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1 Rhythms during the polar night: Evidence of clock-gene oscillations

2 in the Arctic scallop *Chlamys islandica*

3

4

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16

17 **Abstract**

18 Arctic regions are highly impacted by climate change and are characterized by drastic  
19 seasonal changes in light intensity and duration with extended periods of permanent light or  
20 darkness. Organisms use cyclic variations in light to synchronize daily and seasonal biological  
21 rhythms to anticipate cyclic variations in the environment, to control phenology and to  
22 maintain fitness. In this study, we investigated the diel biological rhythms of the Arctic  
23 scallop, *Chlamys islandica*, during the autumnal equinox and polar night. Putative circadian  
24 clock genes and putative light perception genes were identified in the Arctic scallop. Clock  
25 gene expression oscillated in the three tissues studied (gills, muscle, mantle edge). The  
26 oscillation of some genes in some tissues shifted from daily to tidal periodicity between the  
27 equinox and polar night periods and was associated with valve behaviour. These results are  
28 the first evidence of the persistence of clock gene expression oscillations during the polar  
29 night and might suggest that functional clockwork could entrain rhythmic behaviours in polar  
30 environments.

## 31 **Introduction**

32 Biological rhythms are a fundamental property of life. These mechanisms regulate most  
33 metabolic, physiological and behavioural activities and enable organisms to anticipate cyclic  
34 changes in the environment [1,2]. The importance of biological rhythms under different  
35 cycles (e.g., daily, seasonal) is evidenced by their ubiquity among all phyla [1,3]. Among  
36 biological rhythms, circadian processes are well described and have a molecular origin  
37 characterized by endogenous transcriptional–translational negative and positive feedback  
38 loops with a period close to 24 h [4]. Although rhythmicity could persist under constant  
39 darkness, the clock needs to be entrained by external cues, known as zeitgeber, to remain  
40 synchronized with the environment. Light, by mediating the expression of visual and  
41 nonvisual light-sensitive genes such as cryptochromes and opsins, is by far the major cue used  
42 for this entrainment [5]. The circadian clock is also involved in the measurement of the  
43 photoperiod and provides information about the seasonal cycle [6]. Cyclic change in  
44 photoperiod is a determinant cue for the phenology of many organisms, providing the optimal  
45 timing for seasonal life cycle events such as migration, reproduction or diapause [7,8]. Thus,  
46 in polar environments where light rhythmicity is drastically dampened, the interest and  
47 significance of maintaining physiological and behavioural rhythmic expressions remains an  
48 important unanswered question [9].

49 Arctic regions are facing major changes, including a rapid decline in ice cover and a faster  
50 warming rate than other latitudes [10]. These changes are likely to have numerous ecological  
51 consequences on trophic interactions and ecosystem functions [11,12]. Polar environments are  
52 characterized by increasing seasonal changes in light intensity and duration with extended  
53 periods of permanent light (polar day) and darkness (polar night, PN). The absence of light  
54 during the PN in the Arctic was previously associated with a period of reduced activity in  
55 marine ecosystems due to the limitation of primary production [13]. However, studies have

56 revealed high levels of biological activities and trophic interactions during the darkest period  
57 of the PN in marine ecosystems [14,15]. Similarly, the behavioural rhythmicity of polar  
58 organisms was investigated and revealed some discrepancies according to species, with some  
59 organisms exhibiting behavioural arrhythmicity during polar days and PNs while other studies  
60 identified behavioural or physiological rhythms during polar days and nights [9 for  
61 review,16]. However, to date, no studies have demonstrated circadian clock gene oscillations  
62 in Arctic organisms during the PN, which would support the hypothesis of an active  
63 clockwork responsible for rhythmic processes during this period.

64 Circadian clocks have been largely investigated in terrestrial species, but knowledge of  
65 marine clock systems is still scarce despite the ecological importance and complexity of  
66 marine ecosystems [17]. This is particularly true for polar ecosystems, with few studies  
67 devoted to understanding the behavioural or molecular rhythms during the polar day or night  
68 [15,18–20].

69 In the present study, we associated molecular and behavioural approaches to decipher the  
70 importance and function of endogenous clocks and light perception gene rhythmicity in the  
71 Arctic scallop *Chlamys islandica* in an Arctic fjord (Kongsfjorden, Spitsbergen, Svalbard, 78°  
72 56' N). Scallops were monitored during distinct periods of the year in polar regions, i.e., the  
73 short period of light-dark alternation during the autumnal equinox (Eq) and the darkest period  
74 of the PN. The aim of this study was to gain insight into the ability to maintain a functioning  
75 clockwork in polar regions through an example of an Arctic bivalve. The objectives were to  
76 identify putative circadian clock genes of *C. islandica* and determine whether, especially  
77 during the PN, it maintains rhythmic expression in several tissues in relation to scallop  
78 behaviour. The results of this study provide information on the clock system adaptation of  
79 polar organisms to their environment.

