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Abstract

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

Introduction

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

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

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

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

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

Materials and Methods

(a) Study area and general conditions

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

(b) Identification of light perception and clock gene candidates

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

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

(c) Phylogenetic reconstruction

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

(d) Sampling procedure

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

(e) Gene candidate expressions

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

(f) Behavioural monitoring of scallops

At the same location, the behaviour of 7 scallops was monitored during the PN and equinox 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 ± 2 h, and daily periodicities were defined by a significant period range of 24 ± 4
175 h. To account for multiple testing of genes, only Benjamini-Hochberg adjusted *p*-values <
176 0.05 were considered significant.

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

Results

(a) Identification of clock and light perception gene candidates

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

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

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

(b) Gene expression during the equinox and PN

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

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

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

(c) Scallop valve behaviour and environmental parameters

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

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

Discussion

We showed for the first time the persistence of putative circadian clock genes expression oscillations in a polar organism during the PN. Previous studies in vertebrate and invertebrate organisms failed to exhibit the rhythmicity of the molecular clock in polar environments [37,38]. The absence of clock gene expression rhythmicity during the PN in the copepod *Calanus finmarchicus* was related to the physiological transition to diapause rather than the lack of entrainment by the diel light cycle [20]. Several studies reported rhythmic behaviour during the polar day and night [15,16,39]. For example, in Arctic reindeers, controversial findings were found about the existence of circadian clock and rhythmic outputs [37,40]. However, the occurrence of apparent behavioural rhythms could not necessarily indicate a functional clockwork system underlying these rhythms. Indeed, animals could develop adaptive strategies and respond directly to external cues, a phenomenon known as "masking" [9]. In the present work and despite the lack of expression of some genes in some tissues, which is likely related to the sensitivity of quantitative PCR, the results clearly suggested that peripheral clocks in scallops are characterized by a complex network. While the mammalian circadian system is highly hierarchically organized, several tissues in insects have autonomous peripheral clocks that are directly entrained by environmental cycles independently of the central clock [41,42], as might be the case for *C. islandica*. Despite the absence of a well-defined central system in bivalves, further research efforts are required to disentangle the complex interaction and function among peripheral and central clock systems.

The results also showed that the expression of putative circadian clock genes oscillated during the PN in *C. islandica*, which might suggest a functional clock. Persistent daily oscillations in continual darkness may be adaptive due to interdependence between circadian clock function and homeostatic processes [9]. The behavioural rhythms observed also might support the hypothesis that rhythmic behaviour is under the control of an endogenous clock. However, 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

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

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

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

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

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

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

Competing interests. Authors have no competing interests.

Authors' contribution. Study design, D.T., M.P., C.B., L.C. and H.A.; fieldwork, M.P., D.T., C.B. and H.A.; molecular analysis: M.P. L.B.; behavioral analysis, D.T.; interpretation, M.P., D.T., manuscript writing, M.P.; Funding: D.T., L.C., H.A. All authors contributed critically to the drafts, and gave final approval for publication.

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373

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Figure legends

Figure 1. Cyclic expression of *C. islandica* light perception and core clock gene candidates during equinox and polar night. Relative transcription levels (mean ± SEM, n = 5) of *Cimelanopsin*, *Cirhdopsin*, *Cicry1*, *Cicry2*, *Ciclock*, *Cibmal*, *Ciper* and *Ciror* RNA in muscle, gill and mantle edge tissues of *C. islandica* during Eq and PN. Only candidates exhibiting significant rhythmicity were presented. Full set of results is available in electronic supplemental material, figure S3. Dotted lines refer to tide cycles. Yellow and dark areas indicated photophase and scotophase during equinox; dark and grey areas referred to the night and nautical twilight periods respectively during PN. Significant oscillations, using RAIN algorithm, were denoted T for ultradian rhythm in the tidal range (12 ± 2 h) and D for daily rhythm (24 ± 4 h). Exact adjusted *p*-values were presented in electronic supplemental material, table S4.

