Supplementary Information

Bivalve mollusk circadian clock genes can run at tidal frequency.

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Journal : Proceedings of the Royal Society B: Biological Sciences

DOI: 10.1098/rspb.2016.0049

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The following items are provided:

Methods S1: Supplementary method information

Experimental model and subject details

All animal studies were conducted in accordance with local legislation. All investigations were performed on Pacific oysters, *C. gigas*, of comparable age (1.5 years old, 65-75 mm shell length). All oysters were from natural recruitment in Arcachon Bay, in southwest France, and cultivated in the same area by the oyster farm "Port du Rocher" (La Teste de Buch, Arcachon Bay, France). Before each experiment, oysters were translocated and kept either at the study site (Eyrac pier, Arcachon Bay, France, latitude 44.66°, longitude -1.16°) or at the Marine station of Arcachon (Arcachon Bay, France) with a continuous flow of natural sea water from the bay and a natural photoperiod.

Site characteristics of the field study

Field experiments were performed at Eyrac pier, located in Arcachon Bay, on the western coast of France (latitude 44.66°, longitude -1.16°). The site is connected to the open Atlantic Ocean and exposed to semidiurnal tide regimes, i.e., 2 tides per day, with a maximum amplitude of ± 2.4 m and a tidal flow at midtide reaching 0.6 $m.s^{-1}$ during spring tides [1]. Oysters were placed at a depth of 4 m (± tides), i.e. always in subtidal conditions, with at least 1.6 m of seawater above them. Animals were in close proximity to wild oysters. Temperature (°C) and light intensity in Lux (n.b. the conversion to light irradiance in µmol m^{-2} s^{-1} was not possible) were measured all along the field study every 10 minutes by a data logger (HOBO Pendant® Temperature/Light Data Logger, Onset Computer Corporation, Bourne, MA, USA) fixed to the bag with oysters

equipped for valve activity study and were provided figure S1. Variations in water height at Eyrac pier were provided by the French Marine Service of Oceanography and Hydrography (SHOM, www.shom.fr). The astronomical data related to sun, earth, and moon positions were retrieved from the website www.timeanddate.com and are available table S1.

Valve activity behavior in the field study

For the valve activity behavior in the field experiment, 15 oysters were placed in a bag fixed on a permanently immersed oyster table on March 5, 2018. The valve activity behavior of these oysters was recorded for 18 days (March 5 to 23, 2018, 00 corresponds to 17 h, local time, UTC +1) using a high-frequency noninvasive (HFNI) valvometer field technology [2]. Briefly, a pair of lightweight electrodes designed to minimize disturbance to bivalve behavior were glued on each half shell. These electrodes were connected to the valvometer by flexible wires, which allowed the oysters to move their valves without constraints. Between the electrodes, an electromagnetic current was generated, which allowed measurements of the amount of valve opening and closing. The signal was recorded using a custom acquisition card every 0.1 sec, and the data were automatically transmitted daily to a data processing center at the Arcachon Marine Biological Station (France) using cellular and Internet networks.

Sampling for gene expression in the field study

For the gene-expression field experiment, 112 oysters were placed in the study site on March 5, 2018, in 16 oysters bags (7 oysters per bag). Gill tissues were sampled from 7 oysters every 3.1 h from 14 March 2018 at 18 h 48 to 16 March 2018 at 17 h 18 (local time, UTC +1), i.e., 16 sampling

times for 46.5 h. The experimental sampling details are in Table S1. The oysters were quickly dissected (2-3 min) in a shelter on the Eyrac pier under dim red light during the night. Gill tissues were immediately and individually transferred into 700 µL of RNA lysis buffer (Kit SV Total RNA Isolation System, Promega) with 0.5 g of ceramic beads and homogenized before storage at -80°C.

