhythm would be a real consequence of the circadian clock disruption and not only
343 a direct reaction of light perception in presence of ALAN. ALAN also affects essential clock-
344 associated genes (*CgHiomt*, *CgOctβ2*, *CgRhodopsin-like 1*) involved in physiological
345 processes regulation. Thus, we can imagine that, by cascading effect, the expression of other
346 genes involved in several physiological functions can also be disrupted by ALAN. For
347 example, ALAN at 0.1–0.3 lux using a fluorescent bulbs induces the suppression of the
348 increase of melatonin during nighttime in *Salvelinus* (Liu et al., 2019). Moreover, in the
349 European perch *Perca fluviatilis* and the roach *Rutilus rutilus* the impairment of circadian
350 melatonin pattern by ALAN (HPS lamps) is associated with a decrease of the mRNA
351 expression of gonadotropins, which could disrupt their reproduction (Bruning et al., 2018).

352

353 **Conclusion**

354 This study is the first to investigate ALAN effects at environmentally realistic intensities on
355 oysters, a key species of the benthic coastal ecosystems. Our results reveal deep impacts on
356 the daily behavioral rhythm of *C. gigas*, from ALAN intensities starting from 0.1 lux,
357 comparable to the skyglow intensities. We also found that the expression of some of the clock
358 and clock-associated genes of the oyster's molecular clock was affected by ALAN, suggesting
359 a disruption of the endogenous clock and potential cascading physiological consequences.
360 However, studies focusing on the ALAN's effects on oysters' biological rhythms and the
361 consequences of their disruption on their fitness and physiology are needed. Furthermore,
362 given that ALAN spreads fast in coastal environments in which oysters are a key species,
363 ALAN's effects on this organism could have ecological consequences that should be
364 investigated to get a global picture of how ALAN will affect coastal ecosystems.

365

1
2 366 **Ethics:** All experiments complied with the laws in effect in France and they conformed to
3 international ethical standards.

4
5 368 **Data accessibility:** The data underlying this study are available on Supplementary data.

6
7
8 369 **Author contributions:** Study design and methodology, D.T., A.B., L.P.; experimentation,
9 A.B., D.T.; molecular analysis: A.B., L.P.; data treatment, A.B.; interpretation, A.B., D.T.,
10 370 L.P.; manuscript writing, A.B.; review and editing, all authors; funding, D.T. All authors
11 371 contributed critically to the drafts, and gave final approval for publication.
12
13
14
15
16 372

17
18
19 373 **Competing interests:** We have no competing interests.
20
21

22 374 **Funding**

23
24 375 This work was supported by the financial support of the French Nation Research Agency
25 (ANR), LUCIOLE project (ANR-22-CE34-0010-01) and a Bordeaux University PhD
26 376 scholarship for A. Botté.
27
28
29
30
31

32 378 **Acknowledgments**

33
34
35 379 We kindly thank Pierre Ciret, Christian Portier, and Christelle Teillet for their help during the
36 setup of the experiments.
37 380
38
39
40
41 381

382 **References**

- 1
2
3 383 Andrade, H., Massabuau, J.-C., Cochrane, S., Ciret, P., Tran, D., Sow, M., Camus, L., 2016.
4 384 High Frequency Non-invasive (HFNI) Bio-Sensors As a Potential Tool for Marine
5 385 Monitoring and Assessments. *Front. Mar. Sci.* 3. 10.3389/fmars.2016.00187
6
7
8 386 Ayalon, I., Rosenberg, Y., Benichou, J. I. C., Campos, C. L. D., Sayco, S. L. G., Nada, M. A.
9
10 387 L., Baquiran, J. I. P., Ligson, C. A., Avisar, D., Conaco, C., Kuechly, H. U., Kyba, C.
11
12 388 C. M., Cabaitan, P. C., Levy, O., 2021. Coral Gametogenesis Collapse under Artificial
13
14 389 Light Pollution. *Curr. Biol.* 31(2), 413-419 e413. 10.1016/j.cub.2020.10.039
15
16 390 Bernard, I., Massabuau, J. C., Ciret, P., Sow, M., Sottolichio, A., Pouvreau, S., Tran, D., 2016.
17 391 In situ spawning in a marine broadcast spawner, the Pacific oyster *Crassostrea gigas*:
18 392 Timing and environmental triggers. *Limnol. Oceanogr.* 61(2), 635-647.
19 393 10.1002/lno.10240
20
21
22 394 Bierman, A., 2012. Will switching to LED outdoor lighting increase sky glow? *Light. Res.*
23 395 *Technol.* 44(4), 449-458. 10.1177/1477153512437147
24
25
26 396 Bingham, C., Arbogast, B., Guillaume, G. C., Lee, J. K., Halberg, F., 1982. Inferential statistical
27 397 methods for estimating and comparing cosinor parameters. *Chronobiologia* 9, 397-439.
28
29
30 398 Bradshaw, W. E., Holzapfel, C. M., 2010. Light, time, and the physiology of biotic response to
31 399 rapid climate change in animals. *Annu. Rev. Physiol.* 72, 147-166. 10.1146/annurev-
32 400 *physiol*-021909-135837
33
34
35 401 Bruning, A., Kloas, W., Preuer, T., Holker, F., 2018. Influence of artificially induced light
36 402 pollution on the hormone system of two common fish species, perch and roach, in a
37 403 rural habitat. *Conserv. Physiol.* 6(1), coy016. 10.1093/conphys/coy016
38
39
40 404 Cermakian, N., Sassone-Corsi, P., 2000. Multilevel regulation of the circadian clock. *Nat. Rev.*
41 405 *Mol. Cell. Biol.* 1(1), 59-67. 10.1038/35036078
42
43
44 406 Christoforou, E., 2022. Effects of microfiber and light pollution on coastal ecosystem services
45 407 provided by the blue mussel, *Mytilus edulis*. University of Glasgow.
46 408 <https://theses.gla.ac.uk/82885/>
47
48
49
50 409 Davies, T. W., Duffy, J. P., Bennie, J., Gaston, K. J., 2014. The nature, extent, and ecological
51 410 implications of marine light pollution. *Front. Ecol. Environ.* 12(6), 347-355.
52 411 10.1890/130281
53
54
55 412 Davies, T. W., Duffy, J. P., Bennie, J., Gaston, K. J., 2016. Stemming the Tide of Light
56 413 Pollution Encroaching into Marine Protected Areas. *Conserv. Lett.* 9(3), 164-171.
57 414 10.1111/conl.12191
58
59
60
61
62
63
64
65

- 415 Davies, T. W., Smyth, T., 2018. Why artificial light at night should be a focus for global change
 1 416 research in the 21st century. *Glob. Chang. Biol.* 24(3), 872-882. 10.1111/gcb.13927
- 3 417 Falchi, F., Cinzano, P., Elvidge, C. D., Keith, D. M., Haim, A., 2011. Limiting the impact of
 4 418 light pollution on human health, environment and stellar visibility. *J Environ. Manage.*
 5 419 92(10), 2714-2722. 10.1016/j.jenvman.2011.06.029
- 8 420 Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C., Elvidge, C. D., Baugh, K., Portnov, B. A.,
 9 421 Rybnikova, N. A., Furgoni, R., 2016. The new world atlas of artificial night sky
 10 422 brightness. *Sci. Adv.* 2(6), e1600377. 10.1126/sciadv.1600377
- 13 423 Gaston, K. J., Bennie, J., 2014. Demographic effects of artificial nighttime lighting on animal
 14 424 populations. *Environ. Rev.* 22(4), 323-330. 10.1139/er-2014-0005
- 17 425 Gaston, K. J., Davies, T. W., Nedelec, S. L., Holt, L. A., 2017. Impacts of Artificial Light at
 18 426 Night on Biological Timings. *Annu. Rev. Ecol. Evol. Syst.* 48(1), 49-68.
 19 427 10.1146/annurev-ecolsys-110316-022745
- 22 428 Gaston, K. J., 2018. Lighting up the nighttime. *Science* 362(6416), 744-746.
 23 429 10.1126/science.aau8226
- 26 430 Gouthière, L., Mauvieux, B., 2003. Etapes essentielles dans l'analyse des rythmes: qualité des
 27 431 données expérimentales, recherche de périodes par analyses spectrales de principes
 28 432 divers, modélisation. XXXVème Congrès Société Francoph Chronobiol, Université de
 29 433 Saint-Etienne.
- 31 434 Grubisic, M., 2018. Waters under Artificial Lights: Does Light Pollution Matter for Aquatic
 32 435 Primary Producers? *Limnol. Oceanogr. Bull.* 27(3), 76-81. 10.1002/lob.10254
- 34 436 Grubisic, M., Haim, A., Bhusal, P., Dominoni, D. M., Gabriel, K. M. A., Jechow, A., Kupprat,
 35 437 F., Lerner, A., Marchant, P., Riley, W., Stebelova, K., van Grunsven, R. H. A., Zeman,
 36 438 M., Zubidat, A. E., Hölker, F., 2019. Light Pollution, Circadian Photoreception, and
 37 439 Melatonin in Vertebrates. *Sustainability* 11(22). 10.3390/su11226400
- 38 440 Helm, B., Visser, M. E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T., Bloch,
 39 441 G., 2017. Two sides of a coin: ecological and chronobiological perspectives of timing
 40 442 in the wild. *Philos. Trans. R. Soc. Lond., B., Biol. Sci.* 372(1734).
 41 443 10.1098/rstb.2016.0246
- 42 444 Hölker, F., Moss, T., Griefahn, B., Kloas, W., Voigt, C. C., Henckel, D., Hänel, A., Kappeler,
 43 445 P. M., Völker, S., Schwöpe, A., Franke, S., Uhrlandt, D., Fischer, J., Klenke, R., Wolter,
 44 446 C., Tockner, K., 2010. The Dark Side of Light: A Transdisciplinary Research Agenda
 45 447 for Light Pollution Policy. *Ecol. Soc.* 15(4). 10.5751/es-03685-150413

- 448 Jones, T. M., Durrant, J., Michaelides, E. B., Green, M. P., 2015. Melatonin: a possible link
1 449 between the presence of artificial light at night and reductions in biological fitness.
2
3 450 *Philos. Trans. R. Soc. Lond., B., Biol. Sci.* 370(1667). 10.1098/rstb.2014.0122
4
- 5 451 Kummu, M., de Moel, H., Salvucci, G., Viviroli, D., Ward, P. J., Varis, O., 2016. Over the hills
6
7 452 and further away from coast: global geospatial patterns of human and environment over
8
9 453 the 20th–21st centuries. *Environ. Res. Lett.* 11(3). 10.1088/1748-9326/11/3/034010
10
- 11 454 Liu, Q., Manning, A. J., Duston, J., 2019. Light intensity and suppression of nocturnal plasma
12
13 455 melatonin in Arctic charr (*Salvelinus alpinus*). *Comp. Biochem. Physiol. A Mol. Integr.*
14
15 456 *Physiol.* 228, 103-106. 10.1016/j.cbpa.2018.11.012
- 16 457 Livak, K. J., Schmittgen, T. D., 2001. Analysis of relative gene expression data using real-time
17
18 458 quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25(4), 402-408.
19
20 459 10.1006/meth.2001.1262
- 21
22 460 Longcore, T., Rich, C., 2004. Ecological light pollution. *Front. Ecol. Environ.* 2(4), 191-198.
23
24 461 10.1890/1540-9295(2004)002[0191:Elp]2.0.Co;2
- 25 462 Longcore, T., Rodriguez, A., Witherington, B., Penniman, J. F., Herf, L., Herf, M., 2018. Rapid
26
27 463 assessment of lamp spectrum to quantify ecological effects of light at night. *J. Exp.*
28
29 464 *Zool. A: Ecol. Integr. Physiol.* 329(8-9), 511-521. 10.1002/jez.2184
- 30
31 465 Luarte, T., Bonta, C. C., Silva-Rodriguez, E. A., Quijon, P. A., Miranda, C., Farias, A. A.,
32
33 466 Duarte, C., 2016. Light pollution reduces activity, food consumption and growth rates
34
35 467 in a sandy beach invertebrate. *Environ. Pollut.* 218, 1147-1153.
36
37 468 10.1016/j.envpol.2016.08.068
- 38 469 Luginbuhl, C. B., Boley, P. A., Davis, D. R., 2014. The impact of light source spectral power
39
40 470 distribution on sky glow. *J. Quant. Spectrosc. Radiat. Transf.* 139, 21-26.
41
42 471 10.1016/j.jqsrt.2013.12.004
- 43
44 472 Lynn, K. D., Tummon Flynn, P., Manriquez, K., Manriquez, P. H., Pulgar, J., Duarte, C.,
45
46 473 Quijon, P. A., 2021. Artificial light at night alters the settlement of acorn barnacles on
47
48 474 a man-made habitat in Atlantic Canada. *Mar. Pollut. Bull.* 163, 111928.
49
50 475 10.1016/j.marpolbul.2020.111928
- 51 476 Manriquez, K., Quijon, P. A., Manriquez, P. H., Miranda, C., Pulgar, J., Quintanilla-Ahumada,
52
53 477 D., Duarte, C., 2021. Artificial Light at Night (ALAN) negatively affects the settlement
54
55 478 success of two prominent intertidal barnacles in the southeast Pacific. *Mar. Pollut. Bull.*
56
57 479 168, 112416. 10.1016/j.marpolbul.2021.112416
- 58 480 Manriquez, P. H., Jara, M. E., Diaz, M. I., Quijon, P. A., Widdicombe, S., Pulgar, J., Manriquez,
59
60 481 K., Quintanilla-Ahumada, D., Duarte, C., 2019. Artificial light pollution influences
61
62
63
64
65

