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Keywords

High Arctic, polar day, mussel, behavior, daily rhythm, lunar rhythm, semi-lunar rhythm, tidal rhythm.

Abstract

Marine species exhibit a multitude of biological rhythms, in accordance with their complex ecosystem governed by sun, earth and moon trajectories. Because of the inclination of the earth's axis, the high Arctic ecosystem is characterized by several months of permanent illumination during the polar day. The persistence of biological rhythms in this photic context remains unclear. Yet, this information is crucial for the understanding of polar ecosystems functioning, as well as to predict the impact of future climate changes. Particularly, the impact of extreme photoperiods on recent invasive species remains largely unknown. Here, we investigate how environmental cycles shape the behavior of a re-emerging polar resident, the mussel *Mytilus sp.* during polar day (17 April to 26 August 2020; Svalbard, Ny-Ålesund, 78°56' N, 11°56' E). Our results show that in the high Arctic polar day, mussels' behavior is shaped by both the photoperiod and the diel sun trajectories above the horizon. Additionally, mussels also exhibit tidal, semi-lunar, and lunar rhythms of valve opening amplitude. We argue that these rhythms may have ecosystems functioning implications, and that the mussels' ability to deal with drastic light regimes may explain their northward expansion and new resettlement in high Arctic.

Introduction

The high Arctic is undergoing major changes, including a fast decline in ice cover and warming at a rate that is two to four times faster than that the rest of the planet (2021; Chylek et al. 2022). These drastic changes are likely to have numerous ecological consequences, such as species poleward expansion, shift in trophic interactions, and reorganization of biological communities (Post et al. 2018; Beaugrand et al. 2019). In order to understand how the Arctic and its ecosystems are changing, focus needs to be directed toward a more factual understanding of today's biology in polar areas (Schmal et al. 2020).

Living organisms have evolved in complex biotopes governed by environmental cycles related to astronomical trajectories and interactions (Tessmar- Raible et al. 2011). The temporal coordination of biological processes with these cycles is crucial, from molecular to behavioral activities (Mermet et al. 2017; Helm et al. 2017). Endogenous clocks allow organisms to anticipate changes in their environment by using the highly predictable cycles as zeitgebers, such as the light / dark cycle. Due to the axis of rotation of the earth, the light / dark alternation disappears at polar latitudes during polar day and polar night, characterized by several months of permanent illumination and darkness respectively. Thus, the interest of the persistence of daily rhythms in polar ecosystems is questionable (Schmal et al. 2020). Biological clocks are highly adaptive, but robust behavioral rhythms could be a drawback for adaptation to constant photic environments (Bloch et al. 2013; Abhilash et al. 2017; Bertolini et al. 2019; Schmal et al. 2020). The issue of the maintenance of biological rhythms during the polar day remains crucial for the understanding of polar ecosystems functioning, but also to predict the impact of future changes (Schmal et al. 2020; Huffeldt 2020; Hüppe et al. 2020; Perrigault et al. 2020; Payton et al. 2021). Indeed, the northward expansion of organisms originally located at more southern latitudes implies substantial daily changes in photic conditions. The impact of extreme photoperiods on invasive species remains unknown.

The blue mussel *Mytilus sp*. is recently resettled in the high Arctic after a 1000-year absence (Berge et al. 2005). It succeeded in Svalbard archipelago as a hybrid species, resulting from the mix of three species: *Mytilus edulis*, *Mytilus Galloprovincialis*, and *Mytilus trossulus* (Mathiesen et al. 2017; Leopold et al. 2019). The blue mussel is frequently used as an environmental indicator, as it is a semi-sessile organism, widely distributed in coastal regions in both hemispheres, with a relatively long-life span. As it has the potential to dominate benthic habitats in intertidal and shallow subtidal areas, the blue mussel plays an important ecological role as an ecosystem engineer, and also a commercial role in the shellfish industry

(Leopold et al. 2019). However, close to their northern limit of distribution, *Mytilus* rarely occur in the intertidal areas because of cold winter aerial temperatures, but rather occur in subtidal areas at depths down to approximately 5 m (Mathiesen et al. 2017). The blue mussel possesses a functional endogenous circadian clock machinery (Chapman et al. 2017, 2020). As a bivalve organism, it shows valve opening behavioral rhythms, closely related to physiological processes such as breathing and nutrition, synchronized by environmental factors (García-March et al. 2008; Tran et al. 2011). A recent study has shown that both moonlight and sunlight oscillations below the horizon shape the blue mussel's valve behavior during the high Arctic polar night (Tran et al. 2023). However, how do mussels control their valve behavior during the high Arctic polar day remains unknown.

The objective of this work was to better understand the ability of *Mytilus sp*. to maintain, or not, functional biological rhythms in polar regions, with a focus on polar day period when the sun is permanently above the horizon. First, we investigated the pattern of valve behavior according to seasons and then focusing on polar day. Then, we investigated the existence of a valve behavior daily rhythm, as well as the presence of tidal, semilunar, and lunar rhythms of *Mytilus sp.* during polar day. The results of this study provide key knowledge on the adaptation of temperate species that currently colonize polar environments.