80

## 81 **Materials and Methods**

### 82 **(a) Study area and general conditions**

83 We studied behaviour and gene expression in the Arctic scallop *C. islandica* at Ny-Alesund  
84 (78° 56' N, 11°56' E), Kongsfjorden, Spitsbergen Island, Svalbard. Two sets of experiments  
85 were carried out. The first experiment was performed during a dark period of the PN, from 27  
86 to 28 January 2017, when the maximum sun angle below the horizon was between -6 and -  
87 12°, which corresponded to nautical twilight. A second experiment was performed during the  
88 Eq, from 22 to 23 September 2017, corresponding to a photoperiod close to 12 hr daylight and  
89 12 hr darkness. Specimens (n = 127, 68 ± 8 mm in length, 63.4 ± 8.1 mm in width and  
90 21.7 ± 3.4 mm in thickness) were collected in a scallop bed at a depth of 50 m located  
91 southwest of Moffen Island and north of Svalbard (79° 84' N, 12°77' E) using a dredge  
92 deployed from the research vessel RV Helmer Hanssen. Scallops were placed in ballasted  
93 cages (20 × 50 × 100 cm/7 individuals per cage) and acclimated on the sea floor at a minimal  
94 depth of 3 m during low tide under the old pier at Ny-Alesund, 4 months before the series of  
95 sampling times. Geophysical data were retrieved from the site  
96 <https://www.timeanddate.com/> and environmental parameters (temperature, chlorophyll a,  
97 photosynthetic active radiation) were obtained on site from the AWIPEV-COSYNA Svalbard  
98 Underwater Observatory ([https://www.awipev.eu/awipev-observatories/underwater-](https://www.awipev.eu/awipev-observatories/underwater-observatory/)  
99 [observatory/](https://www.awipev.eu/awipev-observatories/underwater-observatory/)), placed at a depth of 11 m close to the sampling site.

100

### 101 **(b) Identification of light perception and clock gene candidates**

102 Briefly (details in electronic supplementary material, methods S1), total RNA was extracted  
103 from each sample using TRIzol (Ambion, AM9738) according to the manufacturer's  
104 instructions. Extracts were reverse transcribed using Moloney murine leukaemia virus (M-

105 MLV) reverse transcriptase (Promega, Madison, WI, USA). The candidate partial cDNA  
106 sequences were amplified using specific primers (electronic supplementary material, table S1),  
107 cloned and sequenced by GATC Biotech (GATC Biotech SARL, Marseille, France). Forward  
108 and reverse sequences were assembled using BioEdit 7.0 software. The cDNA and deduced  
109 amino acid partial sequences of candidate genes were analysed and compared using the BLAST  
110 algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequences were deposited into GenBank  
111 (<https://www.ncbi.nlm.nih.gov/genbank/>), and accession numbers are provided (electronic  
112 supplementary material, table S2).

113

#### 114 **(c) Phylogenetic reconstruction**

115 To investigate whether the putative light perception and clock genes cloned from *C. islandica*  
116 were orthologues of known genes of these functions from other organisms, phylogenetic  
117 reconstructions were performed as follows. Amino acid sequences were aligned using  
118 MUSCLE implemented in Geneious Prime 2019.1.1 (<https://www.geneious.com>) and then  
119 processed in Gblocks (version 0.91b)  
120 ([http://molevol.cmima.csic.es/castresana/Gblocks\\_server.html](http://molevol.cmima.csic.es/castresana/Gblocks_server.html)) to remove gaps. Phylogenetic  
121 reconstructions were performed with the maximum likelihood method using PhyML 3 [22] and  
122 validated with 1000 bootstrap replicates. The best-fitting model of evolution was selected via  
123 the Akaike information criterion with SMS [23]. All information (final length, best model,  
124 outgroup) for each phylogenetic reconstruction is provided (electronic supplemental material,  
125 table S3). Trees were visualized using FigTree v 1.4.4  
126 (<http://tree.bio.ed.ac.uk/software/figtree/>).

127

#### 128 **(d) Sampling procedure**

129 Sampling consisted of the retrieval of 5 individual scallops collected every 2 h for 26 h and 22  
130 h during the Eq and PN, respectively. Detailed information on the sampling frequency,  
131 environmental parameters and tissue sampling selection are available in the electronic  
132 supplementary material (figure S1, methods S1). At each sampling time, three tissues (mantle  
133 edge, gills, muscle) from individuals were quickly excised on site under red light during the  
134 night, kept at 4°C in RNAlater overnight and stored at -80°C until processing for gene  
135 expression assays, as described in detail in the electronic supplementary material (methods  
136 S1).

137

#### 138 **(e) Gene candidate expressions**

139 Sequencing of candidate fragments provided the design of specific primers for the  
140 measurement of the mRNA expression levels of clock genes: cryptochrome 2 (*Cicry2*), clock  
141 (*Ciclock*), *bmal* (*Cibmal*), period (*Ciper*), *ror* (*Ciror*) ; and genes putatively involved in light  
142 perception: cryptochrome 1 (*Cicry1*), rhodopsin-like (*Cirhodopsin*) and melanopsin-like  
143 (*Cimelanopsin*). mRNA expression was assessed by qPCR on a LightCycler 480 System  
144 using SYBR green chemistry. Reference genes (elongation factor 1 – *Cief1* and  
145 glyceraldehyde 3-phosphate dehydrogenase – *Cigapdh*) were sequenced and used as  
146 endogenous controls (electronic supplementary material, methods S1, table S2). All qPCR  
147 analyses were run in duplicate for each sample, and the relative mRNA expression level was  
148 calculated by the comparative Ct method ( $2^{-\Delta\Delta Ct}$  method) [21].