- 482 behavioral and physiological traits in a keystone predator species, *Concholepas*
1 483 *concholepas*. *Sci. Total Environ.* 661, 543-552. 10.1016/j.scitotenv.2019.01.157
2
3 484 Mat, A. M., Massabuau, J. C., Ciret, P., Tran, D., 2012. Evidence for a plastic dual circadian
4
5 485 rhythm in the oyster *Crassostrea gigas*. *Chronobiol. Int.* 29(7), 857-867.
6
7 486 10.3109/07420528.2012.699126
8
9 487 Mat, A. M., Massabuau, J.-C., Ciret, P., Tran, D., 2014. Looking for the clock mechanism
10
11 488 responsible for circatidal behavior in the oyster *Crassostrea gigas*. *Mar. Biol.* 161(1),
12
13 489 89-99. 10.1007/s00227-013-2317-2
14
15 490 Navara, K. J., Nelson, R. J., 2007. The dark side of light at night: physiological,
16
17 491 epidemiological, and ecological consequences. *J. Pineal Res.* 43(3), 215-224.
18
19 492 10.1111/j.1600-079X.2007.00473.x
20
21 493 Nunez, J. D., Bas, C. C., Perez Garcia, M., Ocampo, E. H., Ribeiro, P. D., Luppi, T. A., 2021.
22
23 494 Artificial light at night may increase the predation pressure in a salt marsh keystone
24
25 495 species. *Mar Environ Res* 167, 105285. 10.1016/j.marenvres.2021.105285
26
27 496 Partch, C. L., Green, C. B., Takahashi, J. S., 2014. Molecular architecture of the mammalian
28
29 497 circadian clock. *Trends Cell Biol.* 24(2), 90-99. 10.1016/j.tcb.2013.07.002
30
31 498 Payton, L., Perrigault, M., Hoede, C., Massabuau, J. C., Sow, M., Huvet, A., Boullot, F.,
32
33 499 Fabioux, C., Hegaret, H., Tran, D., 2017a. Remodeling of the cycling transcriptome of
34
35 500 the oyster *Crassostrea gigas* by the harmful algae *Alexandrium minutum*. *Sci. Rep.* 7(1),
36
37 501 3480. 10.1038/s41598-017-03797-4
38
39 502 Payton, L., Sow, M., Massabuau, J. C., Ciret, P., Tran, D., 2017b. How annual course of
40
41 503 photoperiod shapes seasonal behavior of diploid and triploid oysters, *Crassostrea gigas*.
42
43 504 PLoS One 12(10), e0185918. 10.1371/journal.pone.0185918
44
45 505 Payton, L., Tran, D., 2019. Moonlight cycles synchronize oyster behaviour. *Biol. Lett.* 15(1),
46
47 506 20180299. 10.1098/rsbl.2018.0299
48
49 507 Perrigault, M., Tran, D., 2017. Identification of the Molecular Clockwork of the Oyster
50
51 508 *Crassostrea gigas*. PLoS One 12(1), e0169790. 10.1371/journal.pone.0169790
52
53 509 Pevet, P., Balemans, M. G., Legerstee, W. C., Vivien-Roels, B., 1980. Circadian rhythmicity
54
55 510 of the activity of hydroxyindole-O-methyl transferase (HIOMT) in the formation of
56
57 511 melatonin and 5-methoxytryptophol in the pineal, retina, and harderian gland of the
58
59 512 golden hamster. *J. Neural Transm.* 49(4), 229-245. 10.1007/BF01252128
60
61 513 Pulgar, J., Zeballos, D., Vargas, J., Aldana, M., Manriquez, P. H., Manriquez, K., Quijon, P.
62
63 514 A., Widdicombe, S., Anguita, C., Quintanilla, D., Duarte, C., 2019. Endogenous cycles,
64
65 515 activity patterns and energy expenditure of an intertidal fish is modified by artificial

- 516 light pollution at night (ALAN). *Environ. Pollut.* 244, 361-366.
 1 517 10.1016/j.envpol.2018.10.063
 2
 3 518 Rich, C., Longcore, T., 2006. *Ecological Consequences of Artificial Night Lighting*. Island
 4 Press.
 5 519
 6
 7 520 Roeder, T., 2020. The control of metabolic traits by octopamine and tyramine in invertebrates.
 8
 9 521 *J. Exp. Biol.* 223(Pt 7). 10.1242/jeb.194282
 10
 11 522 Sanders, D., Frago, E., Kehoe, R., Patterson, C., Gaston, K. J., 2021. A meta-analysis of
 12 biological impacts of artificial light at night. *Nat Ecol Evol* 5(1), 74-81.
 13 523 10.1038/s41559-020-01322-x
 14 524
 15
 16 525 Scargle, J. D., 1982. *Studies in astronomical time series analysis. II - Statistical aspects of*
 17 *spectral analysis of unevenly spaced data.* *Astrophys. J.* 263. 10.1086/160554
 18 526
 19
 20 527 Thomas, J. R., James, J., Newman, R. C., Riley, W. D., Griffiths, S. W., Cable, J., 2016. The
 21 impact of streetlights on an aquatic invasive species: Artificial light at night alters signal
 22 528 crayfish behaviour. *Appl. Anim. Behav. Sci.* 176, 143-149.
 23 529 10.1016/j.applanim.2015.11.020
 24 530
 25
 26 531 Tidau, S., Whittle, J., Jenkins, S. R., Davies, T. W., 2022. Artificial light at night reverses
 27 monthly foraging pattern under simulated moonlight. *Biol. Lett.* 18(7), 20220110.
 28 532 10.1098/rsbl.2022.0110
 29 533
 30
 31 534 Tran, D., Nadau, A., Durrieu, G., Ciret, P., Parisot, J. P., Massabuau, J. C., 2011. Field
 32 chronobiology of a molluscan bivalve: how the moon and sun cycles interact to drive
 33 535 oyster activity rhythms. *Chronobiol. Int.* 28(4), 307-317.
 34 536 10.3109/07420528.2011.565897
 35 537
 36
 37 538 Tran, D., Perrigault, M., Ciret, P., Payton, L., 2020. Bivalve mollusc circadian clock genes can
 38 run at tidal frequency. *Proc. Biol. Sci.* 287(1918), 20192440. 10.1098/rspb.2019.2440
 39 539
 40
 41 540 Wu, C., Jiang, Q., Wei, L., Cai, Z., Chen, J., Yu, W., He, C., Wang, J., Guo, W., Wang, X.,
 42 541 2018. A Rhodopsin-Like Gene May Be Associated With the Light-Sensitivity of Adult
 43 Pacific Oyster *Crassostrea gigas*. *Front. Physiol.* 9, 221. 10.3389/fphys.2018.00221
 44 542
 45
 46 543 Xie, F., Xiao, P., Chen, D., Xu, L., Zhang, B., 2012. miRDeepFinder: a miRNA analysis tool
 47 for deep sequencing of plant small RNAs. *Plant. Mol. Biol.* 10.1007/s11103-012-9885-
 48 544 2
 49 545
 50
 51 546 Yerushalmi, S., Green, R. M., 2009. Evidence for the adaptive significance of circadian
 52 rhythms. *Ecol. Lett.* 12(9), 970-981. 10.1111/j.1461-0248.2009.01343.x
 53 547
 54
 55 548 Zissis, G., Bertoldi, P., 2018. Status of LED-Lighting world market in 2017.
 56
 57
 58
 59
 60
 61
 62
 63
 64
 65

549

1

2

3 550

4

5

6 551

7

8

9 552

10

11

12 553

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

554 **Figures & legends**

1
2
3 555 **Figure 1.** Behavioral daily pattern of oysters ($n = 15\text{--}16$ oysters) in control condition (0 lux)
4
5 556 and exposed to different ALAN intensities (0.1 lux, 1 lux, 10 lux, and 25 lux). Mean hourly
6
7
8 557 VOD data are shown as mean \pm SE ($n = 7$ days). White backgrounds indicate daytime and
9
10 558 yellow and black backgrounds indicate nighttime. Arrows indicate the VOD peak during the
11
12
13 559 day.

14
15
16 560 **Figure 2.** Effect of ALAN intensity on (A) the mean daily valve opening duration (VOD),
17
18 561 and (B) the mean VOD during the daytime and the nighttime. Data are expressed in mean \pm
19
20
21 562 SE ($n = 7$ days). Different letters indicate significant differences between ALAN conditions
22
23 563 and between daytime and nighttime ($p < 0.05$).

24
25
26 564 **Figure 3.** Effect of ALAN intensity on oysters daily rhythm characteristics: (A) the period at
27
28
29 565 group level, (B) the percent rhythm (PR) at group level, (C) amplitude of the rhythm at group
30
31 566 level, (D) the percentage of oysters having a daily rhythm, (E), period of individual rhythmic
32
33
34 567 oysters, dotted lines define the daily range, (F) the PR at individual level of oysters having a
35
36 568 daily rhythm, (G) amplitude of the rhythm at the individual level of oysters having a daily
37
38
39 569 rhythm. Data are expressed in mean \pm SE. For A-C, the SE shows the daily variability of the
40
41 570 group's significant rhythm parameters ($n = 7$ days). For E-G the SE shows the individual
42
43 571 variability ($n = 15\text{--}16$ oysters). Different letters indicate significant differences ($p < 0.05$).

44
45
46 572 **Figure 4.** Effect of ALAN intensity on the difference in relative mRNA expression level
47
48
49 573 (mean \pm SE, $n = 8$) on oyster gills tissues, between daytime and nighttime of five clock and
50
51 574 clock-associated genes. White bars indicate the gene level during daytime and, colored bars
52
53
54 575 during nighttime. Asterisks indicate significant differences between daytime and nighttime (p
55
56 576 < 0.05).

57
58
59 577
60
61
62
63
64
65

578 **Table 1.** Two-Way Analysis of Variance testing the effect of Daytime/Nighttime and the
 579 ALAN intensity (0 lux, 0.1 lux, 1 lux, 10 lux, 25 lux) on the relative mRNA expression level
 580 on gill tissues of 13 clock and clock-associated genes. In bold, significant p -values ($p < 0.05$).

Source of variation	p -value	df	F
Daytime/Nighttime	0.046	1	3.996
ALAN intensity	0.886	4	0.288
Daytime/Nighttime x ALAN intensity	0.462	4	0.902
Daytime/Nighttime within 0 lux	0.014		
Daytime/Nighttime within 0.1 lux	0.743		
Daytime/Nighttime within 1 lux	0.652		
Daytime/Nighttime within 10 lux	0.903		
Daytime/Nighttime within 25 lux	0.261		

1

1

2

3 2

Artificial light at night at environmental intensities disrupts daily rhythm of the oyster

4

5 3

Crassostrea gigas

6

7

8 4

9

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

10

11 5

12

13

14 6

15

16

17 7

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

18

19 8

20

21

22 9

*Corresponding author: Damien Tran

23

24 10

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

25

26 11

27

28 12

29

30 13

31

32 14

33

34 15

35

36 16

37

38 17

39

40 18

41

42 19

43

44 20

45

46 17

47

48 18

49

50 19

51

52 20

53

54

55

56

57

58

59

60

61

62

63

64

65

21 **Abstract**

1
2
3 22 Artificial Light At Night (ALAN) masks the natural light cycles and thus can disturb the
4
5 23 synchronization of organisms' biological rhythms with their environment. Although
6
7
8 24 coastlines are highly exposed to this growing threat, studies concerning the impacts of ALAN
9
10 25 on coastal organisms remain scarce. In this study, we investigated the ALAN exposure effects
11
12
13 26 at environmentally realistic intensities (0.1, 1, 10, 25 lux) on the oyster *Crassostrea gigas*, a
14
15 27 sessile bivalve subject to light pollution on shores. We focused on the effects on oyster's daily
16
17 28 rhythm at behavioral and molecular levels. Our results showed that ALAN disrupts the
18
19
20 29 oyster's daily rhythm by increasing valve activity and annihilating day / night differences of
21
22 30 expression of circadian clock and clock-associated genes. ALAN effects occur starting from
23
24
25 31 0.1 lux, in the range of artificial skyglow illuminances. We concluded that realistic ALAN
26
27 32 exposure affects oysters' biological rhythm, which could lead to severe physiological and
28
29
30 33 ecological consequences.

31
32
33 34 Keywords: ALAN, biological rhythm, circadian clock, *Crassostrea gigas*, behavior
34
35
36 35
37
38
39 36
40
41
42 37
43
44
45 38
46
47
48 39
49
50 40
51
52
53 41
54
55
56 42
57
58
59 43
60
61
62
63
64
65

44 Introduction

1
2
3 45 Natural light cycles play an important role in biological timings (Gaston et al., 2017). Indeed,
4
5 46 predictable variations of light intensity over a day, a month, or a year are used as signals by
6
7 47 organisms to synchronize their biological rhythms with their environment (Bradshaw and
8
9 48 Holzapfel, 2010; Gaston et al., 2017). These biological rhythms are ubiquitous and find their
10
11 49 origin in each cell with an endogenous clock, which uses environmental cues, such as natural
12
13 50 light cycles, to synchronize organisms' physiological processes and behavior with their
14
15 51 environment. This synchronization enables organisms to be fully adapted to their environment
16
17 52 and to anticipate its changes (Cermakian and Sassone-Corsi, 2000; Partch et al., 2014).
18
19 53 However, Artificial Light At Night (ALAN) affects the natural nocturnal lighting levels,
20
21 54 which can disrupt the organisms' perception of natural variations of light, and affect
22
23 55 organisms' biological rhythms (daily, lunar, seasonal) and their physiology (Falchi et al.,
24
25 56 2011; Gaston and Bennie, 2014). For example, it can disrupt migration, reproduction,
26
27 57 foraging, or prey/predator interactions (Davies et al., 2014; Gaston et al., 2017; Longcore and
28
29 58 Rich, 2004; Navara and Nelson, 2007). These ALAN's effects on organisms' physiology and
30
31 59 behavior occur in a large range of species, including diurnal ones, and at low intensities
32
33 60 (below 1 lux) (Sanders et al., 2021). Thus ALAN can have consequences at the individual,
34
35 61 population, and ecosystem scale. Nocturnal artificial lighting affects the whole world (Falchi
36
37 62 et al., 2016) and spreads fast with a 6% increase in the sky luminosity per year (Hölker et al.,
38
39 63 2010). The negative effects of ALAN on organism behavior or physiology become
40
41 64 increasingly studied over the years (Davies and Smyth, 2018) but they remain widely
42
43 65 investigated for terrestrial organisms. However, ALAN effects on marine organisms, and
44
45 66 especially benthic organisms, recently received more attention. Studying the impacts of
46
47 67 ALAN on species living in coastal ecosystems is of great importance considering that already
48
49 68 22.2 % of the world's coasts are exposed to ALAN as well as 35% of marine protected areas
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

69 (Davies et al., 2014, 2016), percentages that certainly will increase since the human
70 population living in coastal areas is predicted to grow (Kummu et al., 2016). Furthermore,
71 ALAN's negative effects could be worsened by the increasing use of LED (Light-Emitting
72 Diode) in public lighting (Gaston, 2018; Zisis and Bertoldi, 2018). Indeed, LED lights
73 spectrum contains more short-wavelength than spectra of other lighting types such as HPS
74 (High-Pressure Sodium) lighting (Bierman, 2012; Falchi et al., 2011; Longcore et al., 2018;
75 Luginbuhl et al., 2014). Considering that short-wavelength, such as blue wavelength, go
76 further into seawater (Davies et al., 2014; Grubisic et al., 2019) and that marine organisms
77 would be highly sensitive to blue wavelength (Grubisic, 2018), the negative effects of ALAN
78 could be amplified on the coastal fauna. Therefore, ALAN becomes over the years a growing
79 threat to marine life.