Material and methods

Animals, study area and data collection

This study analyzes valve behavior data of 15 *Mytilus sp.* mussels (67.1 ± 2.7 mm shell length) recorded over a 15-month period (from 01/09/2019 to 27/11/2020), with a focus on polar day, from 17 April to 26 August 2020 (132 days). The study was conducted in accordance with local legislation. The mussels were collected manually by a diver from natural recruitment in the Isfjorden, near Longyearbyen (latitude 78°13'N, longitude 15°38'E), Svalbard. They were equipped with electrodes to record their behavior at 10 Hz using a high-frequency non-invasive (HFNI) valvometer biosensor (Payton et al. 2017a). Mussels were placed at the seafloor, always in subtidal conditions, at a depth of 3 m (± tides) in a ballasted cage (50 x 50 x 100 cm) under an old pier in Kongsfjorden near Ny-Ålesund (western coast of Spitsbergen Island, Svalbard; 78°56' N, 11°56' E) the 01/09/2019 (Fig. 1a).

Environmental data acquisition

Water temperature was measured every 10 sec, near the mussels, by our HFNI valvometer biosensor using a temperature sensor (ADT7420 sensor, Analog Devices). The astronomical data related to sun, earth, and moon positions during polar day were retrieved from the site https://www.timeanddate.com for the lunar cycles and https://planetcalc.com for the sun angle elevation. Tidal data measured in Ny-Ålesund (78°55' N, 11°56' E) were obtained from https://www.kartverket.no. Astronomical positions and environmental parameters are available in Supplementary Information S1. The map of the study area (Fig. 1a) was created in RStudio (R Core Team 2013) using the package ggOceanMaps (Vihtakari 2022). All times are expressed in UTC.

Valve behavior

Principle of measure to record mussel valve activity

Two lightweight electrode sensors designed to minimize disturbance to mussel behavior were glued on both shells (Tran et al. 2003; Andrade et al. 2016). These sensors were connected to the valvometer biosensor by flexible wires, which allowed the mussels to move their shells without constraints. The measurement is magnetic principle based. The sensors are made with small self-inductance coils (material: ferrite; size: 3.2 mm x 2.5 mm x 2 mm; weight: ~0.06 g), whose specifications are: inductance: 470 µH; rated current: 45 mA; self-resonance frequency: 5 mHz. Thanks to these sensor specificities, a very low electromagnetic field (~1-2 nT) was generated between the electrodes by the biosensor, allowing to measure valve opening amplitude. The signal was recorded at 10 Hz using custom acquisition cards (Nanog manufacturer, Pessac, France), and the data were automatically transmitted daily to a data processing center at the Arcachon Marine Station (France) using internet network (54 000 data / day / mussel).

Valve behavior quantification

Field valve activity data were analyzed using LabView 8.0 software (National Instruments). The valve behavior endpoints were expressed as the hourly valve opening amplitude (VOA, %) of each individual (individual hourly valve opening amplitude data during polar day 2020 are available in Supplementary Information S2). The hourly VOA was reported as a percentage, with 100 % indicating that the valves were opened at their maximum amplitude during the entire hour, and 0 % indicating that the valves were closed during the entire hour. All times are expressed in UTC.

Temporal data set

Seasonal time cutting

The mean valve opening amplitude (VOA, %) of 15 *Mytilus sp.* was calculated for each polar season (here, the use of "season" term is not in its classical acceptation, but adapted to polar environment) (Fig. 1b): period of diel light dark alternation around autumn equinox (from 01/10/2019 to 24/10/2019 and from 26/08/2020 to 24/10/2020), polar night (when the sun is permanently below the horizon, from 25/10/2019 to 17/02/2020 and from 25/10/2020 to 27/11/2020), period of diel light dark alternation around spring equinox (from 18/02/2020 to 16/04/2020), and polar day (when the sun is permanently above the horizon, from 17/04/2020 to 26/08/2020). Resulting data are available in Supplementary Information S3.

Polar day investigation

The investigation of mussels' behaviour focused on the polar day, when the sun was permanently above the horizon, from 17 April 2020 to 26 August 2020 (132 days), meaning a dataset of 3 168 hourly VOA data per individual (n = 15 mussels, i.e. 47 520 data in total, available in Supplementary Information S2). From hourly VOA, daily VOA were calculated (n = 15 mussels, i.e. 1 980 data).

Time cutting of the polar day

To go deeper in the investigation, the polar day was -divided into 8 parts of 14-16 days, each part corresponding to a neap-spring tidal cycle (Supplementary Information S1).