149

#### 150 **(f) Behavioural monitoring of scallops**

151 At the same location, the behaviour of 7 scallops was monitored during the PN and equinox  
152 periods. More specifically, the valve activity behaviour of animals was recorded using high-



153 frequency noninvasive (HFNI) valvometer field technology [24]. Briefly, a pair of lightweight  
154 electrodes designed to minimize disturbance to bivalve behaviour were glued on each half  
155 shell. These electrodes were connected to the valvometer by flexible wires, avoiding any  
156 valve movement constraints. The electromagnetic current generated between the electrodes  
157 allowed for variations in valve opening and closing to be measured. The signal was recorded  
158 every 0.1 sec using a custom acquisition card, and data were automatically transmitted daily  
159 to a data processing centre at the Marine Biological Station of Arcachon (France) using  
160 internet networks.

161 Data were analysed with LabView 8.0 software (National Instruments). The valve behaviour  
162 endpoints were expressed as the valve opening amplitude (VOA) of each individual and the  
163 group. A VOA equal to 100 % indicated that the scallop's valve was open at its maximum  
164 gaping amplitude for the entire time studied, whereas a VOA equal to 0 % indicated that the  
165 scallop's valve was closed.

166

#### 167 **(d) Statistical analysis**

168 The gene expression and valve behaviour datasets were investigated for ultradian and daily  
169 periodicities in R (32-bit, version 3.2.2) using the RAIN [25] package. The RAIN algorithm is  
170 a robust nonparametric method used for the detection of rhythms of specified periods in  
171 biological data that can detect arbitrary wave forms. Different peak shapes were tested for  
172 each dataset, and the model providing the most significant fit was selected to explain the  
173 variation in the data. Ultradian periodicities in the tidal range were defined by a significant  
174 period of  $12 \pm 2$  h, and daily periodicities were defined by a significant period range of  $24 \pm 4$   
175 h. To account for multiple testing of genes, only Benjamini-Hochberg adjusted  $p$ -values  $<$   
176 0.05 were considered significant.

177 Differences in gene expression between the Eq and PN were identified using analysis of  
178 variance (one-way ANOVA) after checking assumptions (normality and homoscedasticity of  
179 the error term). When assumptions were not met, the nonparametric Kruskal–Wallis test was  
180 performed. If the null hypothesis was rejected, the Student–Newman-Keuls method was  
181 applied to determine significant differences between conditions. For all statistical results, a  
182 probability of  $p < 0.05$  was considered significant. Statistical analyses were performed using  
183 Sigma Stat software (Version 13.0, SYSTAT, Chicago, USA). Correlations of transcript  
184 expression among clock candidates and among tissues were analysed during the Eq and PN  
185 using Spearman. Differences were considered statistically significant at  $p < 0.05$ .

186

## 187 **Results**

### 188 **(a) Identification of clock and light perception gene candidates**

189 Molecular approaches allowed for the identification of putative light perception and clock  
190 gene orthologues known in invertebrates and mammals. Phylogenetic analyses confirmed the  
191 clustering of each putative light perception and core clock gene (electronic supplementary  
192 material, figure S2 A-F). All the sequences of the gene candidates were closely clustered in  
193 the phylogenetic trees, with good support, to those corresponding to the scallop species  
194 *Myzohopecten yessoensis*. For instance, proteins translated from the putative clock genes  
195 *Cibmal*, *Ciper* and *Ciclock* belong to the family of PAS-bHLH transcription factors [26,27],  
196 and phylogenetic reconstructions allowed the generation of three different trees with a close  
197 molecular relationship with other identified core clock genes in bivalves. Discrimination was  
198 also observed among members of the nuclear receptors and cryptochrome family. *C. islandica*  
199 possesses both putative light-sensitive cryptochromes, insect-like CiCRY1 and putative  
200 vertebrate-type CiCRY2, whose proteins act as transcriptional repressors on the CLOCK-

201 BMAL complex [26,28]. Among the multitude of nuclear receptors, CiROR clustered closely  
202 with nuclear receptor 1F(NR1F), the retinoic acid receptor-related orphan receptors (ROR)  
203 orthologue in the oyster *C. gigas* [29].

204 Scallops possess numerous eyes along the external border of the mantle. These ocular  
205 apparatuses act as defence mechanisms against predators and allow for light perception with  
206 the involvement of specific opsin genes, similar to vertebrates [30,31]. Our analyses led to the  
207 identification of two different opsin-like proteins clustered in a group of mollusc visual r  
208 opsins (*Cirhodopsin*) and a group of mollusc melanopsins (*Cimelanopsin*), respectively [32].

209

#### 210 **(b) Gene expression during the equinox and PN**

211 Due to the lack of a well-defined central clock in bivalves, we sampled three tissues (mantle  
212 edge, muscle, gills) where autonomous peripheral clocks [33] might be present (see electronic  
213 supplementary material, methods S1, for a description of the choice of these specific tissues).  
214 Transcriptional variations in putative light perception and circadian clock genes in the three  
215 tissues of *C. islandica* were investigated during the equinox and PN periods (figure 1, figure  
216 2, electronic supplementary material, figure S3). For some gene candidates, the transcription  
217 levels were below the threshold of PCR quantification with the applied methodology.