80 The oyster *Crassostrea gigas* is a marine bivalve of commercial importance with a large
81 distribution. Oysters are also sessile organisms living in benthic areas, thus inevitably exposed
82 to ALAN through direct or indirect sources of artificial light. The direct sources of lighting on
83 the coast are for example streetlights on a pier, harbors, etc. The indirect source of ALAN is
84 called skyglow, with a lower light intensity but much more spatially extensive than direct
85 lighting. It takes its source mostly in big cities from which artificial lights are diffused into the
86 atmosphere, resulting in a global lightening of the sky at night (Gaston, 2018). Both of these
87 ALAN sources could disrupt oysters' biological rhythms. These oysters' biological rhythms
88 related to daily (Mat et al., 2012), tidal (Tran et al., 2011), lunar (Payton and Tran, 2019), and
89 seasonal (Payton et al., 2017b) natural cycles have already been described. Concerning their
90 daily rhythm, oysters have a plastic endogenous circadian rhythm generated by a molecular
91 clock (Mat et al., 2012; Mat et al., 2014; Payton et al., 2017a; Perrigault and Tran, 2017; Tran
92 et al., 2020). This plasticity enables oysters to easily adapt to new environments but it also

93 could make them vulnerable to disruption by ALAN, which could have consequences on their
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 106
94 physiology and ecology.

95 The objective of this study is to evaluate the effects of several ALAN intensities encountered
96 in the environment on the daily rhythm of the oyster *C. gigas*. To achieve that, valve behavior
97 rhythm and circadian clock genes expression were studied. We exposed oysters during 7 days
98 to ALAN intensities ranging from 0.1 to 25 lux with the lowest intensity representing a
99 skyglow intensity (Gaston, 2018) and the highest intensity being in the range of direct ALAN
100 due to a lighted parking lot (Rich and Longcore, 2006). We hypothesized that ALAN would
101 disrupt oysters' daily rhythm at the behavioral and molecular levels. We assumed that ALAN
102 exposure disrupts the oysters' circadian clock machinery, impairing the clock's outputs such
103 as clock-associated genes' expression and rhythmic behavior, and may also lead to a direct
104 behavioral response during nighttime. Finally, we hypothesized that some of these effects
105 would occur in an intensity-dependent manner.

107 **Materials and methods**

108 *General conditions*

109 The experiment was conducted from January to April 2021 in the Marine Station of Arcachon
110 on 160 oysters (85.8 ± 0.7 mm shell length; 48.4 ± 0.5 mm shell width; mean \pm SE) coming
111 from an oyster's farm source of the Arcachon bay (France). During the acclimation and
112 experimental duration, oysters were placed into tanks (L x W x H: 74.8 x 54.8 x 40.8 mm)
113 continuously supplied with natural seawater from the Arcachon bay, which was filtered (<
114 $1\mu\text{m}$), oxygenized, and the temperature was monitored ($T = 15.0 \pm 0.1$ °C). Tanks were
115 placed in an isolated room and equipped with an antivibration bench to minimize external
116 disturbances to animal behavior. The oysters were not fed during the experiment.

117 *Experimental protocol*

1
2 118 Oysters were maintained under a L:D 10:14 cycle with daytime from 7:00 to 17:00 h (all
3
4
5 119 times noted in local time, UTC +1) (Fig. S1A). The light intensity during daytime varied
6
7 120 gradually to mimic the natural cycle of light using programmable white (413–688 nm, peak at
8
9 121 551 nm; Fig. S2A) LED light bars (MH3SP3 DSunY). The maximum intensity during
10
11 122 daytime was 1473.42 ± 106.21 lux (mean \pm SE) (Tab. S.1) between 11:30 to 12:30 h. After 7
12
13 123 to 9 days of acclimation to the experimental setup, the proper experiment started for a
14
15 124 duration of 7 days. There were 5 conditions: a control condition and 4 conditions where
16
17 125 oysters were exposed to several ALAN intensities (Tab. S1): 0.1, 1, 10, and 25 lux. In the 4
18
19 126 ALAN conditions, oysters were exposed to ALAN from 17:30 to 6:30 h (Fig. S1B) using
20
21 127 white (411–687 nm, peak at 563 nm; Fig. S2B–S2E) LED strips (MiBoxer Mi-Light WL5).
22
23 128 Illuminances underwater were measured at five positions in the tank (Fig. S3) using a
24
25 129 handheld spectroradiometer (Blue-Wave UVN-100, StellarNet Inc.). The control group was in
26
27 130 the complete dark at night, with a light intensity inferior to the detection limit of the
28
29 131 spectroradiometer (0.05 lux), mentioned in the study as 0 lux for convenience. For each of the
30
31 132 5 experimental conditions, 32 oysters were placed in the same tank, and the valve activity of
32
33 133 16 of them was continuously measured using a High Frequency–Non Invasive (HFNI)
34
35 134 valvometer technology (Andrade et al., 2016). For each experimental conditions, 32 different
36
37 135 oysters were used. On the 7th day of the experiment, the gill tissue of 8 oysters were sampled
38
39 136 during daytime at 12:00 h and during nighttime at 19:00 h and stored in Tri Reagent
40
41 137 (Invitrogen) at -80 °C for further molecular analysis.
42
43
44
45
46
47
48
49
50
51

52 138 *Behavioral measurements*

53
54 139 For valve activity measurement using HFNI valvometer technology, lightweight's
55
56 140 electromagnets were glued on each valve of oysters and linked to a valvometer device using
57
58 141 flexible wires (Andrade et al., 2016). The electromagnetic current generated between the two
59
60
61
62
63
64
65

142 electromagnets allows to measure oyster valve activity. For each individual, the signal was
1 recorded every 4.8s. Data were processed using Labview 8.0 (National Instrument, Austin,
2 143 TX, USA). The study focused on the hourly Valve Opening Duration (VOD) meaning that we
3
4 144 determined the percentage of time each individual spent with its valve open for each hour. For
5
6 145 example, an individual with its valves open during a whole hour corresponds to a VOD of 100
7
8 146 %, while an individual with its valves closed during one hour corresponds to a VOD of 0 %.
9
10
11
12 147

148 *Chronobiological analyses*

149 Chronobiological analysis of the hourly VOD data were performed at groups and individual
150 levels using the software Time Series Analysis Serie Cosinor 8.0 (Expert Soft Technologies).
151 First, data quality was evaluated using the autocorrelation diagram to control the absence of
152 random repartition of data, and the Partial Autocorrelation Function (PACF) calculation to
153 assess the absence of stationary character (Gouthière and Mauvieux, 2003). Then the Lomb
154 and Scargle periodogram was used to search for periodicity in the data (Scargle, 1982). A
155 significant period was accepted for $p > 0.95$. Data rhythmicity was then modeled using the
156 Cosinor model, which uses a cosine function calculated by regression (Bingham et al., 1982).
157 For a given period, the model is written as: $Y(t) = A \cos\left(\frac{2\pi t}{\tau} + \phi\right) + M + \varepsilon(t)$ where A is
158 the amplitude (difference between the average level and the highest value of the rhythm), ϕ
159 the acrophase (the highest value of the rhythm), τ the given period (interval between two
160 identical events), M the mesor (average level of the rhythm), and $\varepsilon(t)$ the relative error. Then
161 the calculated model and the existence of rhythmicity were validated by two tests: the ellipse
162 test had to be rejected, and the probability for the null amplitude hypothesis had to be lower
163 than 0.05. The percent rhythm (PR), a chronobiometric parameter, had been calculated and
164 represents the percentage of cyclic behavior explained by the model, meaning the strength of
165 the rhythm.

166 *Total RNA extraction and cDNA synthesis*

1
2 167 Total RNA was extracted from gills using Tri reagent (Invitrogen) and an SV Total RNA
3
4
5 168 Isolation System kit (Promega). The total RNA quantity and quality were assessed by
6
7 169 spectrophotometry (OD230, OD260, OD280). RNA reverse transcription was realized using
8
9
10 170 Goscript™ Reverse Transcription System kits (Promega).

13 171 *mRNA expression analysis by Real-Time PCR*

15 172 Real-Time qPCR was realized using GoTaq® qPCR Master Mix kit (Promega). Primers sets
16
17
18 173 of clock and clock-associated genes (*CgClock*, *CgBmal*, *CgCry*, *CgPer*, *CgTim1*, *CgCry1*,
19
20 174 *CgRev-erb*, *CgRor*, *CgHiomt-like*, *CgOctβ2*, *CgRhodopsin-like 1*, *CgRhodopsin-like 2*,
21
22
23 175 *CgRhodopsin-like 3*), and housekeeping genes (*CgEfl*, *Cg28S*, *CgGadph*) are listed in the
24
25 176 table S2. qPCR reactions were realized as follows: 95 °C for 2 min to activate the GoTaq
26
27
28 177 polymerase, 40 cycles of 95 °C for 15 seconds (denaturation), and 60 °C for 1 minute
29
30 178 (annealing and extension) for amplification of target cDNA, followed by 2 minutes at 60 °C
31
32
33 179 for the final elongation. In the end, melting curves were generated by gradually decreasing the
34
35 180 temperature from 95 °C to 60 °C to control the primer specificity. The comparative Ct method
36
37 181 $2^{-\Delta Ct}$ (Livak and Schmittgen, 2001) was used to determine the relative transcript level of clock
38
39
40 182 and clock-associated genes, where $\Delta Ct = Ct(\text{target gene}) - Ct(\text{housekeeping gene})$. Gene's
41
42 183 expression was normalized with the geometric mean of the housekeeping genes *CgEfl* and
43
44
45 184 *Cg28S*, based on stability values (Xie et al., 2012).

48 185 *Statistical analysis*

50
51 186 All statistical analysis were performed using SigmaPlot software (version 13.0; Systat
52
53 187 Software, USA). T-tests were performed for two groups' comparisons after checking
54
55
56 188 assumptions (normality of data and equal variance) and if they were not validated the non-
57
58 189 parametric Mann-Whitney rank sum test was performed. Two groups' comparisons were used
59
60 190 for molecular analysis results. For multiple comparisons, one-way ANOVA were used after
61
62
63
64
65

191 checking assumptions (normality of data and equal variance). When assumptions were not
192 validated, the non-parametric Kruskal-Wallis One-Way ANOVA on ranks test was
193 performed. In case of significant differences, a Tukey test was realized for all pairwise
194 multiple comparisons unless the groups did not have equal size, in which case the Dunn's test
195 was realized. Multiple comparisons were performed for behavioral analyses. On the whole
196 molecular data, a two-way ANOVA was also performed after checking assumptions
197 (normality of data and equal variance), and in case of significant differences, a Tuckey test
198 was used for all pairwise multiple comparisons. For all test results, a difference was
199 considered significant when $p < 0.05$.