Different phases of significant biological rhythms tested

When a significant daily, tidal, semi-lunar or lunar rhythm was identified (see "Chronobiological analysis" below), the VOA oscillations during the corresponding geophysical cycles were identified. For individuals with a significant daily rhythm, mean VOA was calculated individually at 4 phases (6 h ranges per phase) of daily cycles (n = 132 cycles): midnight (21h-3h), AM (3h-9h), midday (9h-15h) and PM (15h-21h). For individuals with a significant tidal rhythm, mean VOA was calculated individually at 4 phases (3h ranges per phase) of tidal cycles (n = 255 cycles): ebb tide, flow tide, high tide, and low tide (Supplementary Information S1). For individuals with a significant semi-lunar rhythm, mean VOA was calculated individually according to 4 tidal coefficient categories: highest, intermediate / decreasing, lowest, and intermediate / increasing tidal coefficients (Supplementary Information S1). In individuals with a significant lunar rhythm, mean VOA was calculated individually at 4 phases (7-9 days ranges per phase) of synodic lunar cycles (n = 4 cycles): new moon, first quarter of the moon, full moon and third quarter of the moon

(Supplementary Information S1). All times are expressed in UTC. Resulting data are available in Supplementary Information S3.

Statistical analyses

VOA differences between season

VOA differences between season were tested on mean seasonal individual VOA values (Supplementary Information S3). Significant differences between the rhythms' phases were tested using ANOVA on ranks (because of the non-normality of the data) for repeated measures (Friedman Repeated Measures Analysis of Variance on Ranks), followed by Student-Newman-Keuls method, using Sigma Plot software (Version 13.0; Systat Software, USA).

Random components mixed model for repeated measures

A random components mixed model for repeated measures was applied, knowing that the VOA data were not independent, to test the effects and the interactions of the maximum sun elevation angle (around midday), the water temperature, the direction of sun elevation (i.e. increasing sun max. elevation from the beginning of polar day to the summer solstice; or decreasing sun max. elevation from summer solstice to the end of the polar day) and the lunar phases (new moon / 1st quarter moon / full moon / 3rd quarter moon) on individual daily VOA values, using XLSTAT 2022 software (Addinsoft. New York, USA).

VOA differences between phases of significant biological rhythms

When a significant daily, tidal, semi-lunar or lunar rhythm was identified (see "Chronobiological analysis" below), VOA differences between the corresponding phases were tested on mean individual VOA values, only in mussels with a significant rhythm (Supplementary Information S3). Significant differences between the rhythms' phases were tested using ANOVA on ranks (because of the non-normality of the data) for repeated measures (Friedman Repeated Measures Analysis of Variance on Ranks), followed by Student-Newman-Keuls method, using Sigma Plot software (Version 13.0; Systat Software, USA).

Chronobiological analysis

In order to determine the existence of biological rhythms in mussel's behavior, chronobiological analysis were done on individual hourly VOA data, either on the whole polar day period, or on each of the 8 neap-spring tidal cycles of the polar day. Results are summarized in Supplementary Information S4.

198 **Determination of tested periods**

199 Four biological rhythms were investigated: daily rhythm, tidal rhythm, semi-lunar rhythm, and
200 lunar rhythm. The corresponding geophysical cycles susceptible to synchronize these biological
201 rhythms were (respectively): daily cycle (24h), tidal cycle (12.4h), neap-spring tidal cycles
202 (14.7 d), and synodic moon cycle (29.5 d). Thus, rhythms were searched in the period ranges
203 of: 24 ± 4 h for daily rhythm, 12.4 ± 2 h for tidal rhythm, 14.7 ± 2.5 d for semi-lunar rhythm,
204 and 29.5 ± 5 d for lunar rhythm.

205 **Determination of significant rhythms**

206 Chronobiological analyses were performed using TSA Serial Cosinor 8.0 software. Several
207 steps were required to validate a significant rhythm (Gouthiere et al. 2005; Tran et al. 2011).
208 Four steps must be validated. First, the quality of the data set was assessed by controlling for
209 the absence of randomness using the autocorrelation diagram (Box et al. 2015). Second, the
210 absence of a stationary phenomenon was checked by using a partial autocorrelation function
211 (PACF) calculation (Box et al. 2015). Third, the recorded data were tested for periodicities by
212 the spectral method of the Lomb and Scargle periodogram, which combines the principle of a
213 regression analysis and Fourier transformations (Scargle 1982). This method gives a threshold
214 of probability ($p = 0.95$) defining the limit below which the signal can be regarded as "noise".
215 Fourth, the rhythmicity was validated and modeled with the Cosinor model, which uses a cosine
216 function calculated by regression (Nelson et al. 1979; Bingham et al. 1982). For a given period,
217 the model is written as $Y(t) = A\cos(\pi t/\tau + \phi) + M + \varepsilon(t)$ where Y (t) is an observation of the
218 mean VOA at time t, A is the amplitude, ϕ is the acrophase, τ is the period, M is the mesor and
219 ε is the relative error. Two key tests validated the calculated model and the existence of a
220 rhythm: the elliptic test had to be rejected, and the probability for the null amplitude hypothesis
221 had to be < 0.05. For a set of data, several significant periodicities could occur. To identify
222 significant secondary periodicities, we reinjected the previously calculated residues of the
223 Cosinor model to remove the trend related to the first statistical period and then repeated the
224 entire procedure (1-4 steps). This entire procedure was necessary to validate secondary
225 periodicities. In this study, the procedure was repeated up to four times to reveal significant
226 rhythmicity in the range of the studied rhythms.
227