218 The results showed the presence and persistence of significant molecular rhythms during the  
219 Eq and PN (figure 1, electronic supplementary material, table S4). Chronobiological analyses  
220 by RAIN led to the identification of both significant daily (~24 h) and ultradian in the tidal  
221 range (~12 h) oscillations of gene expression. Surprisingly, more genes exhibiting significant  
222 daily rhythmicity were identified during the PN than during the Eq: *Ciclock* in the gills;  
223 *Cimelanopsin*, *Cicry1* and *Ciror* in the mantle edge; and *Cibmal* and *Ciror* in the muscle. In  
224 contrast, during the Eq, more gene ultradian oscillations were found than during the PN,

225 mostly peaking at the high and ebb of the tides: *Cirrhodopsin*, *Cicry1* and *Ciror* in the mantle  
226 edge and *Cimelanopsin*, *Cicry2* and *Ciror* in the muscle. The tidal rhythmicity in gene  
227 expression during the Eq tended to peak during high tides, whereas opposite trends were  
228 observed during the PN. Gene oscillations were also tissue specific. In the gills, only *Ciclock*  
229 was rhythmic in both periods. In the mantle edge, except for *Ciror*, which oscillated in both  
230 periods, only putative light perception genes oscillated at a daily frequency. During the Eq,  
231 *Cimelanopsin* and *Cicry1* expression increased during daylight, while *Cirrhodopsin* peaked at  
232 sunrise and sunset. During the PN, *Cimelanopsin* and *Cicry1* increased at the end of the night  
233 phase. An additional peak in *Cimelanopsin* expression was also observed at solar noon, which  
234 corresponded to the sun position the closest below to the horizon line. Finally, in the muscle,  
235 *Cicry2* and *Ciror* oscillated in both periods. The expression of light perception gene  
236 candidates did not oscillate during the PN, and only *Cimelanopsin* oscillated during the Eq in  
237 the muscle. Moreover, gene expression profiles differed according to the tissues and the  
238 sampling period. For instance, clock genes such as *Cicry2* in the muscle ( $p = 0.0042$ ) and  
239 *Ciclock* in the gills ( $p = 0.0278$ ) maintained significant tidal and daily rhythms, respectively,  
240 during the Eq and PN, while *Ciror* shifted from tidal oscillations in the mantle edge and  
241 muscle during the Eq to daily oscillations during the PN. Finally, *Ciper* expression oscillated  
242 only in the muscle during the PN. A comparison of the mean expression levels of the studied  
243 genes revealed differences according to the tissue and period tested (figure 2). Overall, gene  
244 expression was higher during the Eq than the PN, with a drastic increase in *Cibmal* in the  
245 muscle ( $p < 0.001$ ) and in putative light perception genes (*Cimelanopsin*, *Cicry1*,  $p < 0.001$ )  
246 in the gills. A three-way ANOVA performed on the three genes expressed in all tissues and  
247 periods (*Cimelanopsin*, *Cicry1* and *Ciror*) showed highly significant effects of tissues,  
248 seasons, genes and interactions (table insert in figure 2).

249

### 250 (c) Scallop valve behaviour and environmental parameters

251 The behaviour of *C. islandica* was monitored during the Eq and PN sampling times, and  
252 relationships with environmental parameters such as temperature, chlorophyll *a* concentration  
253 and photosynthetically active radiation were assessed (figure 3). Stable temperature values of  
254  $2.3 \pm 0.5^{\circ}\text{C}$  and  $5.8 \pm 0.1^{\circ}\text{C}$  were measured during the PN and equinox sampling periods,  
255 respectively. In Arctic regions such as Svalbard, phytoplankton blooms do not occur during  
256 the PN, which lasts 4 months. Higher chlorophyll *a* concentrations were observed during the  
257 equinox than during the PN, with a maximum during the night. Such diel patterns in  
258 chlorophyll *a* were previously reported and associated with the diel periodicity of  
259 picoplankton, which are characterized by a lower abundance at midday under high irradiance  
260 and a maximum at night [34,35]. Picophytoplankton are the main primary producers in  
261 oligotrophic oceans and have been reported to dominate chlorophyll biomass in Arctic regions  
262 [36].

263 The VOA results did not reveal direct relationships between scallop behaviour and  
264 temperature or phytoplankton abundance. Similar to the molecular results, a daily and  
265 ultradian rhythm in VOA were found. We showed (figure 3, electronic supplementary  
266 material, figure S4) significant mean VOA rhythms in the tidal range ( $p = 0.006$  (PN),  $p =$   
267  $0.030$  (Eq)) associated with daily VOA rhythms ( $p = 0.010$  (PN),  $p > 0.001$  (Eq)). The daily  
268 rhythm was more significant during the Eq, while the tidal rhythm was predominant during  
269 the PN. The mean VOA was minimal during the PN low tides and maximal during the Eq low  
270 tides. Daily characteristics showed an increase in VOA at midnight in the PN, whereas in the  
271 Eq, the daily peak was greater at sunset.