200

201 **Results**

202 Figure 1 shows the behavioral mean daily pattern of oysters in the control condition, and
203 exposed to ALAN ranging from 0.1 to 25 lux. In the control condition, we show a diurnal
204 pattern with a maximal VOD at 11–12 h (peak showed by the black arrow). When exposed to
205 ALAN, the overall daily pattern of oysters' behavior is modified, with an increase of the
206 hourly VOD during nighttime and a 7-hours shift of the daily VOD peak, delayed from
207 daytime to nighttime, for all ALAN intensities starting from 0.1 lux. Statistical analysis of the
208 mean daily VOD shows a significant increase from 0.1 lux, of the mean VOD when oysters
209 are exposed to ALAN for all of the tested intensities ($p = 0.002$; Fig. 2A), without an intensity
210 effect. The figure 2B shows no significant differences in VOD during daytime between all
211 conditions. However, ALAN causes a significant increase of the mean VOD during nighttime
212 starting from 0.1 lux. The significant difference of VOD between daytime and nighttime
213 observed in the control condition decreases at 0.1 lux and significantly disappears at 1, 10,
214 and 25 lux. Figure 3 shows chronobiological analysis, characterizing the effects of ALAN
215 exposure on the oysters' daily behavioral rhythm at the group and individual levels (detailed

216 results in Table S3 and Table S4). At the group level, a significant daily rhythm persists in all
1
2 217 conditions with no significant differences of period length between the conditions (Fig. 3A).
3
4 218 However, ALAN affected several parameters of this rhythm. Indeed, there is a significant
5
6
7 219 decrease ($p < 0.001$) of the percent rhythm (PR, %) of the daily rhythm when oysters are
8
9
10 220 exposed to ALAN starting from 1 lux (Fig. 3B). Furthermore, the amplitude of the daily
11
12 221 rhythm decreases significantly ($p < 0.001$) in all ALAN conditions, starting from 0.1 lux and
13
14 222 without significant ALAN intensity-effect (Fig. 3C). At the individual level, results show an
15
16 223 ALAN intensity-dependent decrease of the percentage of oysters having a significant daily
17
18 224 rhythm (Fig. 3D). Figure 3E shows a decrease ($p < 0.001$) of the individual rhythmic period
19
20
21 225 lengths, getting out the daily range for many individuals exposed to ALAN (Table S3). The
22
23
24 226 mean period of oysters exposed to 1 lux and 25 lux are 17.9 ± 2.1 and 15.2 ± 1.7 h
25
26 227 respectively, under the circadian range (20-28 h), explaining the global loss of rhythmicity at
27
28
29 228 the daily scale shown in figure 3D. Furthermore, the PR of oysters having a daily rhythm
30
31 229 tends to decrease starting from 0.1 lux, and it decreases significantly ($p = 0.015$) when they
32
33
34 230 are exposed to 10 lux (Fig. 3F). Finally, the amplitude of oysters having a daily rhythm show
35
36 231 no significant differences ($p = 0.169$) but a trend to decrease when oysters are exposed to
37
38
39 232 ALAN (Fig 3G). Figures 4 and S4 shows the mRNA level during daytime and nighttime of
40
41 233 thirteen circadian clock (*CgClock*, *CgBmal*, *CgCry*, *CgPer*, *CgTim1*, *CgCry1*, *CgRev-erb*,
42
43 234 *CgRor*) and clock-associated (*CgHiomt-like*, *CgOctβ2*, *CgRhodopsin-like 1*, *CgRhodopsin-*
44
45 235 *like 2*, *CgRhodopsin-like 3*) genes for all of the tested ALAN intensities. No significant
46
47
48 236 differences of gene expression are observed between the control and the ALAN conditions
49
50
51 237 neither at daytime nor at nighttime. However, results show significant differences of mRNA
52
53 238 expression levels between daytime and nighttime in the control condition for five genes (two
54
55 239 circadian clock genes: *CgTim1* and *CgRev-erb*; and three clock-associated genes: *CgHiomt-*
56
57 240 *like*, *CgOctβ2* and *CgRhodopsin-like 1*), with an increase of expression during nighttime (Fig.

241 4). This daytime / nighttime difference of gene expression significantly disappears in all of the
242 five genes for all ALAN intensities tested. Finally, the table 1 shows the results of a two-way
243 ANOVA, testing the effects of the period of the day (daytime and nighttime), ALAN
244 intensities, and their interaction on the overall relative mRNA levels of the thirteen genes
245 studied. These results reveal a significant difference ($p = 0.046$) of the overall mRNA level
246 between daytime and nighttime only for the control condition ($p = 0.014$). In all ALAN
247 conditions, the daytime / nighttime difference of gene expression is abolished.

249 Discussion

250 Our study shows that ALAN affects the daily behavioral rhythm and the molecular clock of
251 oysters starting from the lowest ALAN intensity tested, 0.1 lux, in the range of artificial
252 skyglow. Multiples ALAN's effects on oyster's behavioral rhythm are observed: a decrease of
253 the percentage of oysters having a daily rhythm, a decrease of the rhythm's robustness, a
254 decrease of the rhythmic amplitude, an increase of the mesor (mean VOD), and a shift from a
255 diurnal to a nocturnal peak activity. At the molecular level, the overall difference of clock and
256 clock-associated gene expression observed in control condition between daytime and
257 nighttime is abolished when oysters are exposed to ALAN. While a clear ALAN intensity-
258 effect is observed at the individual level regarding the percentage of oysters having a daily
259 behavioral rhythm, most of ALAN's effects at both behavioral and molecular levels occur
260 from 0.1 to 25 lux without significant intensity-effect.

261 We showed that ALAN exposure leads to an increase of oysters' VOD, explained by an
262 increase of activity during nighttime leading to a decrease (at 0.1 lux) or a loss (at 1, 10, and
263 25 lux) of daytime / nighttime differences of valve behavior and a shift of peak activity from
264 daytime to nighttime. Activity increase during nighttime as an effect of ALAN has been also
265 shown in other marine organisms, such as the diurnal fish *Girella laevis* exposed to 70 lux

266 at night (Pulgar et al., 2019). On the contrary, the nocturnal crayfish *Pacifastacus leniusculus*
267 showed a decrease of activity during nighttime when exposed to ALAN at 12 lux using a HPS
268 streetlight bulb (Thomas et al., 2016). ALAN effects on organisms' behavior have also been
269 investigated in juvenile gastropods *Concholepas concholepas*, in which ALAN at an intensity
270 of 329.9 lux (LED technology) induces a less efficient search for prey during nighttime
271 (Manriquez et al., 2019). Moreover, some previous results of bivalve behavior disruption by
272 ALAN was shown by Christoforou (2022), which investigated the effects of several ALAN
273 wavelengths at an intensity of 19.86 lux using LED lightings on the behavior of the mussel
274 *Mytilus edulis*. The exposure of mussels to white ALAN induces a decrease during both
275 nighttime and daytime of the open/close frequency valve events. However, in this study, no
276 chronobiological analysis were performed and the relatively invasive experimental device
277 used to record mussels' behavior could have mask ALAN effects on mussels valve activity. In
278 our study, we went further in the investigation of ALAN's impacts on daily behavioral rhythm
279 using chronobiological analysis and lower ALAN intensities, using a non-invasive biosensor
280 (Andrade et al., 2016).

281 Daily rhythms and more generally biological rhythms are of great importance since they
282 enable organisms to anticipate cyclic changes in their environment and regulate accordingly
283 the temporal organization of their physiological processes such as feeding, respiration,
284 immunity, or growth (Helm et al., 2017; Yerushalmi and Green, 2009). Thus, the clear
285 disruption of oysters' daily rhythm by ALAN observed in this study, starting from 0.1 lux,
286 could have deep consequences on organism's physiology and fitness. The oysters were not
287 fed during the experiments to avoid an effect of food supply that may fluctuate, and thus acts
288 as a zeitgeber of daily rhythm. However, during our experimentation duration in the control
289 condition, we did not observe a modification of behavior, showing that food absence did not
290 affect ALAN's effects on oysters' daily rhythm. Furthermore, the experiments were

291 conducted in winter, when the food supply is very low in the environment. Moreover, the
1
2 292 rhythm at the daily scale could not be the only rhythm that can be disrupted by ALAN. It has
3
4
5 293 been shown that oysters have other biological rhythms. For example, oysters showed a lunar
6
7 294 rhythm i.e. their valve behavior is synchronized with lunar cycles by detecting moonlight
8
9
10 295 illumination cycle (Payton and Tran, 2019). This lunar rhythm could be affected by ALAN
11
12 296 since the maximal intensity of the moonlight ranges from 0.1 to 0.3 lux (Rich and Longcore,
13
14 297 2006), thus the natural variations of moonlight intensity can be easily masked by ALAN
15
16
17 298 intensities, such as the skyglow. The masking effect of lunar cycles by ALAN using LED has
18
19 299 been investigated in the corals *Acropora millepora* and *Acropora digitifera*, in which
20
21
22 300 exposition to ALAN delayed or masked their gametogenesis leading to a spawning
23
24 301 desynchronization (Ayalon et al., 2021). Another example of ALAN effect on lunar rhythm is
25
26 302 the disruption of the monthly nocturnal foraging pattern of the gastropod *Nucella lapillus*,
27
28
29 303 based on the lunar cycle, by ALAN produced by LED lighting at an intensity of 10 lux (Tidau
30
31 304 et al., 2022). Moreover, oysters' seasonal rhythms could be disrupted by ALAN. Oysters use
32
33
34 305 the variation of photoperiod through the year to anticipate seasonal changes and synchronize
35
36 306 physiological processes such as behavior, growth, and spawning (Bernard et al., 2016; Payton
37
38
39 307 et al., 2017b). However, ALAN being often present during dusk and dawn, it can mask these
40
41 308 two natural phenomena, preventing oysters to detect the variation of photoperiod along the
42
43
44 309 year, which could affect several seasonal physiological processes and decrease the fitness of
45
46 310 organisms (Gaston et al., 2017). ALAN's effects on organisms' physiological processes and
47
48
49 311 fitness have been shown for example in the amphipod *Orchestoidea tuberculata*, in which
50
51 312 ALAN (halogen light) induces a decrease of growth (Luarte et al., 2016), and in barnacles, in
52
53 313 which ALAN (LED lighting) affects the settlement process (Lynn et al., 2021; Manriquez et
54
55
56 314 al., 2021). Moreover, ALAN's effects on organisms' fitness have also been observed in the
57
58 315 juvenile crabs *Neohelice granulata* since it increases mortality, mainly by increasing
59
60
61
62
63
64
65

316 cannibalistic interactions with adults at an intensity of approximately 40 lux (LED lighting)
1
2 317 (Nunez et al., 2021).
3
4
5 318 In addition to the effects on the oyster's behavioral daily rhythm, this study also showed that
6
7 319 ALAN disturbs the circadian clockwork of *C. gigas* by annihilating differences between
8
9 320 daytime and nighttime in clock and clock-associated genes expression starting from the
10
11 321 intensity 0.1 lux. Genes for which their expression was mainly affected by ALAN are
12
13 322 *CgTim1*, *CgRev-erb*, *CgHiomt-like*, *CgOctβ2*, and *CgRhodopsin-like 1*. *CgTim1* and *CgRev-*
14
15 323 *erb* are both involved in the regulation's feedback loops of the oysters' molecular clock and
16
17 324 thus are core clock genes (Perrigault and Tran, 2017). On the other hand, *CgHiomt-like*,
18
19 325 *CgOctβ2*, and *CgRhodopsin-like 1* are clock-associated genes. The first one is a homologue of
20
21 326 the mammal gene *Hiomt* involved in the final step of the synthesis of melatonin, known to be
22
23 327 a key molecule of the day / night rhythm and a potent antioxidant with a proposed role in
24
25 328 immune function (Jones et al., 2015; Pevet et al., 1980). *CgOctβ2* is the gene of the
26
27 329 octopamine receptor β-2R. Octopamine is a biogenic amine well studied in arthropods and
28
29 330 gastropods where it functions as a neurotransmitter and hormone, fulfilling the roles played
30
31 331 by adrenalin in vertebrates. It is an extremely pleiotropic substance, participating in
32
33 332 invertebrate development such as growth, maturation, and reproduction by activating their
34
35 333 corresponding G protein-coupled receptors (GPCRs), and may interact with the circadian
36
37 334 clock (Roeder, 2020). Finally, the last gene to show a disrupted expression in presence of
38
39 335 ALAN is the gene of the rhodopsin (*CgRhodopsin-like 1*), a photoreceptor involved in
40
41 336 oysters' light-sensitivity (Wu et al., 2018). Thus, ALAN affects five of the thirteen genes
42
43 337 studied here. This relative low transcriptional response observed might be due to the fact that
44
45 338 oysters have been sampled at only two sampling times over the daily cycle. Indeed, a stronger
46
47 339 transcriptional disruption might possibly have been revealed by sampling at another time of
48
49 340 the day. However, our results still show that ALAN can affect genes of the molecular clock of
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

341 *C. gigas* (*CgTim1*, *CgRev-erb*), suggesting that the observed disruption of oyster's daily
342 behavioral rhythm would be a real consequence of the circadian clock disruption and not only
343 a direct reaction of light perception in presence of ALAN. ALAN also affects essential clock-
344 associated genes (*CgHiomt*, *CgOctβ2*, *CgRhodopsin-like 1*) involved in physiological
345 processes regulation. Thus, we can imagine that, by cascading effect, the expression of other
346 genes involved in several physiological functions can also be disrupted by ALAN. For
347 example, ALAN at 0.1–0.3 lux using a fluorescent bulbs induces the suppression of the
348 increase of melatonin during nighttime in *Salvelinus* (Liu et al., 2019). Moreover, in the
349 European perch *Perca fluviatilis* and the roach *Rutilus rutilus* the impairment of circadian
350 melatonin pattern by ALAN (HPS lamps) is associated with a decrease of the mRNA
351 expression of gonadotropins, which could disrupt their reproduction (Bruning et al., 2018).

352

353 **Conclusion**

354 This study is the first to investigate ALAN effects at environmentally realistic intensities on
355 oysters, a key species of the benthic coastal ecosystems. Our results reveal deep impacts on
356 the daily behavioral rhythm of *C. gigas*, from ALAN intensities starting from 0.1 lux,
357 comparable to the skyglow intensities. We also found that the expression of some of the clock
358 and clock-associated genes of the oyster's molecular clock was affected by ALAN, suggesting
359 a disruption of the endogenous clock and potential cascading physiological consequences.
360 However, studies focusing on the ALAN's effects on oysters' biological rhythms and the
361 consequences of their disruption on their fitness and physiology are needed. Furthermore,
362 given that ALAN spreads fast in coastal environments in which oysters are a key species,
363 ALAN's effects on this organism could have ecological consequences that should be
364 investigated to get a global picture of how ALAN will affect coastal ecosystems.

1
2 366 **Ethics:** All experiments complied with the laws in effect in France and they conformed to
3 international ethical standards.

4
5 368 **Data accessibility:** The data underlying this study are available on Supplementary data.