Results

Seasonal behavior

In high Arctic conditions, in Ny-Ålesund, Svalbard (Fig. 1a), the mean valve opening amplitude (VOA, %) of 15 *Mytilus sp.* was significantly higher during polar day; 74.9 ± 0.7 %, mean ± SE) than during the other periods of the year including the polar night (38.8 ± 1.3 %), and the two periods with diel alternation of light and dark centered either on the autumnal equinox (65.3 ± 1.5 %) or on the spring equinox (30.8 ± 1.6 %) (Fig. 1b).

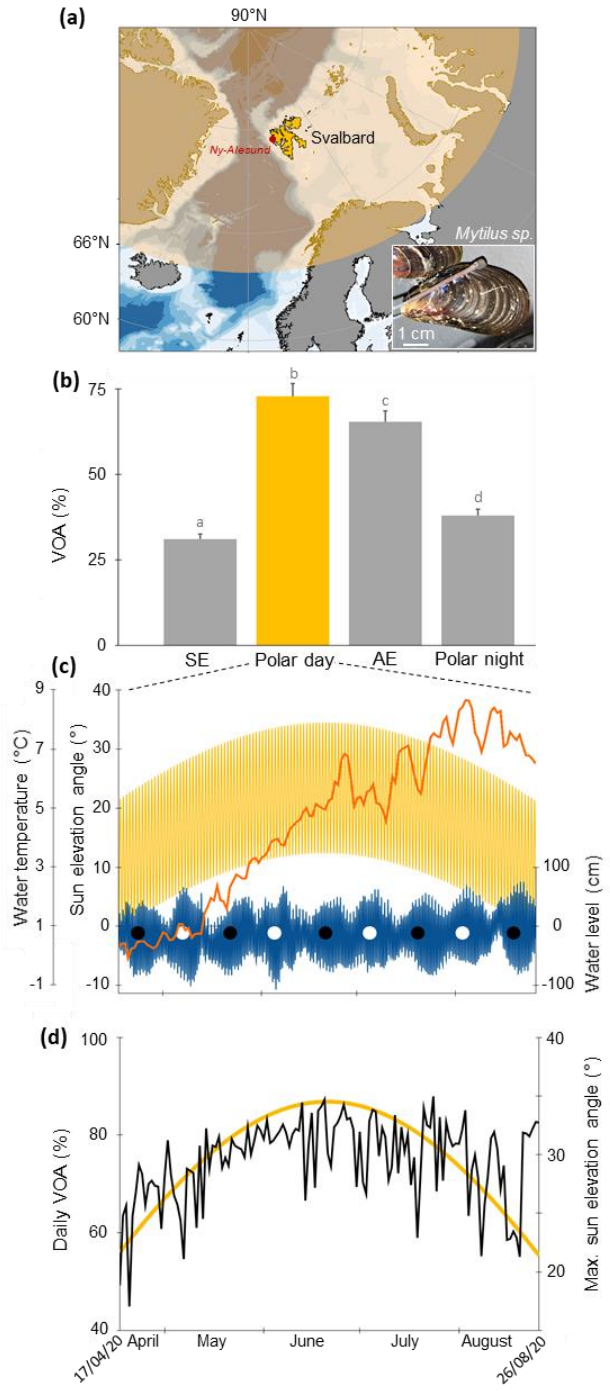


Fig. 1 Context of the study, physical parameters, and mussels valve behavior during polar day. (a) Ny-Ålesund location (78°56′ N, 11°56′ E) and limit of polar day extension (yellow color surface). Insert with one of the 15 *Mytilus sp.* equipped with HFNI valvometer electrodes. (b) Mean hourly valve opening amplitude (VOA, %; mean ± SE; n = 15) during polar day (yellow bar) and other periods of the year, including light / dark alternation periods centered on Spring Equinox (SE) and Autumn Equinox (AE), and polar night. Different letters indicate significant differences ($p < 0.05$). (c) Profiles of physical parameters of the studied site during polar day 2020: hourly sun elevation angle above the horizon (yellow); hourly sea water level (blue) and lunar phases (new moon: black circle; full moon: white circle); daily water temperature (orange). (d) Mean daily VOA (%, n=15) during polar day with the daily maximum sun elevation angle above the horizon in yellow (see Supplementary information S5 for individual variability).