272

### 273 Discussion

274 We showed for the first time the persistence of putative circadian clock genes expression  
275 oscillations in a polar organism during the PN. Previous studies in vertebrate and invertebrate  
276 organisms failed to exhibit the rhythmicity of the molecular clock in polar environments  
277 [37,38]. The absence of clock gene expression rhythmicity during the PN in the copepod  
278 *Calanus finmarchicus* was related to the physiological transition to diapause rather than the  
279 lack of entrainment by the diel light cycle [20]. Several studies reported rhythmic behaviour  
280 during the polar day and night [15,16,39]. For example, in Arctic reindeers, controversial  
281 findings were found about the existence of circadian clock and rhythmic outputs [37,40].  
282 However, the occurrence of apparent behavioural rhythms could not necessarily indicate a  
283 functional clockwork system underlying these rhythms. Indeed, animals could develop  
284 adaptive strategies and respond directly to external cues, a phenomenon known as “masking”  
285 [9]. In the present work and despite the lack of expression of some genes in some tissues,  
286 which is likely related to the sensitivity of quantitative PCR, the results clearly suggested that  
287 peripheral clocks in scallops are characterized by a complex network. While the mammalian  
288 circadian system is highly hierarchically organized, several tissues in insects have  
289 autonomous peripheral clocks that are directly entrained by environmental cycles  
290 independently of the central clock [41,42], as might be the case for *C. islandica*. Despite the  
291 absence of a well-defined central system in bivalves, further research efforts are required to  
292 disentangle the complex interaction and function among peripheral and central clock systems.  
293 The results also showed that the expression of putative circadian clock genes oscillated during  
294 the PN in *C. islandica*, which might suggest a functional clock. Persistent daily oscillations in  
295 continual darkness may be adaptive due to interdependence between circadian clock function  
296 and homeostatic processes [9]. The behavioural rhythms observed also might support the  
297 hypothesis that rhythmic behaviour is under the control of an endogenous clock. However,  
298 functional approaches are necessary to validate these hypotheses.

299 Other surprising results showed that behaviour and gene transcription oscillations showed  
300 tidal and daily rhythms. The capacity of the circadian clock to be entrained by daily and tidal  
301 cues were recently reported in the oyster *C. gigas* [43], suggesting that a single molecular  
302 clock could be able to generate bimodal patterns. Our study showed that this mechanism  
303 might not be restricted to bivalves under temperate latitude but rather ubiquitous to bivalves  
304 across latitudinal gradients.

305 The circadian clock is likely not limited to the regulation of diel rhythmicity but is also  
306 important to measure the photoperiod involved in the timing of seasonal life cycles,  
307 characterizing phenology [6]. For instance, the switch in the tide-dependent peak of *CiCry2*  
308 cyclic expression in the muscle between the Eq (peak at high tides) and the PN (peak at low  
309 tides) was directly antiphase to rhythmic behaviour, since the muscle controls valve opening.  
310 We cannot rule out that the scallops used came from another location, north of Svalbard,  
311 could be less responsive to the actual environmental cues than the native scallops in  
312 Kongfjorden where the sampling was done, although a 4-month acclimation was allowed at  
313 the site prior to the experiments. However, this seasonal change represents temporal niche  
314 switching, an existing but relatively unusual phenomenon in which animals alter their  
315 physiology and behavioural rhythms and occupy a different temporal niche [9]. This  
316 phenomenon has been observed in polar vertebrates [44,45] but also in temperate organisms  
317 such as the oyster *C. gigas* [46], suggesting an important phenologic trait for organisms.  
318 However, the mechanisms that underlie temporal niche switching are not well understood and  
319 deserve further investigation.

320 The results of this study suggested that *C. islandica* could have developed specific  
321 mechanisms to perceive low light intensity to synchronize the clock system during the PN, as  
322 suggested by Tran et al. [15]. Previous research has demonstrated the role of opsins,  
323 especially melanopsin, in circadian responses to light in vertebrates [47,48]. In addition to

324 both opsin members identified in *C. islandica*, further investigations are necessary on the  
325 properties and role of opsins since scallop species possess multiple members of the opsin  
326 family that could react differentially to specific wavelengths [49]. For example, ultraviolet  
327 (UV) wavelengths are common in the light spectrum of polar environments because of the  
328 reflection of light from ice and because of the relative position of the sun on the horizon. UV  
329 radiation provides robust daily cycles at polar latitudes and could be used as zeitgeber by  
330 Arctic organisms [50,51]. Very low light intensity perception could also not be limited to  
331 polar environments since previous studies demonstrated the perception of moonlight by  
332 marine organisms [18,52,53]. Nevertheless, Arctic scallops appeared highly sensitive to light  
333 since the reported mean irradiance during the PN in Svalbard ranges from 1 to  $1.5 \times 10^{-5}$   $\mu\text{mol}$   
334  $\text{photons m}^{-2} \text{ s}^{-1}$  (with a maximum position of the sun at  $-9^\circ$ ). Arctic zooplankton such as  
335 *Calanus spp.* were found to perceive  $10^{-8}$   $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  of blue light [54,55].  
336 Alternatively, we cannot rule out the possibility that the clock system oscillated in free-  
337 running during the PN, despite *Ciclock* expression in gills peaking at the beginning of the  
338 night during the equinox and PN, suggesting entrainment by light cues.