6
7
8 369 **Author contributions:** Study design and methodology, D.T., A.B., L.P.; experimentation,
9 A.B., D.T.; molecular analysis: A.B., L.P.; data treatment, A.B.; interpretation, A.B., D.T.,
10 370 L.P.; manuscript writing, A.B.; review and editing, all authors; funding, D.T. All authors
11 371 contributed critically to the drafts, and gave final approval for publication.
12
13
14
15
16 372

17
18
19 373 **Competing interests:** We have no competing interests.
20
21

22 374 **Funding**

23
24 375 This work was supported by the financial support of the French Nation Research Agency
25 (ANR), LUCIOLE project (ANR-22-CE34-0010-01) and a Bordeaux University PhD
26 376 scholarship for A. Botté.
27
28
29
30
31

32 378 **Acknowledgments**

33
34
35 379 We kindly thank Pierre Ciret, Christian Portier, and Christelle Teillet for their help during the
36 setup of the experiments.
37 380
38
39
40
41 381

382 **References**

- 1
2
3 383 Andrade, H., Massabuau, J.-C., Cochrane, S., Ciret, P., Tran, D., Sow, M., Camus, L., 2016.
4 384 High Frequency Non-invasive (HFNI) Bio-Sensors As a Potential Tool for Marine
5 385 Monitoring and Assessments. *Front. Mar. Sci.* 3. 10.3389/fmars.2016.00187
6
7
8 386 Ayalon, I., Rosenberg, Y., Benichou, J. I. C., Campos, C. L. D., Sayco, S. L. G., Nada, M. A.
9
10 387 L., Baquiran, J. I. P., Ligson, C. A., Avisar, D., Conaco, C., Kuechly, H. U., Kyba, C.
11
12 388 C. M., Cabaitan, P. C., Levy, O., 2021. Coral Gametogenesis Collapse under Artificial
13
14 389 Light Pollution. *Curr. Biol.* 31(2), 413-419 e413. 10.1016/j.cub.2020.10.039
15
16 390 Bernard, I., Massabuau, J. C., Ciret, P., Sow, M., Sottolichio, A., Pouvreau, S., Tran, D., 2016.
17 391 In situ spawning in a marine broadcast spawner, the Pacific oyster *Crassostrea gigas*:
18
19 392 Timing and environmental triggers. *Limnol. Oceanogr.* 61(2), 635-647.
20
21 393 10.1002/lno.10240
22
23 394 Bierman, A., 2012. Will switching to LED outdoor lighting increase sky glow? *Light. Res.*
24
25 395 *Technol.* 44(4), 449-458. 10.1177/1477153512437147
26
27 396 Bingham, C., Arbogast, B., Guillaume, G. C., Lee, J. K., Halberg, F., 1982. Inferential statistical
28
29 397 methods for estimating and comparing cosinor parameters. *Chronobiologia* 9, 397-439.
30
31 398 Bradshaw, W. E., Holzapfel, C. M., 2010. Light, time, and the physiology of biotic response to
32
33 399 rapid climate change in animals. *Annu. Rev. Physiol.* 72, 147-166. 10.1146/annurev-
34
35 400 *physiol*-021909-135837
36
37 401 Bruning, A., Kloas, W., Preuer, T., Holker, F., 2018. Influence of artificially induced light
38
39 402 pollution on the hormone system of two common fish species, perch and roach, in a
40
41 403 rural habitat. *Conserv. Physiol.* 6(1), coy016. 10.1093/conphys/coy016
42
43 404 Cermakian, N., Sassone-Corsi, P., 2000. Multilevel regulation of the circadian clock. *Nat. Rev.*
44
45 405 *Mol. Cell. Biol.* 1(1), 59-67. 10.1038/35036078
46
47 406 Christoforou, E., 2022. Effects of microfiber and light pollution on coastal ecosystem services
48
49 407 provided by the blue mussel, *Mytilus edulis*. University of Glasgow.
50
51 408 <https://theses.gla.ac.uk/82885/>
52
53 409 Davies, T. W., Duffy, J. P., Bennie, J., Gaston, K. J., 2014. The nature, extent, and ecological
54
55 410 implications of marine light pollution. *Front. Ecol. Environ.* 12(6), 347-355.
56
57 411 10.1890/130281
58
59 412 Davies, T. W., Duffy, J. P., Bennie, J., Gaston, K. J., 2016. Stemming the Tide of Light
60
61 413 Pollution Encroaching into Marine Protected Areas. *Conserv. Lett.* 9(3), 164-171.
62
63 414 10.1111/conl.12191
64
65

- 415 Davies, T. W., Smyth, T., 2018. Why artificial light at night should be a focus for global change
1 416 research in the 21st century. *Glob. Chang. Biol.* 24(3), 872-882. 10.1111/gcb.13927
- 3 417 Falchi, F., Cinzano, P., Elvidge, C. D., Keith, D. M., Haim, A., 2011. Limiting the impact of
4 418 light pollution on human health, environment and stellar visibility. *J Environ. Manage.*
5 419 92(10), 2714-2722. 10.1016/j.jenvman.2011.06.029
- 8 420 Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C., Elvidge, C. D., Baugh, K., Portnov, B. A.,
9 421 Rybnikova, N. A., Furgoni, R., 2016. The new world atlas of artificial night sky
10 422 brightness. *Sci. Adv.* 2(6), e1600377. 10.1126/sciadv.1600377
- 13 423 Gaston, K. J., Bennie, J., 2014. Demographic effects of artificial nighttime lighting on animal
14 424 populations. *Environ. Rev.* 22(4), 323-330. 10.1139/er-2014-0005
- 17 425 Gaston, K. J., Davies, T. W., Nedelec, S. L., Holt, L. A., 2017. Impacts of Artificial Light at
18 426 Night on Biological Timings. *Annu. Rev. Ecol. Evol. Syst.* 48(1), 49-68.
19 427 10.1146/annurev-ecolsys-110316-022745
- 22 428 Gaston, K. J., 2018. Lighting up the nighttime. *Science* 362(6416), 744-746.
23 429 10.1126/science.aau8226
- 26 430 Gouthière, L., Mauvieux, B., 2003. Etapes essentielles dans l'analyse des rythmes: qualité des
27 431 données expérimentales, recherche de périodes par analyses spectrales de principes
28 432 divers, modélisation. XXXVème Congrès Société Francoph Chronobiol, Université de
29 433 Saint-Etienne.
- 32 434 Grubisic, M., 2018. Waters under Artificial Lights: Does Light Pollution Matter for Aquatic
33 435 Primary Producers? *Limnol. Oceanogr. Bull.* 27(3), 76-81. 10.1002/lob.10254
- 36 436 Grubisic, M., Haim, A., Bhusal, P., Dominoni, D. M., Gabriel, K. M. A., Jechow, A., Kupprat,
37 437 F., Lerner, A., Marchant, P., Riley, W., Stebelova, K., van Grunsven, R. H. A., Zeman,
38 438 M., Zubidat, A. E., Hölker, F., 2019. Light Pollution, Circadian Photoreception, and
39 439 Melatonin in Vertebrates. *Sustainability* 11(22). 10.3390/su11226400
- 42 440 Helm, B., Visser, M. E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T., Bloch,
43 441 G., 2017. Two sides of a coin: ecological and chronobiological perspectives of timing
44 442 in the wild. *Philos. Trans. R. Soc. Lond., B., Biol. Sci.* 372(1734).
45 443 10.1098/rstb.2016.0246
- 48 444 Hölker, F., Moss, T., Griefahn, B., Kloas, W., Voigt, C. C., Henckel, D., Hänel, A., Kappeler,
49 445 P. M., Völker, S., Schwöpe, A., Franke, S., Uhrlandt, D., Fischer, J., Klenke, R., Wolter,
50 446 C., Tockner, K., 2010. The Dark Side of Light: A Transdisciplinary Research Agenda
51 447 for Light Pollution Policy. *Ecol. Soc.* 15(4). 10.5751/es-03685-150413

- 448 Jones, T. M., Durrant, J., Michaelides, E. B., Green, M. P., 2015. Melatonin: a possible link
1 449 between the presence of artificial light at night and reductions in biological fitness.
2
3 450 *Philos. Trans. R. Soc. Lond., B., Biol. Sci.* 370(1667). 10.1098/rstb.2014.0122
4
- 5 451 Kummu, M., de Moel, H., Salvucci, G., Viviroli, D., Ward, P. J., Varis, O., 2016. Over the hills
6
7 452 and further away from coast: global geospatial patterns of human and environment over
8
9 453 the 20th–21st centuries. *Environ. Res. Lett.* 11(3). 10.1088/1748-9326/11/3/034010
10
- 11 454 Liu, Q., Manning, A. J., Duston, J., 2019. Light intensity and suppression of nocturnal plasma
12
13 455 melatonin in Arctic charr (*Salvelinus alpinus*). *Comp. Biochem. Physiol. A Mol. Integr.*
14
15 456 *Physiol.* 228, 103-106. 10.1016/j.cbpa.2018.11.012
- 16 457 Livak, K. J., Schmittgen, T. D., 2001. Analysis of relative gene expression data using real-time
17
18 458 quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25(4), 402-408.
19
20 459 10.1006/meth.2001.1262
- 21
22 460 Longcore, T., Rich, C., 2004. Ecological light pollution. *Front. Ecol. Environ.* 2(4), 191-198.
23
24 461 10.1890/1540-9295(2004)002[0191:Elp]2.0.Co;2
- 25 462 Longcore, T., Rodriguez, A., Witherington, B., Penniman, J. F., Herf, L., Herf, M., 2018. Rapid
26
27 463 assessment of lamp spectrum to quantify ecological effects of light at night. *J. Exp.*
28
29 464 *Zool. A: Ecol. Integr. Physiol.* 329(8-9), 511-521. 10.1002/jez.2184
- 30
31 465 Luarte, T., Bonta, C. C., Silva-Rodriguez, E. A., Quijon, P. A., Miranda, C., Farias, A. A.,
32
33 466 Duarte, C., 2016. Light pollution reduces activity, food consumption and growth rates
34
35 467 in a sandy beach invertebrate. *Environ. Pollut.* 218, 1147-1153.
36
37 468 10.1016/j.envpol.2016.08.068
- 38 469 Luginbuhl, C. B., Boley, P. A., Davis, D. R., 2014. The impact of light source spectral power
39
40 470 distribution on sky glow. *J. Quant. Spectrosc. Radiat. Transf.* 139, 21-26.
41
42 471 10.1016/j.jqsrt.2013.12.004
- 43
44 472 Lynn, K. D., Tummon Flynn, P., Manriquez, K., Manriquez, P. H., Pulgar, J., Duarte, C.,
45
46 473 Quijon, P. A., 2021. Artificial light at night alters the settlement of acorn barnacles on
47
48 474 a man-made habitat in Atlantic Canada. *Mar. Pollut. Bull.* 163, 111928.
49
50 475 10.1016/j.marpolbul.2020.111928
- 51 476 Manriquez, K., Quijon, P. A., Manriquez, P. H., Miranda, C., Pulgar, J., Quintanilla-Ahumada,
52
53 477 D., Duarte, C., 2021. Artificial Light at Night (ALAN) negatively affects the settlement
54
55 478 success of two prominent intertidal barnacles in the southeast Pacific. *Mar. Pollut. Bull.*
56
57 479 168, 112416. 10.1016/j.marpolbul.2021.112416
- 58 480 Manriquez, P. H., Jara, M. E., Diaz, M. I., Quijon, P. A., Widdicombe, S., Pulgar, J., Manriquez,
59
60 481 K., Quintanilla-Ahumada, D., Duarte, C., 2019. Artificial light pollution influences
61
62
63
64
65

- 482 behavioral and physiological traits in a keystone predator species, *Concholepas*
1 483 *concholepas*. *Sci. Total Environ.* 661, 543-552. 10.1016/j.scitotenv.2019.01.157
2
3 484 Mat, A. M., Massabuau, J. C., Ciret, P., Tran, D., 2012. Evidence for a plastic dual circadian
4
5 485 rhythm in the oyster *Crassostrea gigas*. *Chronobiol. Int.* 29(7), 857-867.
6
7 486 10.3109/07420528.2012.699126
8
9 487 Mat, A. M., Massabuau, J.-C., Ciret, P., Tran, D., 2014. Looking for the clock mechanism
10
11 488 responsible for circatidal behavior in the oyster *Crassostrea gigas*. *Mar. Biol.* 161(1),
12
13 489 89-99. 10.1007/s00227-013-2317-2
14
15 490 Navara, K. J., Nelson, R. J., 2007. The dark side of light at night: physiological,
16
17 491 epidemiological, and ecological consequences. *J. Pineal Res.* 43(3), 215-224.
18
19 492 10.1111/j.1600-079X.2007.00473.x
20
21 493 Nunez, J. D., Bas, C. C., Perez Garcia, M., Ocampo, E. H., Ribeiro, P. D., Luppi, T. A., 2021.
22
23 494 Artificial light at night may increase the predation pressure in a salt marsh keystone
24
25 495 species. *Mar Environ Res* 167, 105285. 10.1016/j.marenvres.2021.105285
26
27 496 Partch, C. L., Green, C. B., Takahashi, J. S., 2014. Molecular architecture of the mammalian
28
29 497 circadian clock. *Trends Cell Biol.* 24(2), 90-99. 10.1016/j.tcb.2013.07.002
30
31 498 Payton, L., Perrigault, M., Hoede, C., Massabuau, J. C., Sow, M., Huvet, A., Boullot, F.,
32
33 499 Fabioux, C., Hegaret, H., Tran, D., 2017a. Remodeling of the cycling transcriptome of
34
35 500 the oyster *Crassostrea gigas* by the harmful algae *Alexandrium minutum*. *Sci. Rep.* 7(1),
36
37 501 3480. 10.1038/s41598-017-03797-4
38
39 502 Payton, L., Sow, M., Massabuau, J. C., Ciret, P., Tran, D., 2017b. How annual course of
40
41 503 photoperiod shapes seasonal behavior of diploid and triploid oysters, *Crassostrea gigas*.
42
43 504 PLoS One 12(10), e0185918. 10.1371/journal.pone.0185918
44
45 505 Payton, L., Tran, D., 2019. Moonlight cycles synchronize oyster behaviour. *Biol. Lett.* 15(1),
46
47 506 20180299. 10.1098/rsbl.2018.0299
48
49 507 Perrigault, M., Tran, D., 2017. Identification of the Molecular Clockwork of the Oyster
50
51 508 *Crassostrea gigas*. PLoS One 12(1), e0169790. 10.1371/journal.pone.0169790
52
53 509 Pevet, P., Balemans, M. G., Legerstee, W. C., Vivien-Roels, B., 1980. Circadian rhythmicity
54
55 510 of the activity of hydroxyindole-O-methyl transferase (HIOMT) in the formation of
56
57 511 melatonin and 5-methoxytryptophol in the pineal, retina, and harderian gland of the
58
59 512 golden hamster. *J. Neural Transm.* 49(4), 229-245. 10.1007/BF01252128
60
61 513 Pulgar, J., Zeballos, D., Vargas, J., Aldana, M., Manriquez, P. H., Manriquez, K., Quijon, P.
62
63 514 A., Widdicombe, S., Anguita, C., Quintanilla, D., Duarte, C., 2019. Endogenous cycles,
64
65 515 activity patterns and energy expenditure of an intertidal fish is modified by artificial