Figure 2. Expression levels of light perception and core clock gene candidates in *C. islandica* during equinox and polar night. Comparison of mean expression levels of light perception and core clock genes of *C. islandica* during Eq daytime and nighttime and PN nautical twilight and night. Letters indicated significant differences at $p < 0.05$ between light regimes and red asterisks denoted significant differences between PN and Eq. Three-way ANOVA analyze (insert) was performed on three genes quantified in all conditions (*Cimelanopsin*, *Cicry1* and *Ciror*).

Figure 3. Scallop valve opening amplitude behavior. Upper panels, chlorophyll *a* concentration and water temperature during the two sampling time periods. Lowed panels, mean hourly VOA (n = 7 scallops) and photosynthetically active radiation (PAR, yellow surface) during Eq (January 27 - 28, 2017) and PN (September 22 - 23, 2017). Dotted lines refer to tide cycles. Significant oscillations, using RAIN algorithm, were denoted T for ultradian rhythm in the tidal range (12 ± 2 h) and D for daily rhythm (24 ± 4 h).

References

1. Yerushalmi S, Green RM. 2009 Evidence for the adaptive significance of circadian rhythms. *Ecol. Lett.* **12**, 970–981. (doi:10.1111/j.1461-0248.2009.01343.x)

2. Dunlap JC, Loros JJ. 2016 Yes, circadian rhythms actually do affect almost everything. *Cell Res.* **26**, 759–760. (doi:10.1038/cr.2016.65)

3. Schwartz W, Helm B, Gerkema MP. 2017 Wild clocks: preface and glossary. *Philos. Trans. R. Soc. B Biol. Sci.* **372**, 20170211. (doi:10.1098/rstb.2017.0211)

4. Dunlap JC. 1999 Molecular bases for circadian clocks. *Cell* **96**, 271–290.

5. Senthilan PR, Grebler R, Reinhard N, Rieger D, Helfrich-Forster C. 2019 Role of rhodopsins as circadian photoreceptors in the *Drosophila melanogaster*. *Biology* **8**. (doi:10.3390/biology8010006)

6. Goto SG. 2013 Roles of circadian clock genes in insect photoperiodism. *Entomol. Sci.* **16**, 1–16. (doi:10.1111/ens.12000)

7. Helm B, Ben-Shlomo R, Sheriff MJ, Hut RA, Foster R, Barnes BM, Dominoni D. 2013 Annual rhythms that underlie phenology: biological time-keeping meets environmental change. *Proc. Biol. Sci.* **280**, 20130016. (doi:10.1098/rspb.2013.0016)

8. Visser ME, Caro SP, van Oers K, Schaper SV, Helm B. 2010 Phenology, seasonal timing and circannual rhythms: towards a unified framework. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **365**, 3113–3127. (doi:10.1098/rstb.2010.0111)

9. Williams CT, Barnes BM, Buck CL. 2015 Persistence, entrainment, and function of circadian rhythms in polar vertebrates. *Physiology* **30**, 86–96. (doi:10.1152/physiol.00045.2014)

10. Bilt W *et al.* 2019 Climate in Svalbard 2100– a knowledge base for climate adaptation. , 105.

11. Wassmann P, Duarte CM, Agusti S, Sejr MK. 2011 Footprints of climate change in the Arctic marine ecosystem. *Glob. Change Biol.* **17**, 1235–1249. (doi:10.1111/j.1365-2486.2010.02311.x)

12. Saikkonen K, Taulavuori K, Hyvönen T, Gundel PE, Hamilton CE, Vänninen I, Nissinen A, Helander M. 2012 Climate change-driven species' range shifts filtered by photoperiodism. *Nat. Clim. Change* **2**, 239.