339 In conclusion, it is assumed that the adaptive value of clock systems and biological rhythms is  
340 to anticipate predictable changes in the environment and appropriately adjust the timing of  
341 biological processes such that they occur at optimal phases of the cycle [9]. Previous studies  
342 reporting permanent or transient absence of rhythms in polar organisms suggested that  
343 organisms specifically adapt to their environment [37]. However, our results showed that  
344 behavioural rhythms of the Arctic scallop were correlated to putative clock gene transcription  
345 oscillations, even during the PN and were likely synchronized by the light and tides, as is the  
346 case in lower latitudes.

347 Finally, numerous studies have reported the impacts of climate change on the phenology of  
348 organisms, leading to phenologic desynchronization between species and trophic resources



349 [56,57]. This phenomenon could be exacerbated in the Arctic, leading to important ecosystem  
350 destabilization [58]. In this context, the existence of a robust clock system in scallops could  
351 handicap them in hampering their adaptation capacities when faced with drastic  
352 environmental changes arriving in polar regions.

353

354

355 Ethics. All procedures were approved and carried out in accordance with international ethical  
356 standards and French guidelines.

357

358 Data accessibility. The mRNA sequences with their partial CDS can be accessed using  
359 GenBank accession numbers as provided in electronic supplementary material, table S2.

360

361 Competing interests. Authors have no competing interests.

362

363 Authors' contribution. Study design, D.T., M.P., C.B., L.C. and H.A.; fieldwork, M.P., D.T.,  
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376 **Figure legends**

377 **Figure 1. Cyclic expression of *C. islandica* light perception and core clock gene**

378 **candidates during equinox and polar night.** Relative transcription levels (mean  $\pm$  SEM, n =  
379 5) of *Cimelanopsin*, *Cirhdopsin*, *Cicry1*, *Cicry2*, *Ciclock*, *Cibmal*, *Ciper* and *Ciror* RNA in  
380 muscle, gill and mantle edge tissues of *C. islandica* during Eq and PN. Only candidates  
381 exhibiting significant rhythmicity were presented. Full set of results is available in electronic  
382 supplemental material, figure S3. Dotted lines refer to tide cycles. Yellow and dark areas  
383 indicated photophase and scotophase during equinox; dark and grey areas referred to the night  
384 and nautical twilight periods respectively during PN. Significant oscillations, using RAIN  
385 algorithm, were denoted T for ultradian rhythm in the tidal range ( $12 \pm 2$  h) and D for daily  
386 rhythm ( $24 \pm 4$  h). Exact adjusted *p*-values were presented in electronic supplemental  
387 material, table S4.

388 **Figure 2. Expression levels of light perception and core clock gene candidates in *C.***

389 ***islandica* during equinox and polar night.** Comparison of mean expression levels of light  
390 perception and core clock genes of *C. islandica* during Eq daytime and nighttime and PN  
391 nautical twilight and night. Letters indicated significant differences at  $p < 0.05$  between light  
392 regimes and red asterisks denoted significant differences between PN and Eq. Three-way  
393 ANOVA analyze (insert) was performed on three genes quantified in all conditions  
394 (*Cimelanopsin*, *Cicry1* and *Ciror*).

395 **Figure 3. Scallop valve opening amplitude behavior.** Upper panels, chlorophyll *a*

396 concentration and water temperature during the two sampling time periods. Lower panels,  
397 mean hourly VOA (n = 7 scallops) and photosynthetically active radiation (PAR, yellow  
398 surface) during Eq (January 27 - 28, 2017) and PN (September 22 - 23, 2017). Dotted lines  
399 refer to tide cycles. Significant oscillations, using RAIN algorithm, were denoted T for  
400 ultradian rhythm in the tidal range ( $12 \pm 2$  h) and D for daily rhythm ( $24 \pm 4$  h).