235 **Astronomical positions and environmental parameters during the polar day**

236 The studied polar day comprised 132 days, 255 tidal cycles, 8 neap-spring tidal cycles, and 4
237 entire synodic lunar cycles (Fig. 1c; Supplementary Information S1). At the location site, the
238 sun remained permanently above the horizon but still showed diel altitude cycles (24 h) with a
239 constant delta of 22.2° between daily min and max sun elevation. The diel maximum sun
240 elevation angle (around midday) increased during polar day and reached a maximum at
241 summer solstice (21/06/2020, 34.5°) before decreasing during the second part of polar day .
242 Ny-Ålesund site exhibited semi-diurnal tidal cycles (12.4 h) of ± 30 cm to ± 80 cm depending
243 on neap-spring tidal cycle (14.7 d). Neap-spring tidal cycles are cycles of tidal amplitudes that
244 occur two times per synodic lunar month cycles (29.5 d). Finally, water temperature recorded
245 near the mussels showed a regular increase during polar day.

246 **Behavior during polar day**

247 Fig. 1d showed that the mean daily valve opening amplitude (VOA) during polar day tended
248 to follow the course of the sun elevation in the sky (individual daily VOA were plotted in
249 Supplementary Information S5). In Table 1, individual daily VOA dataset was used. The
250 mixed model showed a significant random effect for "days" and "mussels" individual
251 parameters. Significant effects on VOA of the fixed parameters "daily sun maximum
252 elevation" and "lunar phases" were shown. No significant effects of "water temperature" or
253 "sun direction" parameters were shown. Finally, there was a significant effect of the
254 interaction between "sun maximum elevation" and "lunar phases" parameters on VOA.

255

256 **Table 1** Random components mixed model for repeated measures applied to test the effects of
257 daily sun maximum elevation, water temperature, sun direction (i.e. increasing (before summer
258 solstice) or decreasing (after summer solstice) sun max. elevation) and lunar phases on
259 individual daily VOA. Asterisks indicate significant *p*-value.

Random components mixed model for repeated measures

Covariance parameters	Z		*p*-value
Random effects			
Days	6.470		< 0.0001***
Individuals	2.434		0.007**
Repeated factors	29.815		< 0.0001***
Source of variation (fixed parameters)	NumDF	F	*p*-value
Quantitative parameters			
Daily sun max. elevation	1	5.987	0.016*

Water temperature	1	2.007	0.159
Qualitative parameters			
Sun direction	1	1.085	0.299
Lunar phases	3	2.809	0.042*
Interactions			
Daily sun max. elevation x Temp.	1	1.912	0.169
Daily sun max. elevation x Sun direction	1	1.016	0.315
Daily sun max. elevation x Lunar phases	3	2.784	0.044*
Sun direction x Temperature	1	0.434	0.511
Sun direction x Lunar phases	3	0.035	0.991
Lunar phase x Temperature	3	0.573	0.634

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Behavioral rhythms during polar day

Daily rhythm

Based on the hourly dataset, individuals chronobiological analyzes showed a significant daily VOA rhythm for 93.3 % of mussels, with a mean period length of 24.13 ± 1.06 h (Fig. 2a). In rhythmic individuals, the VOA was the highest around midday and the lowest around midnight, while intermediate at morning and afternoon (Fig. 2b). To investigate if the daily rhythm evolved along polar day, the 132 days were divided in 8 neap-spring tidal cycles, and new chronobiological analyzes were performed on each of these parts (Fig. 2c). No trends appeared, neither in the percentage of rhythmic individual nor in the period lengths of daily rhythms.

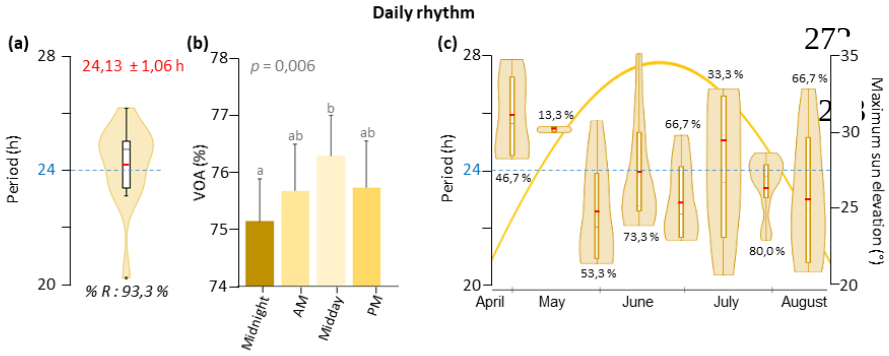


Fig. 2 Chronobiological analysis: daily rhythm of mussels VOA behavior during polar day.