13. Smetacek V, Nicol S. 2005 Polar ocean ecosystems in a changing world. *Nature* **437**, 362–368. (doi:10.1038/nature04161)

14. Berge J *et al.* 2015 Unexpected levels of biological activity during the polar night offer new perspectives on a warming arctic. *Curr. Biol.* **25**, 2555–2561. (doi:10.1016/j.cub.2015.08.024)

15. Tran D, Sow M, Camus L, Ciret P, Berge J, Massabuau J-C. 2016 In the darkness of the polar night, scallops keep on a steady rhythm. *Sci. Rep.* **6**. (doi:10.1038/srep32435)

16. Arnold W, Ruf T, Loe LE, Irvine RJ, Ropstad E, Veiberg V, Albon SD. 2018 Circadian rhythmicity persists through the Polar night and midnight sun in Svalbard reindeer. *Sci. Rep.* **8**, 14466. (doi:10.1038/s41598-018-32778-4)

17. Tessmar-Raible K, Raible F, Arboleda E. 2011 Another place, another timer: Marine species and the rhythms of life. *BioEssays News Rev. Mol. Cell. Dev. Biol.* **33**, 165–172. (doi:10.1002/bies.201000096)

18. Last KS, Hobbs L, Berge J, Brierley AS, Cottier F. 2016 Moonlight drives ocean-scale mass vertical migration of zooplankton during the arctic winter. *Curr. Biol.* **26**, 244–251.

19. Hawley KL, Rosten CM, Haugen TO, Christensen G, Lucas MC. 2017 Freezer on, lights off! Environmental effects on activity rhythms of fish in the Arctic. *Biol. Lett.* **13**. (doi:10.1098/rsbl.2017.0575)

20. Häfker N, Teschke M, Hüppe L, Meyer B. 2018 *Calanus finmarchicus* diel and seasonal rhythmicity in relation to endogenous timing under extreme polar photoperiods. *Mar. Ecol. Prog. Ser.* **603**, 79–92.

21. Livak KJ, Schmittgen TD. 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402–408. (doi:10.1006/meth.2001.1262)

22. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010 New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* **59**, 307–321.

23. Lefort V, Longueville J-E, Gascuel O. 2017 SMS: smart model selection in PhyML. *Mol. Biol. Evol.* **34**, 2422–2424.

24. Andrade H, Massabuau J-C, Cochrane S, Ciret P, Tran D, Sow M, Camus L. 2016 High frequency non-invasive (HFNI) bio-sensors as a potential tool for marine monitoring and assessments. *Front. Mar. Sci.* **3**, 187. (doi:10.3389/fmars.2016.00187)

25. Thaben PF, Westermark PO. 2014 Detecting rhythms in time series with RAIN. *J. Biol. Rhythms* **29**, 391–400. (doi:10.1177/0748730414553029)

26. Perrigault M, Tran D. 2017 Identification of the molecular clockwork of the oyster *Crassostrea gigas*. *PloS One* **12**, e0169790. (doi:10.1371/journal.pone.0169790)

27. Wu D, Rastinejad F. 2017 Structural characterization of mammalian bHLH-PAS transcription factors. *Curr. Opin. Struct. Biol.* **43**, 1–9. (doi:10.1016/j.sbi.2016.09.011)

28. Zhu H, Yuan Q, Briscoe AD, Froy O, Casselman A, Reppert SM. 2005 The two CRYs of the butterfly. *Curr. Biol.* **15**, R953-954. (doi:10.1016/j.cub.2005.11.030)

29. Vogeler S, Galloway TS, Lyons BP, Bean TP. 2014 The nuclear receptor gene family in the Pacific oyster, *Crassostrea gigas*, contains a novel subfamily group. *BMC Genomics* **15**, 369. (doi:10.1186/1471-2164-15-369)

30. Serb JM, Porath-Krause AJ, Pairett AN. 2013 Uncovering a gene duplication of the photoreceptive protein, opsin, in scallops (Bivalvia: Pectinidae). *Integr. Comp. Biol.* **53**, 68–77. (doi:10.1093/icb/ict063)