- 516 light pollution at night (ALAN). *Environ. Pollut.* 244, 361-366.
 1 517 10.1016/j.envpol.2018.10.063
 2
 3 518 Rich, C., Longcore, T., 2006. *Ecological Consequences of Artificial Night Lighting*. Island
 4 Press.
 5 519
 6
 7 520 Roeder, T., 2020. The control of metabolic traits by octopamine and tyramine in invertebrates.
 8 *J. Exp. Biol.* 223(Pt 7). 10.1242/jeb.194282
 9 521
 10 522 Sanders, D., Frago, E., Kehoe, R., Patterson, C., Gaston, K. J., 2021. A meta-analysis of
 11 biological impacts of artificial light at night. *Nat Ecol Evol* 5(1), 74-81.
 12 523 10.1038/s41559-020-01322-x
 13 524
 14 525 Scargle, J. D., 1982. *Studies in astronomical time series analysis. II - Statistical aspects of*
 15 *spectral analysis of unevenly spaced data.* *Astrophys. J.* 263. 10.1086/160554
 16 526
 17 527 Thomas, J. R., James, J., Newman, R. C., Riley, W. D., Griffiths, S. W., Cable, J., 2016. The
 18 impact of streetlights on an aquatic invasive species: Artificial light at night alters signal
 19 crayfish behaviour. *Appl. Anim. Behav. Sci.* 176, 143-149.
 20 528 10.1016/j.applanim.2015.11.020
 21 529
 22 530 Tidau, S., Whittle, J., Jenkins, S. R., Davies, T. W., 2022. Artificial light at night reverses
 23 monthly foraging pattern under simulated moonlight. *Biol. Lett.* 18(7), 20220110.
 24 531 10.1098/rsbl.2022.0110
 25 532
 26 533 Tran, D., Nadau, A., Durrieu, G., Ciret, P., Parisot, J. P., Massabuau, J. C., 2011. Field
 27 chronobiology of a molluscan bivalve: how the moon and sun cycles interact to drive
 28 oyster activity rhythms. *Chronobiol. Int.* 28(4), 307-317.
 29 534 10.3109/07420528.2011.565897
 30 535
 31 536 Tran, D., Perrigault, M., Ciret, P., Payton, L., 2020. Bivalve mollusc circadian clock genes can
 32 run at tidal frequency. *Proc. Biol. Sci.* 287(1918), 20192440. 10.1098/rspb.2019.2440
 33 537
 34 538 Wu, C., Jiang, Q., Wei, L., Cai, Z., Chen, J., Yu, W., He, C., Wang, J., Guo, W., Wang, X.,
 35 2018. A Rhodopsin-Like Gene May Be Associated With the Light-Sensitivity of Adult
 36 Pacific Oyster *Crassostrea gigas*. *Front. Physiol.* 9, 221. 10.3389/fphys.2018.00221
 37 539
 38 540 Xie, F., Xiao, P., Chen, D., Xu, L., Zhang, B., 2012. miRDeepFinder: a miRNA analysis tool
 39 for deep sequencing of plant small RNAs. *Plant. Mol. Biol.* 10.1007/s11103-012-9885-
 40 541 2
 41 542
 42 543 Yerushalmi, S., Green, R. M., 2009. Evidence for the adaptive significance of circadian
 43 rhythms. *Ecol. Lett.* 12(9), 970-981. 10.1111/j.1461-0248.2009.01343.x
 44 544
 45 545 Zissis, G., Bertoldi, P., 2018. Status of LED-Lighting world market in 2017.
 46
 47
 48
 49
 50
 51
 52
 53
 54
 55
 56
 57
 58
 59
 60
 61
 62
 63
 64
 65

549

1

2

3 550

4

5

6 551

7

8

9 552

10

11

12 553

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

554 **Figures & legends**

1
2
3 555 **Figure 1.** Behavioral daily pattern of oysters ($n = 15\text{--}16$ oysters) in control condition (0 lux)
4
5 556 and exposed to different ALAN intensities (0.1 lux, 1 lux, 10 lux, and 25 lux). Mean hourly
6
7
8 557 VOD data are shown as mean \pm SE ($n = 7$ days). White backgrounds indicate daytime and
9
10 558 yellow and black backgrounds indicate nighttime. Arrows indicate the VOD peak during the
11
12
13 559 day.

14
15
16 560 **Figure 2.** Effect of ALAN intensity on (A) the mean daily valve opening duration (VOD),
17
18 561 and (B) the mean VOD during the daytime and the nighttime. Data are expressed in mean \pm
19
20
21 562 SE ($n = 7$ days). Different letters indicate significant differences between ALAN conditions
22
23 563 and between daytime and nighttime ($p < 0.05$).

24
25
26 564 **Figure 3.** Effect of ALAN intensity on oysters daily rhythm characteristics: (A) the period at
27
28
29 565 group level, (B) the percent rhythm (PR) at group level, (C) amplitude of the rhythm at group
30
31 566 level, (D) the percentage of oysters having a daily rhythm, (E), period of individual rhythmic
32
33
34 567 oysters, dotted lines define the daily range, (F) the PR at individual level of oysters having a
35
36 568 daily rhythm, (G) amplitude of the rhythm at the individual level of oysters having a daily
37
38
39 569 rhythm. Data are expressed in mean \pm SE. For A-C, the SE shows the daily variability of the
40
41 570 group's significant rhythm parameters ($n = 7$ days). For E-G the SE shows the individual
42
43 571 variability ($n = 15\text{--}16$ oysters). Different letters indicate significant differences ($p < 0.05$).

44
45
46 572 **Figure 4.** Effect of ALAN intensity on the difference in relative mRNA expression level
47
48
49 573 (mean \pm SE, $n = 8$) on oyster gills tissues, between daytime and nighttime of five clock and
50
51 574 clock-associated genes. White bars indicate the gene level during daytime and, colored bars
52
53
54 575 during nighttime. Asterisks indicate significant differences between daytime and nighttime (p
55
56 576 < 0.05).

57
58
59 577
60
61
62
63
64
65

578 **Table 1.** Two-Way Analysis of Variance testing the effect of Daytime/Nighttime and the
 579 ALAN intensity (0 lux, 0.1 lux, 1 lux, 10 lux, 25 lux) on the relative mRNA expression level
 580 on gill tissues of 13 clock and clock-associated genes. In bold, significant p -values ($p < 0.05$).

Source of variation	p -value	df	F
Daytime/Nighttime	0.046	1	3.996
ALAN intensity	0.886	4	0.288
Daytime/Nighttime x ALAN intensity	0.462	4	0.902
Daytime/Nighttime within 0 lux	0.014		
Daytime/Nighttime within 0.1 lux	0.743		
Daytime/Nighttime within 1 lux	0.652		
Daytime/Nighttime within 10 lux	0.903		
Daytime/Nighttime within 25 lux	0.261		

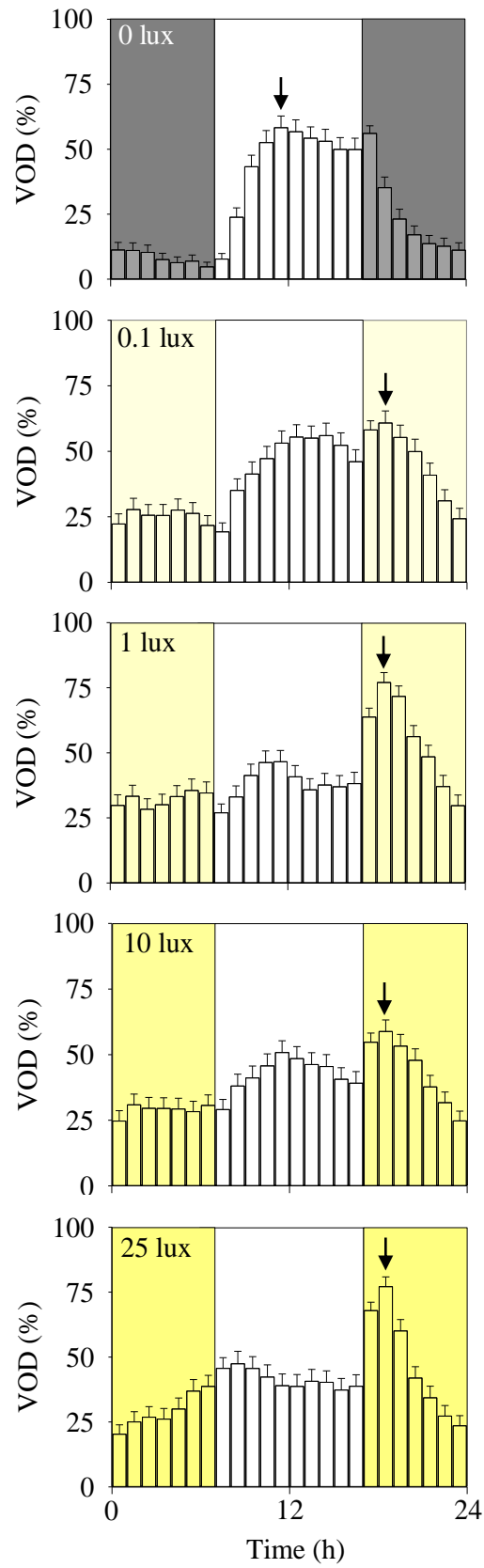


Figure 1. Botté et al.

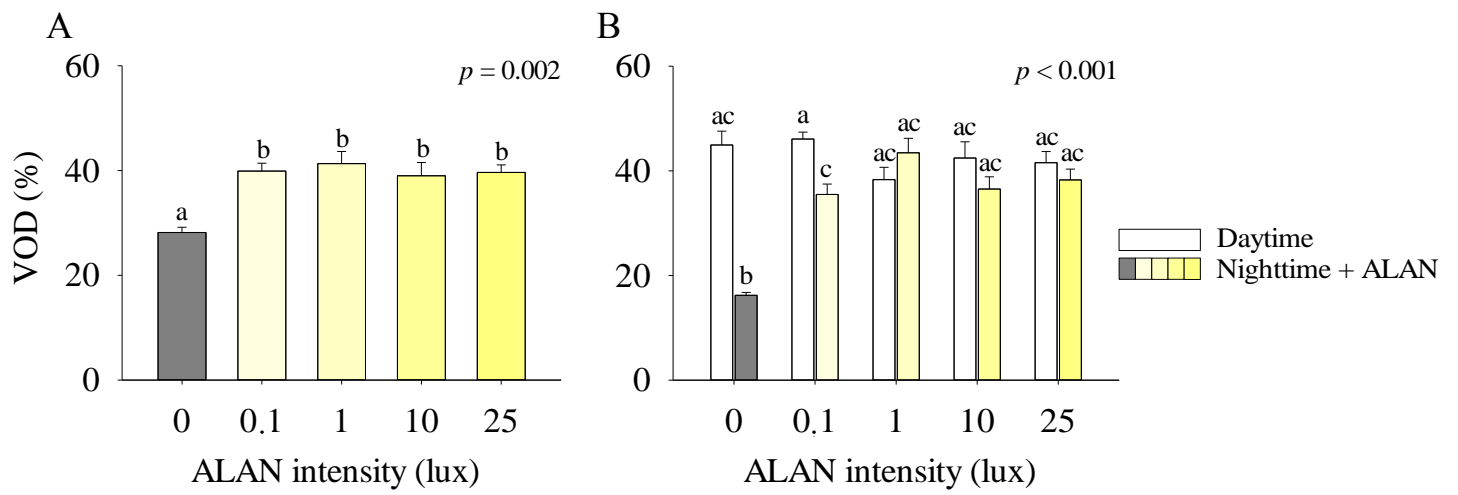


Figure 2. Botté et al.