(a) Individual significant circadian period lengths distribution is shown as a violin plot. The mean (± SE) period length is indicated in red. "% R" indicates the percentage of rhythmic mussels. The period length of the corresponding geophysical cycle is indicated in blue. (b) Mean VOA according to daily phases in rhythmic individuals. (c) Daily rhythm progression along polar day (n = 8 parts / polar day), with the daily maximum sun elevation angle in yellow. Identical letters indicate no significant differences (*p*-value = 0.05).

Tidal rhythm

40 % of mussels exhibited a tidal rhythm, with a mean period length of 12.43 ± 0.04 h (Fig. 3a). For these mussels, VOA was the lowest at high and ebb tides, and the highest at low and flow tides (Fig. 3b). No trends of tidal rhythm's modulation along polar day appeared (Fig. 3c).

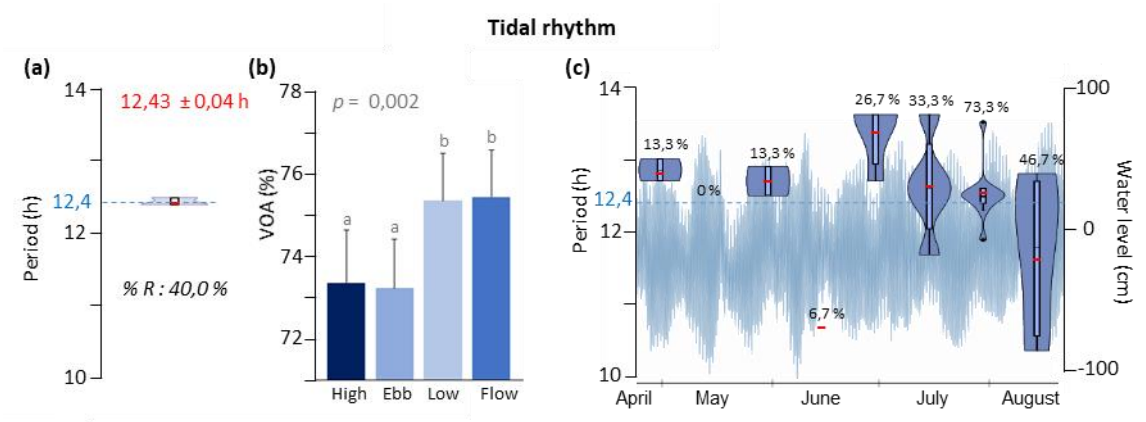
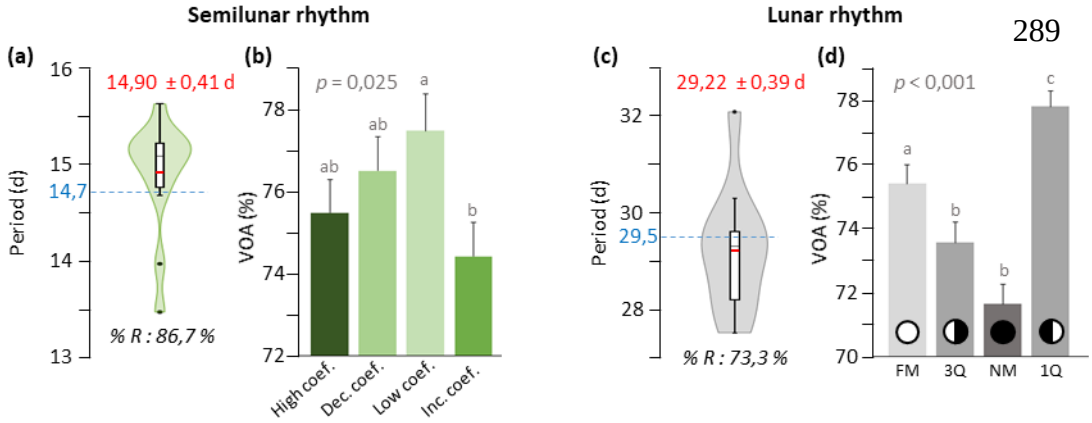


Fig. 3 Chronobiological analysis: tidal rhythm of mussels VOA behavior during polar day.
(a) Individual significant tidal period lengths distribution is shown as a violin plot. The mean (± SE) period length is indicated in red. "% R" indicates the percentage of rhythmic mussels. The period length of the corresponding geophysical cycle is indicated in blue. (b) Mean VOA according to tidal phases in rhythmic individuals. (c) Tidal rhythm progression along polar day (8 parts / polar day) and hourly tidal water level (blue). Identical letters indicate no significant differences (p-value = 0.05).

Semilunar and lunar rhythms

86.7 % of mussels showed a significant semilunar rhythm, with a mean period length of 14.90 ± 0.41 d (Fig. 4a). The highest VOA were observed at low, decreasing, and high tidal coefficients, while the lowest VOA were observed at increasing tidal coefficients (Fig. 4b). Finally, 73.3 % of mussels showed a significant lunar rhythm, with a mean period length of 29.22 ± 0.39 d (Fig. 4c). The mean VOA was the highest at first quarter moon and decreased

287 gradually at full moon before reaching a minimum at third quarter moon and new moon (Fig.
288 4d).