31. Morton B. 2000 The function of pallial eyes within the Pectinidae, with a description of those present in *Patinopecten yessoensis*. *Geol. Soc. Lond. Spec. Publ.* **177**, 247. (doi:10.1144/GSL.SP.2000.177.01.14)

32. Koyanagi M, Terakita A. 2014 Diversity of animal opsin-based pigments and their optogenetic potential. *Biochim. Biophys. Acta* **1837**, 710–716. (doi:10.1016/j.bbabio.2013.09.003)

33. Richards J, Gumz ML. 2012 Advances in understanding the peripheral circadian clocks. *FASEB J.* **26**, 3602–3613. (doi:10.1096/fj.12-203554)

34. Ohi N, Saito H, Taguchi S. 2005 Diel patterns in chlorophyll a specific absorption coefficient and absorption efficiency factor of picoplankton. *J. Oceanogr.* **61**, 379–388. (doi:10.1007/s10872-005-0048-9)

35. Dandonneau Y, Neveux J. 1997 Diel variations of *in vivo* fluorescence in the eastern equatorial Pacific: an unvarying pattern. *JGFOS Process Study Equat. Pac.* **44**, 1869–1880. (doi:10.1016/S0967-0645(97)00020-9)

36. Zhang F, He J, Lin L, Jin H. 2015 Dominance of picophytoplankton in the newly open surface water of the central Arctic Ocean. **38**, 1081–1089. (doi:10.1007/s00300-015-1662-7)

37. Lu W, Meng Q-J, Tyler NJC, Stokkan K-A, Loudon ASI. 2010 A circadian clock is not required in an arctic mammal. *Curr. Biol.* **20**, 533–537. (doi:10.1016/j.cub.2010.01.042)

38. Kobelkova A, Goto SG, Peyton JT, Ikeno T, Lee REJ, Denlinger DL. 2015 Continuous activity and no cycling of clock genes in the Antarctic midge during the polar summer. *J. Insect Physiol.* **81**, 90–96. (doi:10.1016/j.jinsphys.2015.07.008)

39. Berge J *et al.* 2009 Diel vertical migration of Arctic zooplankton during the polar night. *Biol. Lett.* **5**, 69–72. (doi:10.1098/rsbl.2008.0484)

40. Arnold W, Ruf T, Loe LE, Irvine RJ, Ropstad E, Veiberg V, Albon SD. 2018 Circadian rhythmicity persists through the Polar night and midnight sun in Svalbard reindeer. *Sci. Rep.* **8**, 1–12. (doi:10.1038/s41598-018-32778-4)

41. Ito C, Tomioka K. 2016 Heterogeneity of the peripheral circadian systems in *Drosophila melanogaster*: A review. *Front. Physiol.* **7**, 8–8. (doi:10.3389/fphys.2016.00008)

42. Tomioka K, Uryu O, Kamae Y, Umezaki Y, Yoshii T. 2012 Peripheral circadian rhythms and their regulatory mechanism in insects and some other arthropods: a review. *J. Comp. Physiol. B* **182**, 729–740. (doi:10.1007/s00360-012-0651-1)

43. Tran D, Perrigault M, Ciret P, Payton L. 2020 Bivalve mollusc circadian clock genes can run at tidal frequency. *Proc. R. Soc. B Biol. Sci.* **287**, 20192440. (doi:10.1098/rspb.2019.2440)

44. Andreasson S. 1973 Seasonal changes in diel activity of *Cottus poecilopus* and *C. gobio* (Pisces) at the Arctic circle. *Oikos* **24**, 16–23. (doi:10.2307/3543248)

45. Müller K. 1973 Seasonal phase shift and the duration of activity time in the Burbot, *Lota lota* (L.) (Pisces, Gadidae). *J. Comp. Physiol.* **84**, 357–359. (doi:10.1007/BF00696347)