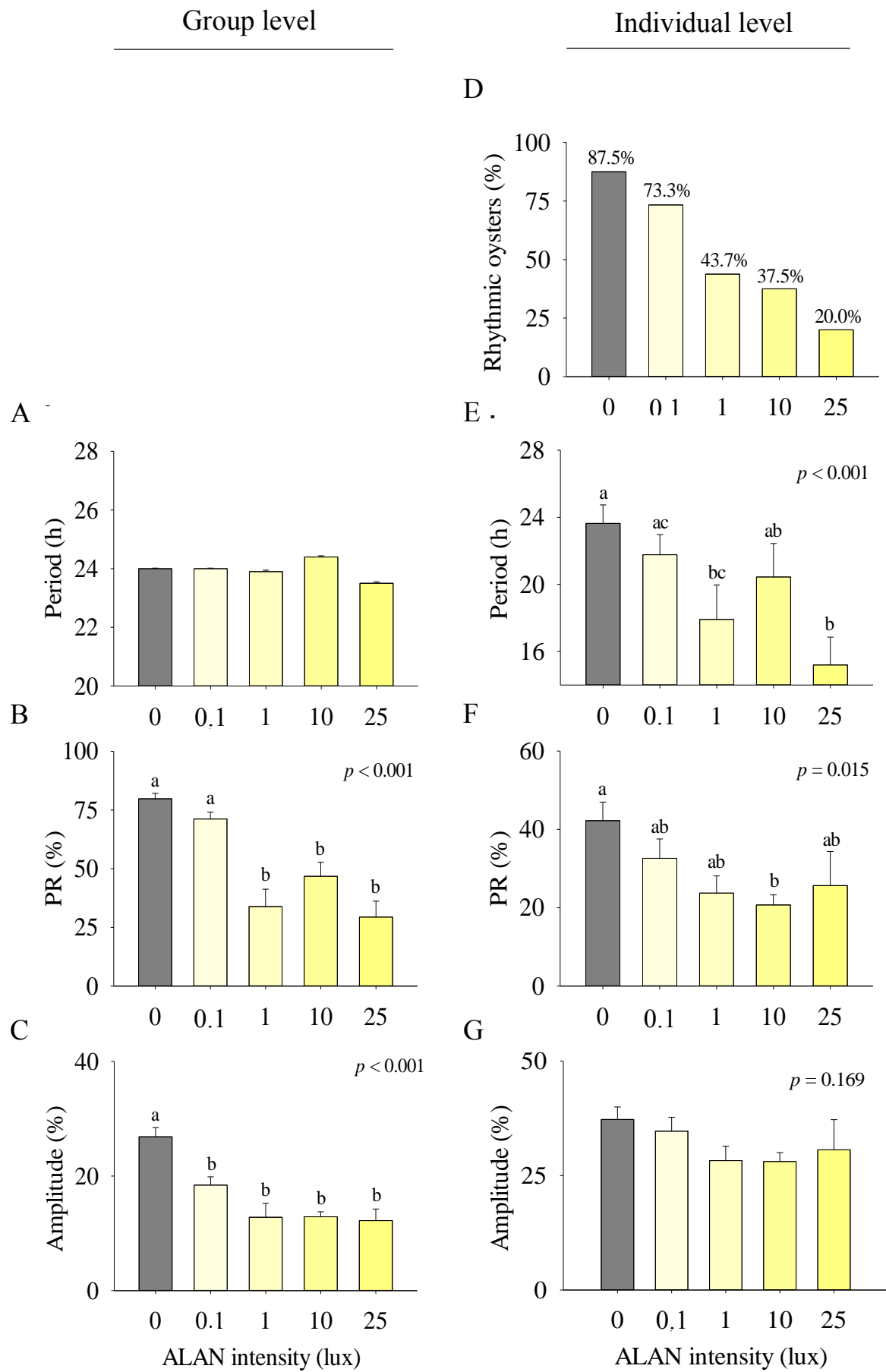


Figure 3. Botté et al.

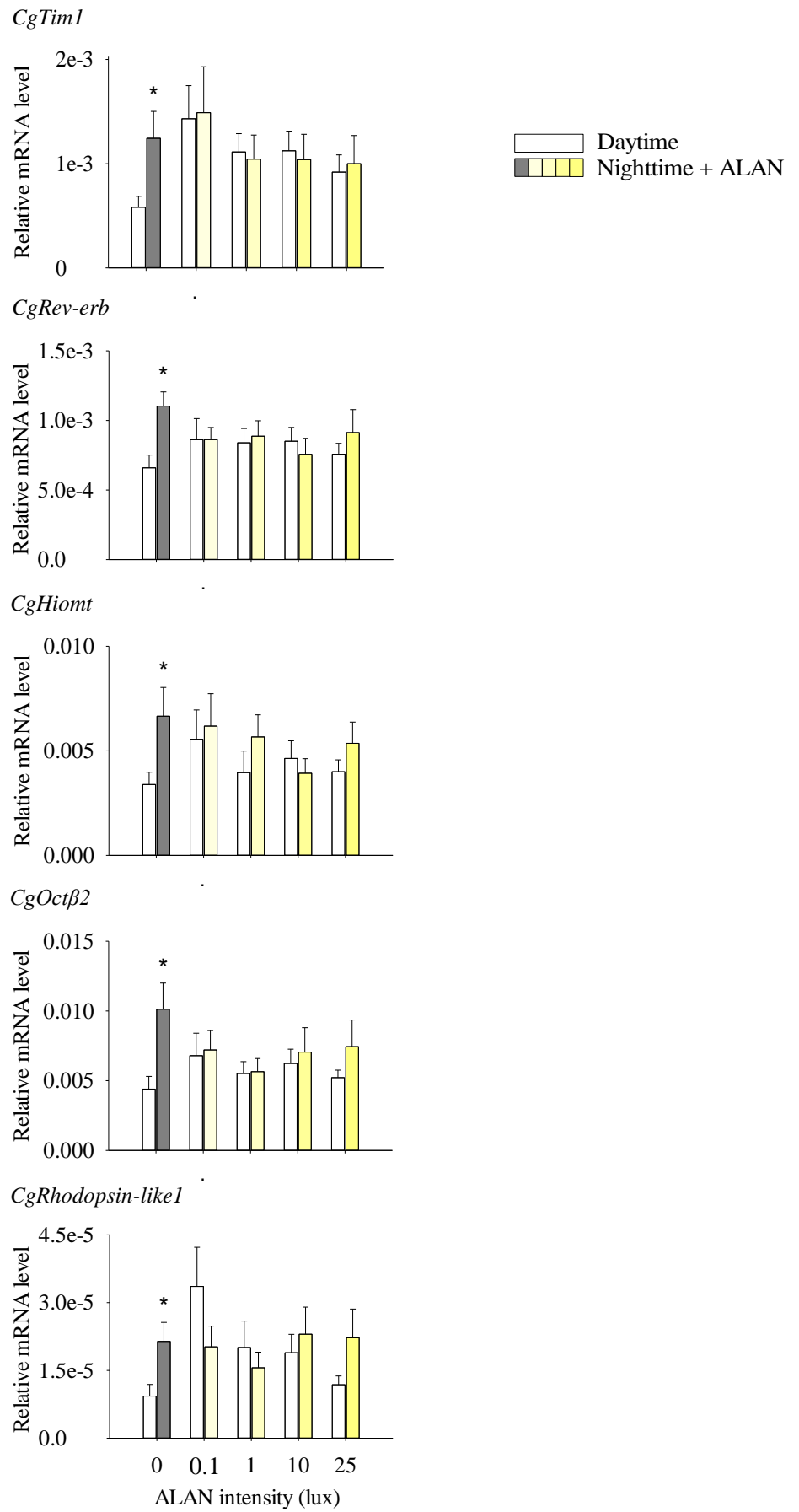


Figure 4. Botté et al.

Supplementary material

Artificial light at night disrupts daily rhythm of the oyster

Crassostrea gigas

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

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

Journal: *Marine pollution bulletin*

Corresponding author: Damien Tran

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

The following items are provided:

Figure S1. Daily light exposure of the control condition (A) and the ALAN conditions (B).

Figure S2. Spectrum of the maximum light intensity during daytime (A), and light spectra of ALAN intensities measured underwater near to the oysters: 0.1 lux (B), 1 lux (C), 10 lux (D), and 25 lux (E) conditions.

Figure S3. Five locations in the experimental tank used for underwater intensity measurements. The depth of the measure is done at the oyster level.

Table S1. Values of underwater measurements of the maximum light intensity during the daytime and ALAN intensities in the tank for each position in the tank, and the mean \pm SE.

Table S2. Forward, reverse primers sequences used for Real-Time PCR analysis in *Crassostrea gigas*.

Table S3. Results of chronobiological analysis for the control condition and ALAN conditions at the group level. Rhythmic parameters are expressed as mean \pm standard error, where the standard error shows the daily variability (n = 7 days).

Table S4. Results of chronobiological analysis for the control condition and ALAN conditions at the individual level (n = 15-16 oysters / condition). The table indicates for the control condition and ALAN conditions: the period of rhythmic individuals (the periods out of the daily range of 24 – 28 h are in italic); and the percent rhythm (PR, %) and rhythm's amplitude of individuals having a daily rhythm.

Figure S4. Relative mRNA expression level (expressed as mean \pm SE) between daytime and nighttime of eight clock and clock-associated genes according to ALAN intensities exposure. White bars indicate the gene level during daytime and, colored bars during nighttime.

Differences between daytime and nighttime are significant for a *p*-value = 0.05.

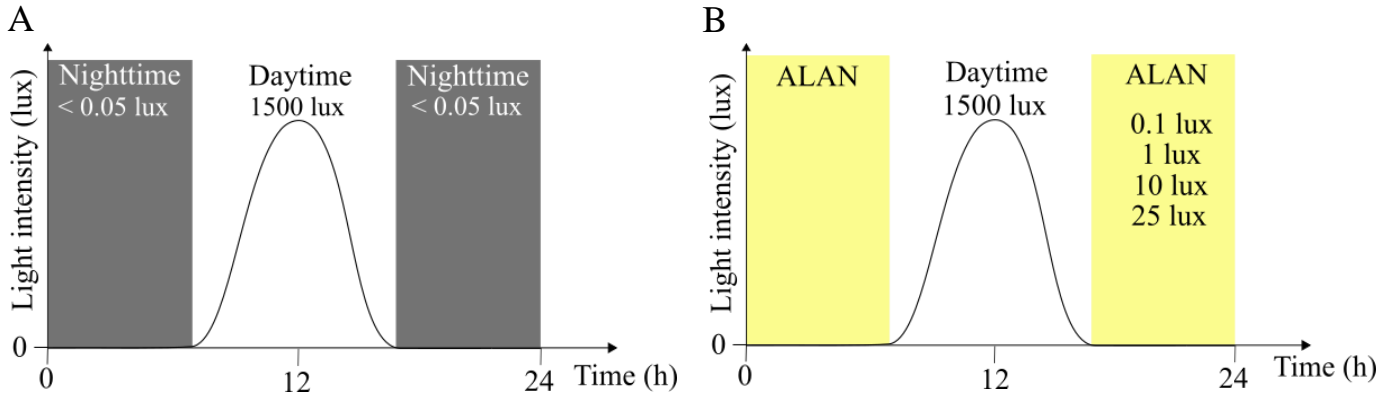


Figure S1. Daily light exposure of the control condition (A) and the ALAN conditions (B).

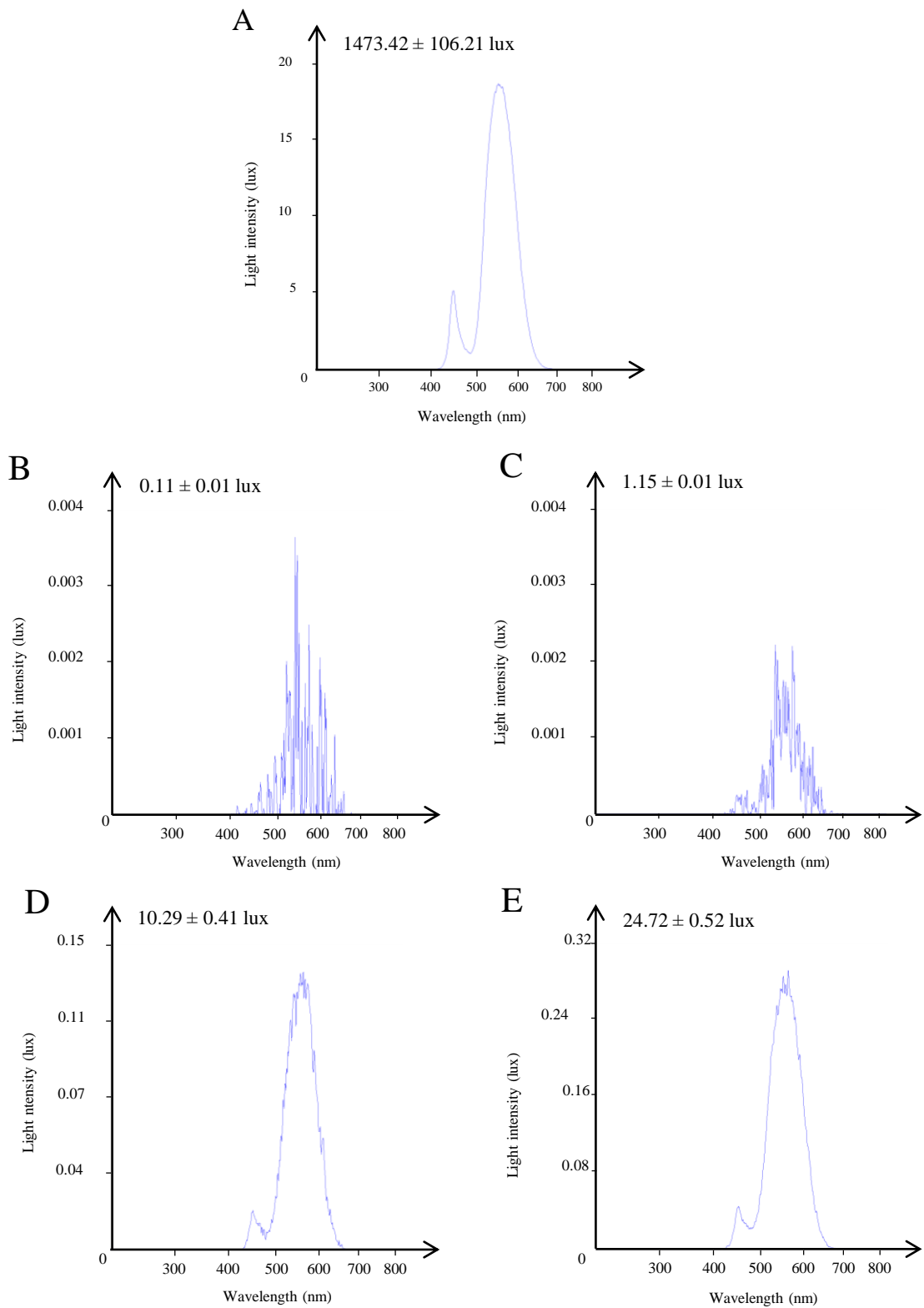


Figure S2. Spectrum of the maximum light intensity during daytime (A), and light spectra of ALAN intensities measured underwater near the oysters: 0.1 lux (B), 1 lux (C), 10 lux (D), and 25 lux (E) conditions.