289

Fig. 4 Chronobiological analysis: semilunar and lunar rhythms of mussels VOA behavior during polar day

(a, c) Individual significant semilunar (a) and lunar (c) period lengths distribution are shown as violin plots. The mean (± SE) period length are indicated in red. "% R" indicate the percentage of rhythmic mussels. The period lengths of the corresponding geophysical cycle are indicated in blue. (b) Mean VOA according to tidal coefficients in individuals with a significant semilunar rhythm. (d) Mean VOA according to lunar rhythm phases in individuals with a significant semilunar rhythm. Identical letters indicate no significant differences (p-value = 0.05).

290 ## Discussion

291 Our findings clearly show that although the sun remains permanently above the horizon, the
292 re-emerging polar resident *Mytilus sp.* exhibit multitude behavioral rhythm periodicities
293 during the high Arctic polar day. First, behavioral valve activity of *Mytilus sp.* follows the sun
294 seasonal trajectory in the sky during polar day. Second, mussels exhibit a daily rhythmic
295 component in their behavior. Finally, mussels also show valve activity rhythms related to the
296 tidal, semi-lunar and lunar cycles.

297

298 The marine ecosystem is governed by a multitude of environmental cycles linked to the
299 celestial movements and interactions of earth, moon and sun. These environmental cycles
300 shape the biology of marine species, that exhibit a variety of biological rhythms already
301 widely documented (Häfker and Tessmar-Raible 2020; Andreatta and Tessmar-Raible 2020;
302 Kaiser and Neumann), from daily and tidal rhythms, to semi-lunar, lunar and seasonal
303 rhythms. Corresponding endogenous clock(s), synchronized by the corresponding predictable
304 and reliable abiotic cues, exist for most of these biological rhythms (Tessmar- Raible et al.
305 2011; Häfker and Tessmar-Raible 2020). Indeed, for all living organisms, an internal tracking

of time offers major advantages compared with solely being able to directly respond to environmental changes (Helm et al. 2017). Intrinsically, organisms benefit from endogenous clock(s) by maintaining a temporal organization of different cellular and physiological processes so that they occur, or not, at the same time. Relatively to their environment, organisms benefit from internal clocks for anticipation of periodic environmental changes (e.g. light availability, temperature…) and synchronization of physiological processes accordingly. Ecologically, internal clocks are crucial for synchronizing biological processes at the population level (e.g. population synchronization of external spawning events, such as observed in oysters (Bernard et al. 2016)), as well as the inter-specific interactions level (e.g. the diel vertical migration of zooplankton, that would be a trade-off between predator avoidance and food supply (Last et al. 2016)). Finally, the biochemistry of a biotope is shaped by the temporal organization of biological processes such as respiration, feeding and excretion of organisms leaving there (Helm et al. 2017).

The influence of the extreme Arctic light regime on bivalves species has already been observed at the annual scale (Ballesta-Artero et al. 2017; Tran et al. 2020b). A previous study on *Mytilus sp.* in the high Arctic showed annual rhythms of its valve behavior and shell growth, with a positive correlation of both parameters with photoperiod (Tran et al. 2020b). The same pattern is observed in our study, with a maximum valve opening amplitude during polar day. Furthermore, our results go further by showing that the annual trajectory of the sun continues to shape mussels valve behavior during polar day, with a positive correlation between valve amplitude and sun elevation angle above the horizon, while no significant effect of water temperature has been observed.

Daily rhythms in bivalve species have been described in temperate species, including *Mytilus sp.*, in both field studies and lab experiments, associated with a functional endogenous circadian clock machinery (Ameyaw-Akumfi and Naylor 1987; García-March et al. 2008; Mat et al. 2012; Payton et al. 2017b; Comeau et al. 2018; Chapman et al. 2020). A recent study has shown the persistence of *Mytilus sp.* daily behavioral rhythms during the high Arctic polar night, synchronized by both lunar day moonlight oscillations, and daily sunlight oscillations below the horizon (Tran et al. 2023). Here, we show for the first time the persistence of daily behavioral rhythm during polar day in a benthic species, *Mytilus sp.*, with a maximum valve amplitude around midday, despite the fact that the sun is always above the horizon, but corresponding to the higher light intensity during the day. These results reinforce previous finding of persistent daily rhythms during Arctic polar day in terrestrial vertebrates and insects (Krüll 1976; Nordtug and Mela 1988; Stelzer and Chittka 2010; Williams et al.