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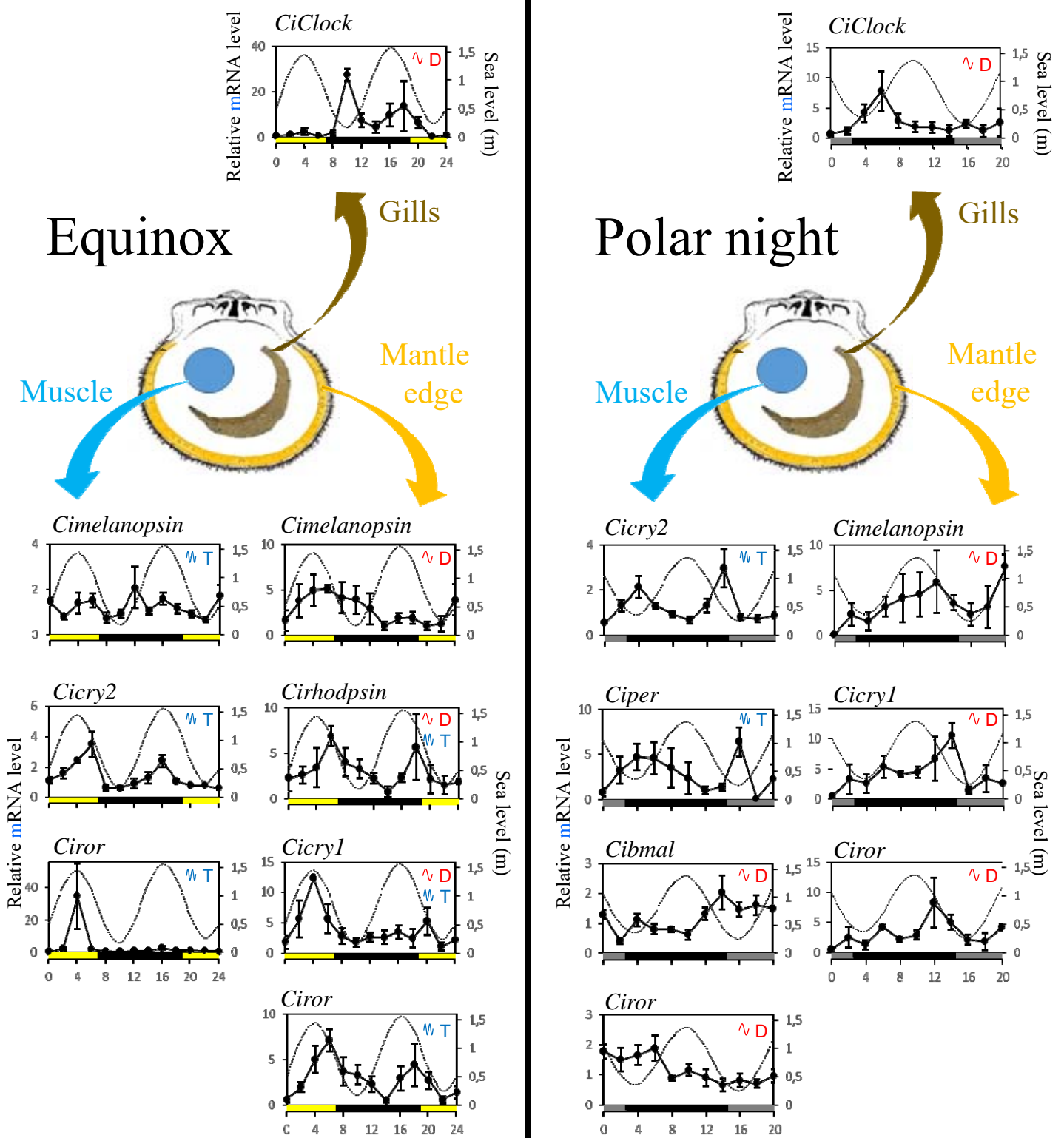


Figure 1.