46. Mat AM, Massabuau J-C, Ciret P, Tran D. 2012 Evidence for a plastic dual circadian rhythm in the oyster *Crassostrea gigas*. *Chronobiol. Int.* **29**, 857–867. (doi:10.3109/07420528.2012.699126)

47. Panda S *et al.* 2003 Melanopsin is required for non-image-forming photic responses in blind mice. *Science* **301**, 525–527. (doi:10.1126/science.1086179)

48. Hankins MW, Peirson SN, Foster RG. 2008 Melanopsin: an exciting photopigment. *Trends Neurosci.* **31**, 27–36. (doi:10.1016/j.tins.2007.11.002)

49. Wang S *et al.* 2017 Scallop genome provides insights into evolution of bilaterian karyotype and development. *Nat. Ecol. Evol.* **1**, 120. (doi:10.1038/s41559-017-0120)

50. Nordtug T, Melø TB. 1988 Diurnal variations in natural light conditions at summer time in Arctic and subarctic areas in relation to light detection in insects. *Holarct. Ecol.* **11**, 202–209.

51. Stelzer RJ, Chittka L. 2010 Bumblebee foraging rhythms under the midnight sun measured with radiofrequency identification. *BMC Biol.* **8**, 93. (doi:10.1186/1741-7007-8-93)

52. Kronfeld-Schor N, Dominoni D, de la Iglesia H, Levy O, Herzog ED, Dayan T, Helfrich-Forster C. 2013 Chronobiology by moonlight. *Proc. Biol. Sci.* **280**, 20123088. (doi:10.1098/rspb.2012.3088)

53. Payton L, Tran D. 2019 Moonlight cycles synchronize oyster behaviour. *Biol. Lett.* **15**, 20180299. (doi:10.1098/rsbl.2018.0299)

54. Cohen JH *et al.* 2015 Is ambient light during the high arctic polar night sufficient to act as a visual cue for zooplankton? *PloS One* **10**, e0126247. (doi:10.1371/journal.pone.0126247)

55. Båtnes AS, Miljeteig C, Berge J, Greenacre M, Johnsen G. 2015 Quantifying the light sensitivity of *Calanus spp.* during the polar night: potential for orchestrated migrations conducted by ambient light from the sun, moon, or aurora borealis? *Polar Biol.* **38**, 51–65. (doi:10.1007/s00300-013-1415-4)

56. Parmesan C. 2006 Ecological and evolutionary responses to recent climate change. *Annu. Rev. Ecol. Evol. Syst.* **37**, 637–669. (doi:10.1146/annurev.ecolsys.37.091305.110100)

57. Poloczanska ES *et al.* 2013 Global imprint of climate change on marine life. *Nat. Clim. Change* **3**, 919.

58. Søreide JE, Leu E, Berge J, Graeve M, Falf-Petersen S. 2010 Timing of blooms, algal food quality and *Calanus glacialis* reproduction and growth in a changing Arctic. *Glob. Change Biol.* **16**, 3154–3163. (doi:10.1111/j.1365-2486.2010.02175.x)