2015; Arnold et al. 2018). Similar observations have been made in pelagic zooplankton species, coinciding with persistent oscillations of the circadian clock gene expression in the copepod *Calanus finmarchicus* (Dale and Kaartvedt 2000; Fortier et al. 2001; Hüppe et al. 2020; Payton et al. 2021). A functional clockwork has also been associated with a persistent rhythmic behavior in the Arctic scallop *Chlamys islandica* during polar night, when the sun is always below the horizon (Tran et al. 2016; Perrigault et al. 2020). Our results suggest that the circadian clock would stay functional in bivalves such as *Mytilus sp.* during the Artic polar day, although a direct response to light cannot be excluded. Indeed, despite a weak synchronization, illustrated by individual daily periods not exactly at 24 h but rather between 20 and 25 h, the common pattern at the group level with a maximal VOA around midday highly suggest that mussels are still able to detect and track sun diel changes during polar day. At least two hypotheses could be suggested. First, *Mytilus sp.* would be able to be synchronized by very weak changes of high sun intensity caused by low diel variations of sun angle in the sky constantly above the horizon. Secondly, *Mytilus sp.* would be able to be synchronized by subtle diel changes in light spectral composition. Indeed, even if the sun is always above the horizon, the light spectrum is redshifted when the sun is closer to the horizon, around midnight, while around midday, light spectrum is blueshifted (Nordtug and Mela 1988). Interestingly, while diel changes of spectral composition are presumably less intense in the middle of polar day, when the sun is permanently far away from the horizon, no clear changes of daily rhythms prevalence occur along polar day. These two hypotheses suggest that *Mytilus sp.* possesses very sensitive molecular light sensors in its tissues, able to detect weak changes in light intensity and/or quality, even at high intensity of sun illumination. These results highlight the need to investigate opsins and cryptochrome families in bivalves species, proteins known to be involved in light reception and clock synchronization, as well as to characterize physically diel light changes in intensity and quality during polar day in the Arctic (Oliveri et al. 2014).

Besides the daily rhythm, we show in our study that other cycles, related to the moon, also shape mussels' behavior in the high Arctic polar day. A very clear tidal rhythm exists but is less commonly expressed than the daily one, probably due to the low tidal amplitude at the study site (< 1 m during polar day). The plasticity between tidal and daily rhythms, with a balance between one or the other relevant to each location, have already been described in bivalve and crustacean species (Enright 1976; García-March et al. 2008; Tran et al. 2020a; Hüppe et al. 2020; Payton et al. 2021). Additionally, as already shown in other bivalve species in temperate areas, mussels also exhibit a semi-lunar rhythm, known to be entrained by neap-

spring tidal cycles that modulates the amplitude of the tidal cycle (García-March et al. 2008; Tran et al. 2011; Payton et al. 2017a; Payton and Tran 2019). Finally, we show that mussels exhibit a lunar rhythm of valve behavior. That result corroborates the recent study showing that *Mytilus sp.* expresses lunar behavioral rhythm at the same location during polar night (Tran et al. 2023). However, if the moonlight entrainment during polar night is intuitively clear, how the mussels are entrained by the lunar cycle in a context of permanent illumination is an open question. This finding could be a strong argument to argue that *Mytilus sp.* possesses an endogenous lunar clock that maintains a strong circalunar rhythm, even in absence of moonlight entrainment, during polar day. Another suggestion would be that other cues than lunar illumination, such as magnetic field, tidal vibrations could synchronize animals with the lunar cycle (Andreatta and Tessmar-Raible 2020). For example, the beat hypothesis developed by Erwin Bünning says that a combination of the circadian clock and the circatidal/circalunidian clock would be able to produce a circa(semi)lunar rhythm (Kaiser and Neumann). Both hypotheses highlight the need to decipher the complexity of lunar rhythms.

All in all, the eyeless bivalve and reemerging polar resident *Mytilus sp.* appears to exhibit multitude behavioral rhythm periodicities despite the extreme photic conditions of Arctic polar day. Interestingly, these valve behavior rhythms are observable despite the overall high valve amplitude opening of mussels during Arctic polar day. Thus, the difference in mean VOA for defined phases of daily, tidal, semilunar and lunar rhythms is narrow, suggesting that small changes of VOA may have a biological implication. Marine bivalves are an essential component of the benthic community. As filter-feeders, they feed on phytoplankton and bacterial communities. The valve behavior rhythms observed in our study are highly probably related to nutrition and respiration rhythms. The multitude of periodicities, synchronized to both sun and moon-related cycles, may confer internal and/or external advantages for mussels, in terms of activity, feeding, predation or UV protection, that remain to be understood. By cascading effects, mussels' biological rhythms may shape species communities and biogeochemical cycles in their close environment, with consequences on the Arctic ecosystems functioning. As a matter of fact, *Mytilus* sp. appears as a very tolerant and adaptive species. Its ability to deal with drastic light regimes may confer internal and external advantages, explaining their northward shift in distribution and new resettlement in high Arctic.

Supplementary Information The online version contains supplementary material available at xxxxxxxxxxxxxxxxxxxxx

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Conflict of interest We have no competing interests.

Ethical approval All experiments complied with the laws in effect in Svalbard and they conformed to international ethical standards.

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