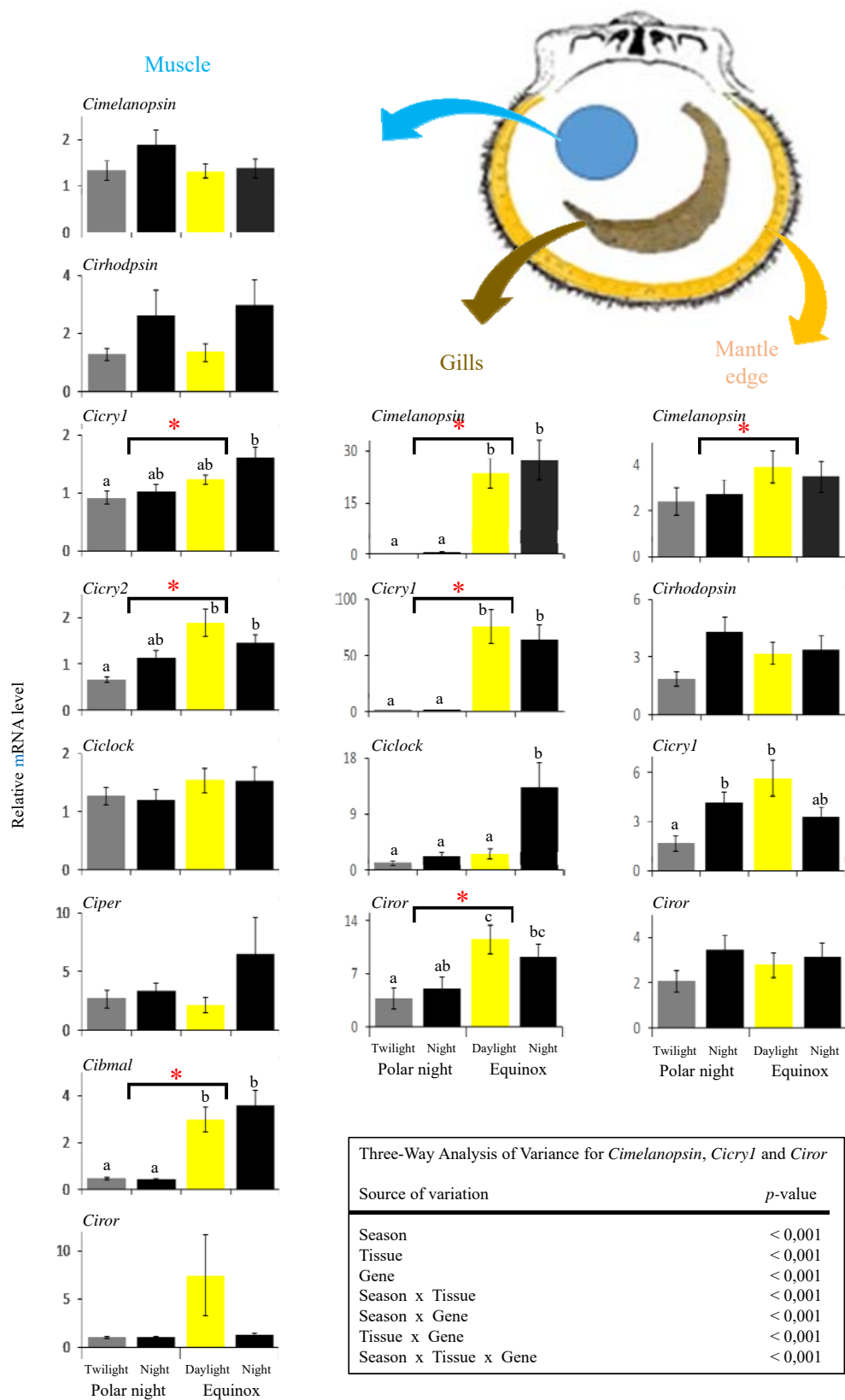


Figure 2

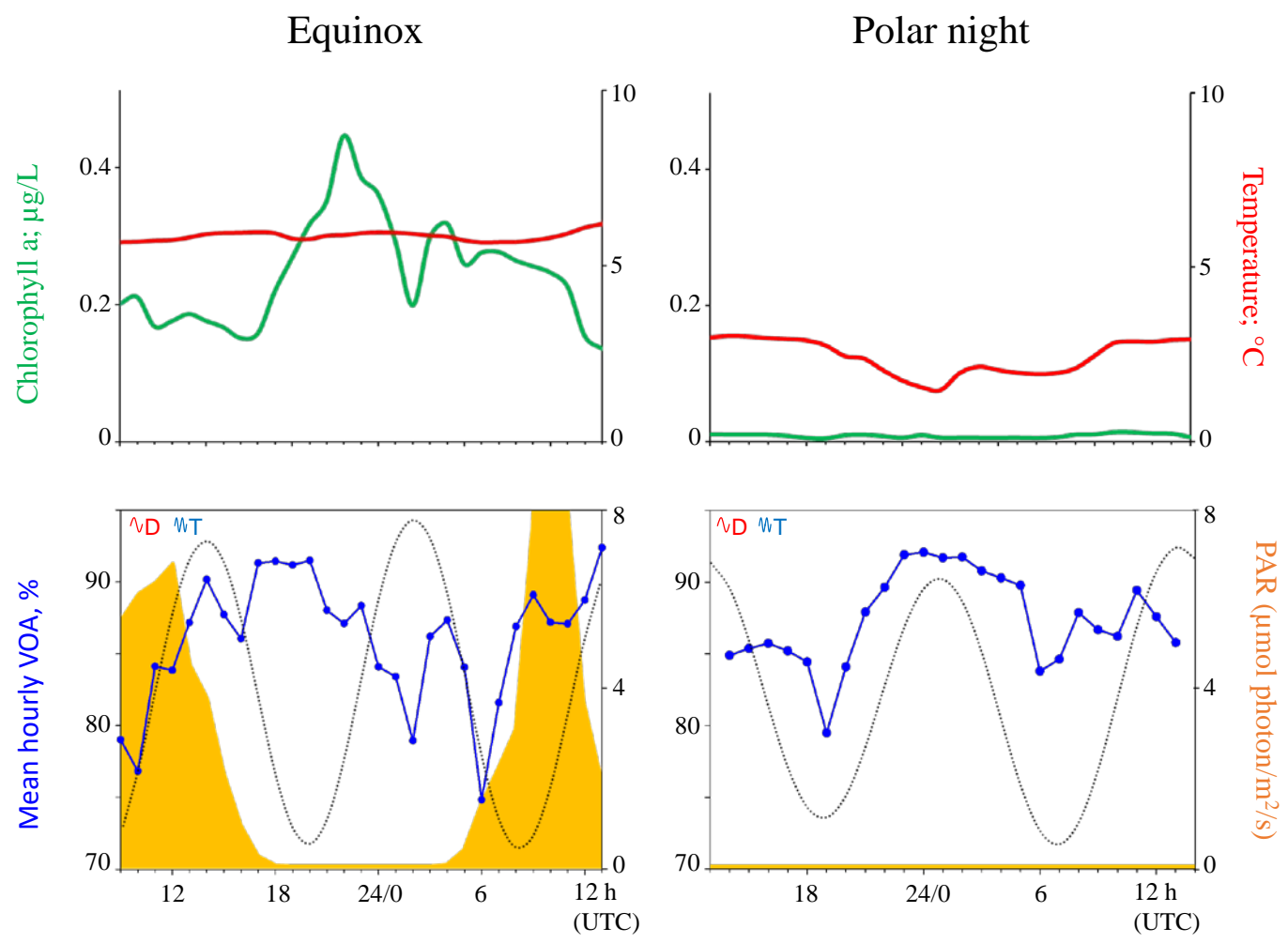


Figure 3.