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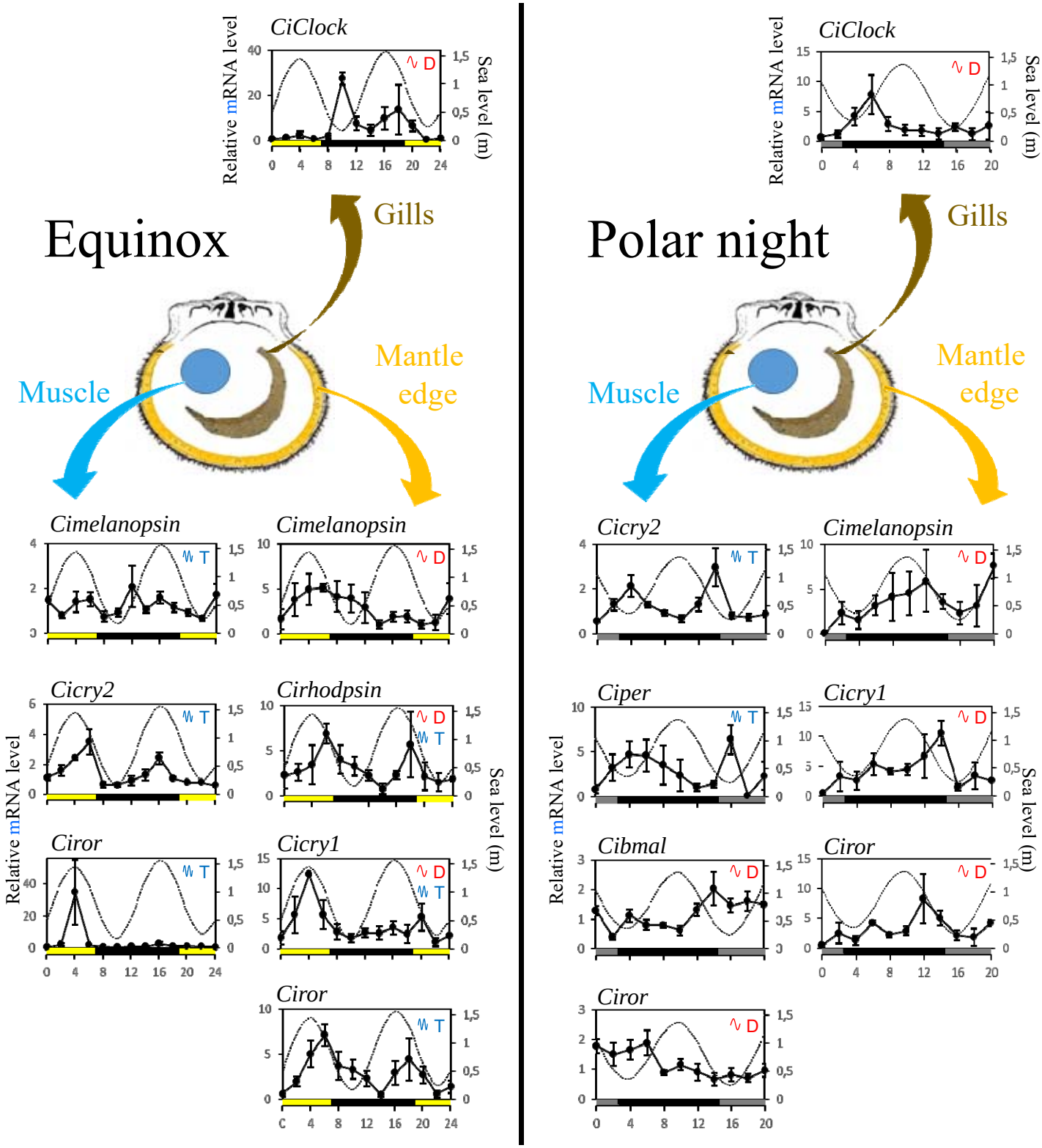
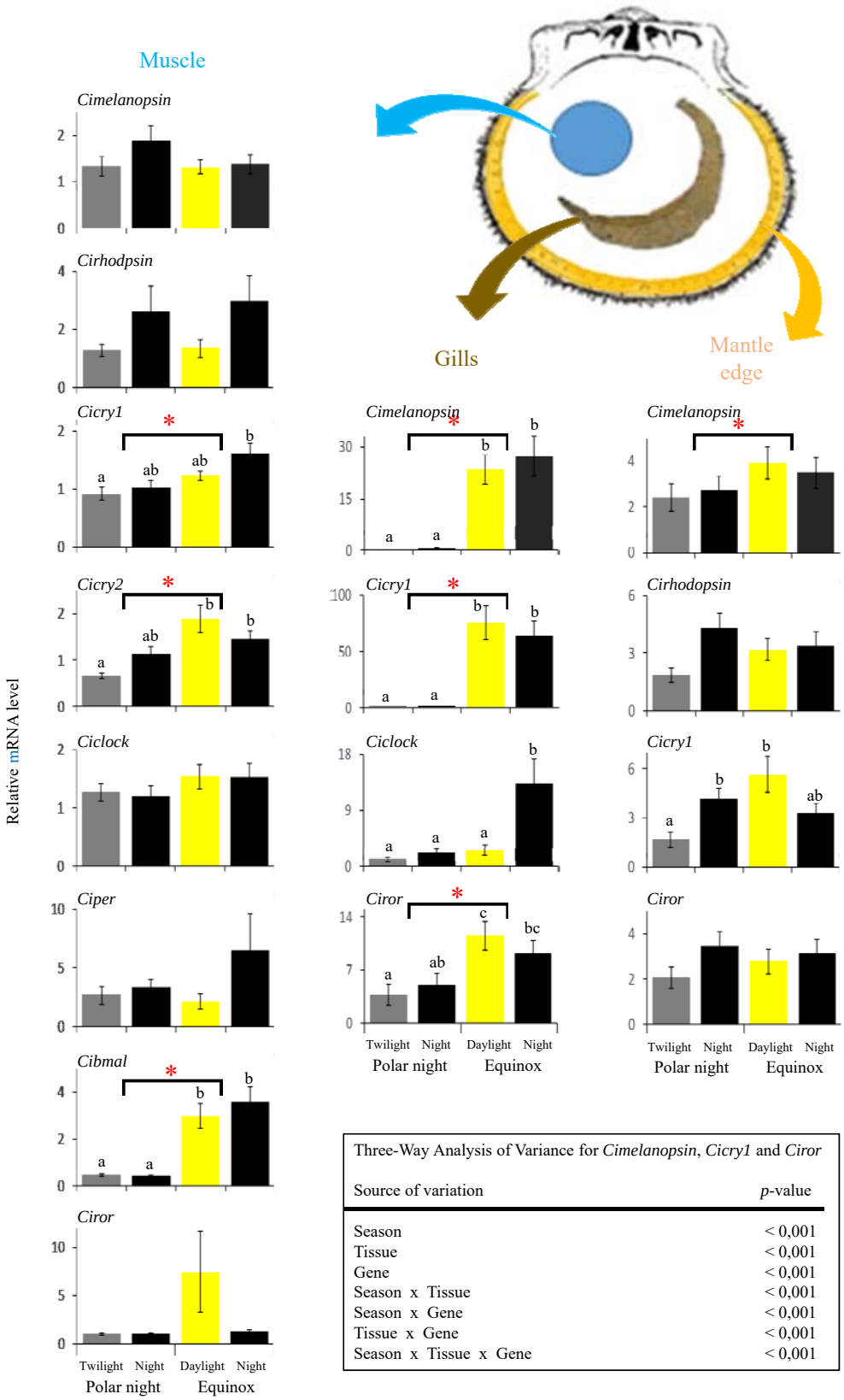