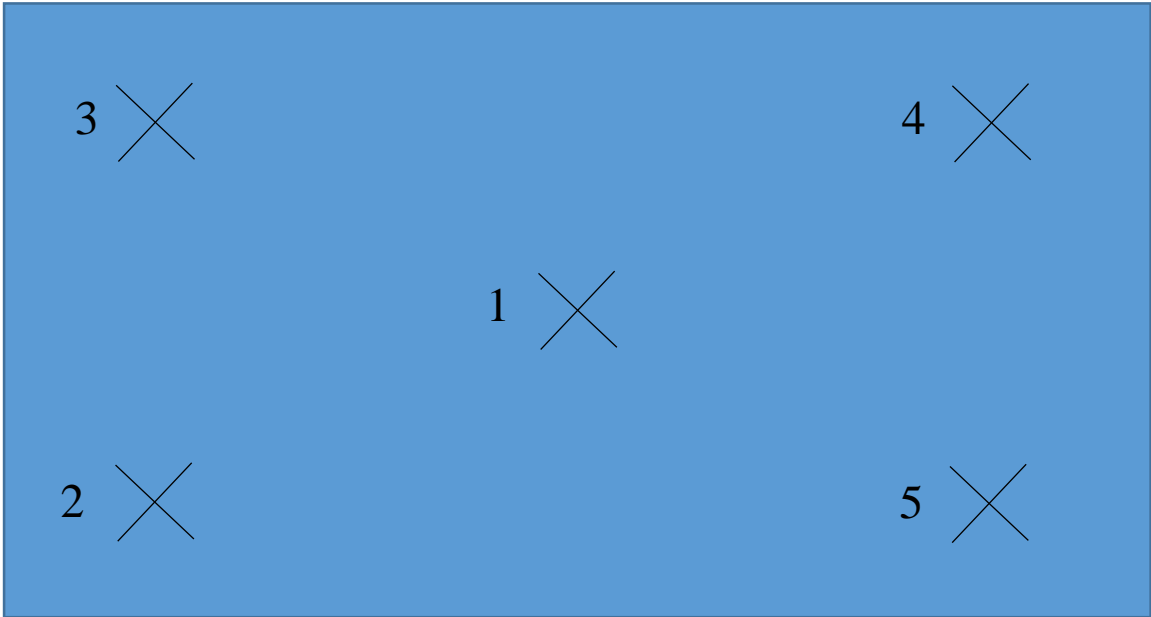


Figure S3. Five locations in the experimental tank used for underwater intensity measurements. The depth of the measure is done at the oyster level.

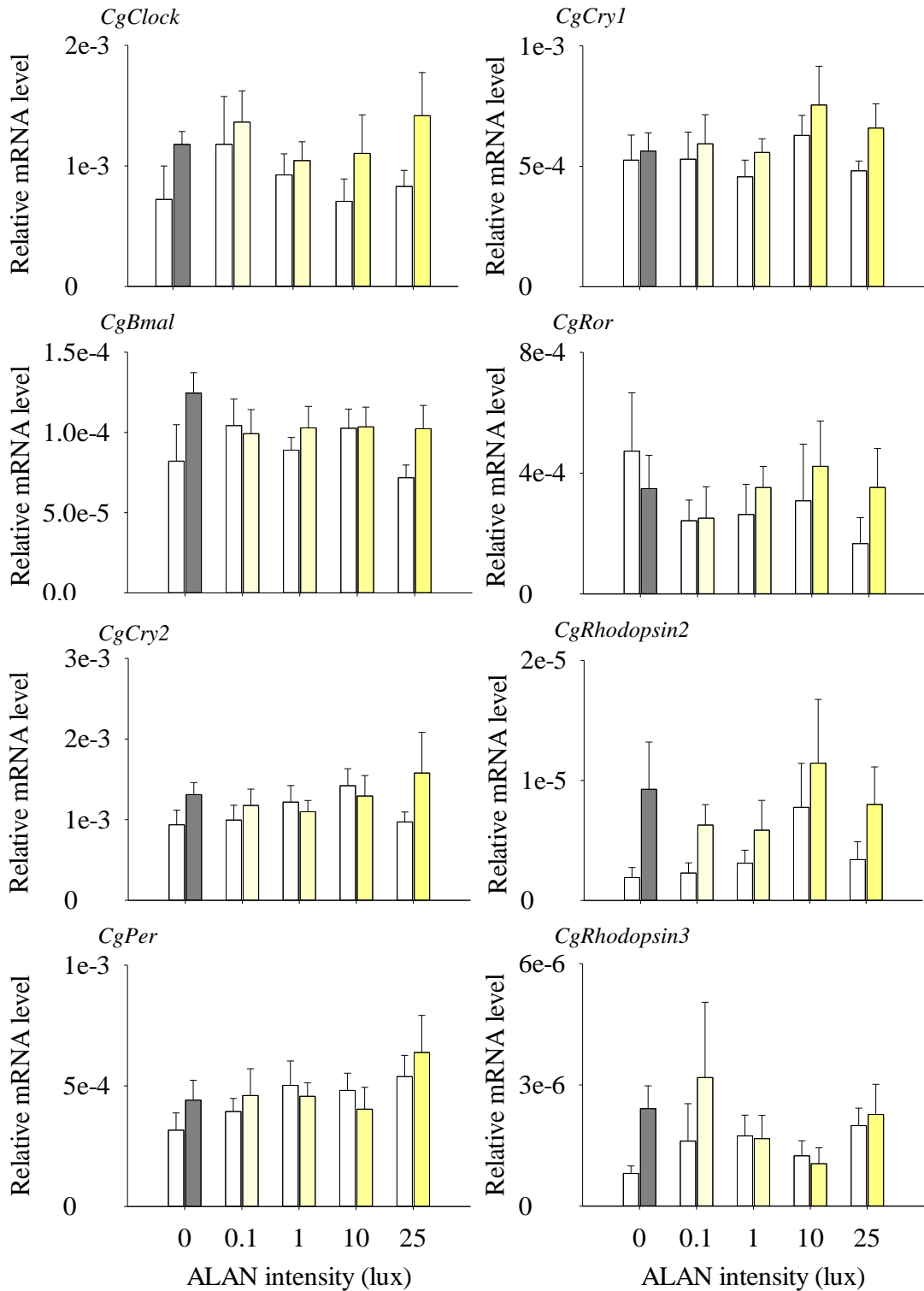


Figure S4. Relative mRNA expression levels (expressed as mean \pm SE) between daytime and nighttime of eight clock and clock-associated genes according to ALAN intensities exposure. White bars indicate the gene level during daytime and, colored bars during nighttime. Differences between daytime and nighttime are significant for a p -value = 0.05.

Location in the tank	ALAN intensity (lux)				Maximum intensity during daytime (lux)
	0.1 lux	1 lux	10 lux	25 lux	
1	0.12	1.46	11.86	26.64	1813.99
2	0.11	1.23	10.33	23.82	1591.36
3	0.12	1.11	9.79	24.87	1192.62
4	0.11	0.84	9.73	23.94	1407.54
5	0.10	1.11	9.75	24.31	1361.59
Mean ± SE	0.11 ± 0.01	1.15 ± 0.01	10.29 ± 0.41	24.72 ± 0.52	1473.42 ± 106.21

Table S1. Values of underwater measurements of the maximum light intensity during the daytime and ALAN intensities in the tank for each position in the tank, and the mean ± SE.

GeneBank access	Gene name	Forward primer	Reverse primer
<i>Crassostrea gigas</i> core clock genes			
KX371073	<i>CgClock (Clock)</i>	5'-TGGGAATGATGTCCAACAGAG-3'	5'-GGTCCATCAATGACAGGAAGT-3'
KX371075	<i>CgBmal (Bmal)</i>	5'-CACAAGTTCAGGTTCAGAGTGTAG-3'	5'-TCACCTGAGGTAGACTGGTTAT-3'
KX371074	<i>CgCryptochrome 2 (Cry2)</i>	5'-AACCTTACAGCAAGCACGAA-3'	5'-TGACATCTGGCTGTGGTTTC-3'
KX371076	<i>CgPeriod (Per)</i>	5'-CCGATGACAGAAATCCCAGTAG-3'	5'-CCATCCTATTCTCCTGCTCTTG-3'
KX371077	<i>CgTimless 1 (Tim1)</i>	5'-AAAGATCCCGGACACAGTATG-3'	5'-TGGAACTCGTTCCTGACTTG-3'
KT991835	<i>CgCryptochrome 1 (Cry1)</i>	5'-TCATGAAGCAGCTCAGATACG-3'	5'-ACCTCCCAGTCAACCAAAG-3'
KJ188106	<i>CgRev-erb (Rev-erb)</i>	5'-GACTTTGCTGATCGCTTCAAC-3'	5'-CTTTCCAAGTCTCCACATTTTC-3'
EKC18621	<i>CgRor (Ror)</i>	5'-CTACGTGAGCAGGTGTTTGA-3'	5'-CGTCCGCTATGTCCTTCAAT-3'
<i>Crassostrea gigas</i> clock-controlled genes			
EKC41768	<i>CgHiomt-like</i>	5'-CGGGTGGATCAGTGTTAGTAATG-3'	5'-TCTCTTGGCCCTGTGATAGA-3'
XM_011433587	<i>CgRhodopsin-like 1</i>	5'-TAGTTCGGCGTCGGAATTTATC-3'	5'-CTGTTTGAATCTCTGCTCTCAC-3'
XM_011448766	<i>CgRhodopsin-like 2</i>	5'-CCCTGAGTCATCCCAAATTCA-3'	5'-GATGTTCTCGGCGTAGCTTTA-3'
XM_020065754	<i>CgRhodopsin-like 3</i>	5'-TGACTTTGACGGCGATACTG-3'	5'-ATAGATCCGCCACCGAAATG-3'
XM_011427386	<i>CgOctβ2</i>	5'-AATCCAGCACACACTCCATAG-3'	5'-TCTGAGTCTCATCTGCGTTTG-3'
<i>Crassostrea gigas</i> housekeeping genes			
AB122066	<i>CgElongation Factor 1 (Ef1)</i>	5'-ACCACCCTGGTGAGATCAAG-3'	5'-ACGACGATCGATTTCTCTT-3'
CAD67717	<i>CgGadph</i>	5'-CGTACCAGTTCAGATGTTTCC-3'	5'-GCCTTGATGGCTGCCTTAATA-3'
Z29546	<i>Cg28S</i>	5'-AAACACGGACCAAGGAGTCT-3'	5'-AGGCTGCCTTCACTTTCATT-3'

Table S2. Forward, reverse primers sequences used for Real-Time PCR analysis in *Crassostrea gigas*.

ALAN intensity (lux)	Period (h)	PR (%)	Amplitude (%)
0 lux	24.00 ± 0.02	79.80 ± 0.02	26.86 ± 1.61
0.1 lux	24.00 ± 0.02	71.20 ± 0.03	18.41 ± 1.45
1 lux	23.90 ± 0.04	33.90 ± 0.07	12.79 ± 2.43
10 lux	24.40 ± 0.04	46.80 ± 0.06	12.88 ± 0.87
25 lux	23.50 ± 0.05	29.40 ± 0.07	12.22 ± 2.01

Table S3. Results of chronobiological analysis for the control condition and ALAN conditions at the group level. Rhythmic parameters are expressed as mean ± standard error, where the standard error shows the daily variability (n = 7 days).

ALAN intensity (lux)														
0 lux			0.1 lux			1 lux			10 lux			25 lux		
Period (h)	PR (%)	Amplitude (%)	Period (h)	PR (%)	Amplitude (%)	Period (h)	PR (%)	Amplitude (%)	Period (h)	PR (%)	Amplitude (%)	Period (h)	PR (%)	Amplitude (%)
23.7	62.62	46.7	24.3	38.78	40.2	23.9	20.46	18	23.8	15.40	24.6	20.9	13.67	21.7
23.3	18.57	22.8	23.7	20.28	29.4	23.5	26.14	31.6	22.4	18.10	26.7	23.8	42.58	43.5
24	75.95	56.9	24	38.74	37.2	24.1	15.37	25.9	23.5	17.51	24.7	22.8	20.74	26.7
25.9	24.84	28.5	23.9	60.09	49.8	22.9	16.00	25.5	25.3	18.12	26.3	<i>12.3</i>	–	–
23.7	47.88	38.8	23.7	18.23	26.4	23.4	15.35	25.2	24	32.67	37.2	7.55	–	–
24	28.21	25.7	24.1	16.64	27.4	23.3	24.82	26.7	24.4	22.51	29	<i>10.1</i>	–	–
24	36.84	38.7	24.2	60.73	51.1	24	48.08	45	8.6	–	–	12.2	–	–
24	61.35	49.9	23.8	26.86	28.5	<i>11.8</i>	–	–	17.4	–	–	17.1	–	–
23.9	62.35	48.7	24	16.71	19.6	<i>7.96</i>	–	–	33.2	–	–	12.1	–	–
25.1	33.97	28.9	24.9	39.95	41.8	<i>9.69</i>	–	–	18.1	–	–	10.9	–	–
24	23.76	30.8	23.1	21.88	30.3	12	–	–	12	–	–	17.4	–	–
24.2	41.95	37.1	<i>16.5</i>	–	–	8.37	–	–	12.6	–	–	n.s.	–	–
24.3	46.16	37.8	<i>12.5</i>	–	–	n.s.	–	–	n.s.	–	–	n.s.	–	–
24	26.95	30.3	<i>12.1</i>	–	–	n.s.	–	–	n.s.	–	–	n.s.	–	–
<i>31.4</i>	–	–	n.s.	–	–	n.s.	–	–	n.s.	–	–	n.s.	–	–
8.7	–	–	/	/	/	n.s.	–	–	n.s.	–	–	/	/	/

Table S4. Results of chronobiological analysis for the control condition and ALAN conditions at the individual level (n = 15-16 oysters / condition). The table indicates for the control condition and ALAN conditions: the period of rhythmic individuals (the periods out of the daily range of 24 – 28 h are in italic); and the percent rhythm (PR, %) and rhythm's amplitude of individuals having a daily rhythm.