Equinox
Polar night
Gills
Muscle
Mantle edge
CiClock
Cimelanopsin
Cicry2
Cirhodpsin
Ciror
Cicry1
Ciper
Cibmal
Relative mRNA level
Sea level (m)
T
D

Figure 1.



Three-Way Analysis of Variance for *Cimelanopsin*, *Cicry1* and *Ciror*	
Source of variation	*p*-value
Season	< 0,001
Tissue	< 0,001
Gene	< 0,001
Season x Tissue	< 0,001
Season x Gene	< 0,001
Tissue x Gene	< 0,001
Season x Tissue x Gene	< 0,001

Figure 2

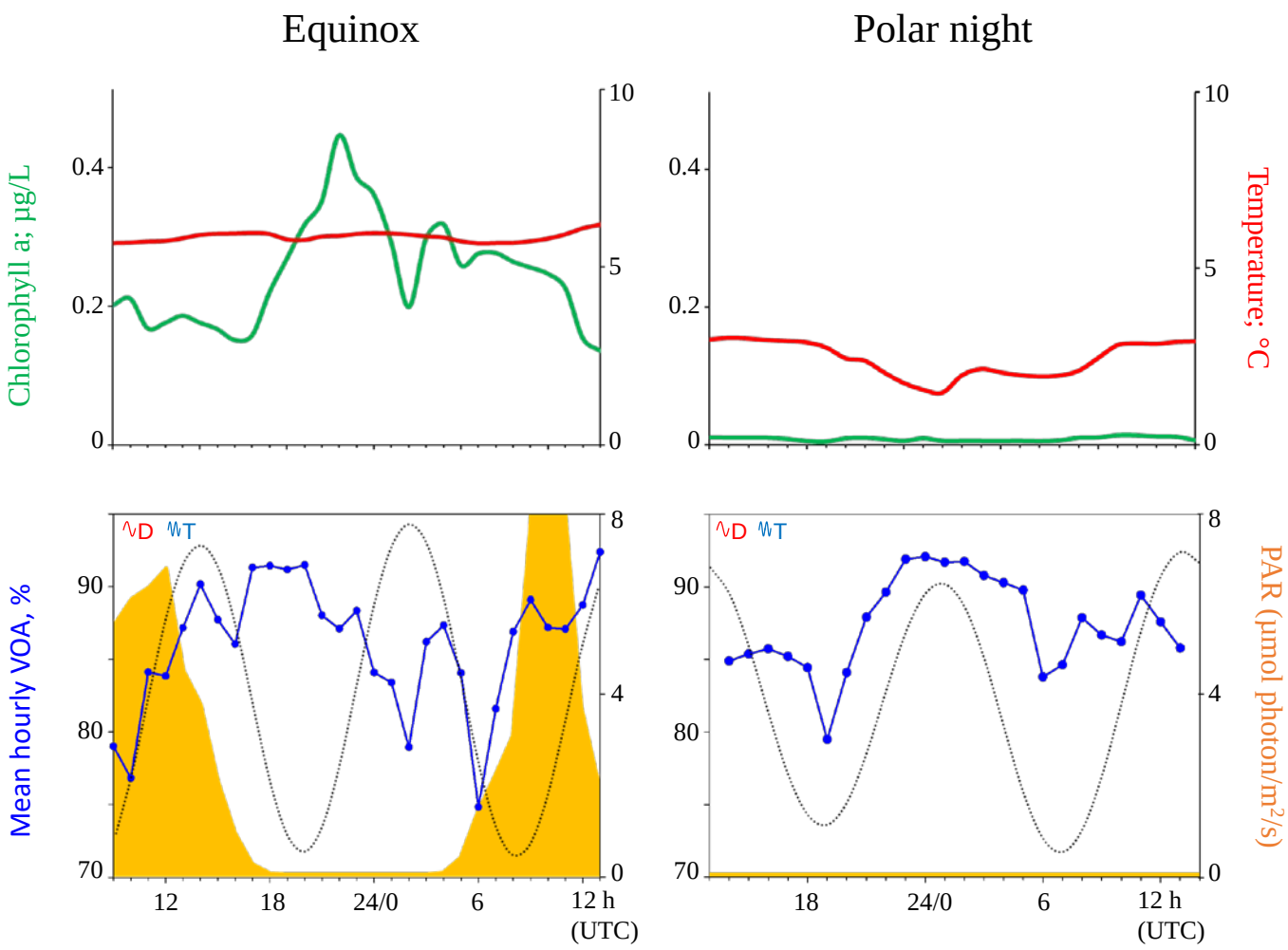

Equinox
Polar night
Chlorophyll a; µg/L
Temperature; °C
Mean hourly VOA, %
PAR (µmol photon/m²/s)
D
T
0.4
0.2
0
10
5
0
90
80
70
8
4
0
12
18
24/0
6
12 h
(UTC)

Figure 3.