Experiments in controlled environments

Experiments (VOA free-run experiment, DD and LD gene expression experiments [3,4]) were performed at the Marine Station of Arcachon (France) in an isolated room. Oysters were placed in tanks isolated from external vibration using an antivibrating bench and continuously supplied with homogenized seawater of constant composition (oxygen, pH and salinity) and at a constant temperature. To prevent from any putative tidal chemosensory cues in the seawater supply, this natural seawater was, first, stored and mixed, in one time, in a 50 m^3 tank. Then, this water was stored in transient tank in the experimental room. In this 150-L tank, the seawater is homogenized with pumps and air-bubbling to break any putative cycles of environmental cues, related to tides for example. The light-dark (LD) regime was rectangular. Photosynthetically active radiation (PAR) during the photophase was produced by neon lights (MASTER TL-DXtra 36 W/865 1SL, Philips) with an irradiance of 19 µmol m^{-2} s^{-1} at the air-water interface (Biospherical Instruments Inc., San Diego, CA, USA), corresponding to a typical overcast day at midday. Fixed average temperature and LD-cycles were defined according to field conditions at the time of the experiments. The sampling of oysters was performed under dim red light during dark phases.

Valve activity behavior record in the laboratory experiment

For the free-running (FR) behavior laboratory experiment, 14 oysters were pre-equipped with lightweight electrodes and placed in the study site (subtidal conditions, Eyrac pier, Arcachon Bay, France) on February 20, 2014 for 3.5 weeks. On March 17, 2014, oysters were placed in the laboratory, divided into 2 tanks under constant darkness (dark-dark, DD), without a food supply. The temperature was fixed at 13 ± 0.5°C. The electrodes were welded for the study of valve behavior with HFNI valvometer laboratory technology over 7 days, from March 18 to March 24, 2014.

Sampling for gene expression in laboratory experiments

The experimental protocols for the FR experiment (DD) and the experiment under LD entrainment are described in [3] and [4], respectively, from where raw data were extracted and used for the RAIN analysis. Briefly, in DD, oysters were exposed to LD entrainment (L:D 10:14 cycle) and fixed temperature at 17.6 ± 0.1°C for 15 days, followed by constant darkness for 15 other days. A total of 8 gill tissue samplings were performed in DD at CT 1, CT5, CT 9, CT 11, CT 15 and CT 23 on day 14 and finally at CT 1 on day 15. In LD experiment, oysters were exposed to LD entrainment (L:D 9:15 cycle, close to the natural photoperiod at that time) and fixed temperature at 15 ± 0.2°C for 13 days. A total of 7 gill tissue samplings were performed every 4 h: ZT 0.5, ZT 4.5, ZT 8.5, ZT 12.5, ZT 16.5 and ZT 20.5 on day 12 and ZT 0.5 on day 13.

Quantification and statistical analysis

Valve behavior quantification

Field and laboratory valve behavior data were manually analyzed with LabView 8.0 software (National Instruments). Individual valve opening amplitudes (VOA) of each oyster were reported each day as a percentage, with 100 % indicating that the valves were opened at their maximum amplitude and 0 % indicating that the valves were closed. Then, the VOA % was reported individually on an hourly basis.

Valve behavior statistical analysis

Chronobiological analyses were performed using TSA Serial Cosinor 6.3 software (Expert Soft Tech). Several steps were required to validate a significant rhythm [1,5]. Four steps must be validated. First, the quality of the data set was assessed by controlling for the absence of randomness using the autocorrelation diagram [6]. Second, the absence of a stationary phenomenon was checked by using a partial autocorrelation function (PACF) calculation [6]. Third, the recorded data were tested for periodicities by the spectral method of the Lomb and Scargle periodogram, which combines the principle of a regression analysis and Fourier transformations [7]. This method gives a threshold of probability ($p = 0.95$) defining the limit below which the signal can be regarded as "noise". Fourth, the rhythmicity was validated and modeled with the cosinor model, which uses a cosine function calculated by regression [8,9]. For a given period, the model is written as $Y(t) = A\cos(\pi t/\tau + \phi) + M + \varepsilon(t)$ where $Y(t)$ is an observation of the mean VOA at time t, A is the amplitude, ϕ is the acrophase, τ is the period, M is the mesor and ε is the relative error. Two key tests validated the calculated model and the existence of a rhythm: the elliptic test had to be rejected, and the probability for the null amplitude hypothesis had to be < 0.05. Then, to account for multiple testing of behavioral expressions, Benjamini-Hochberg adjusted *p*-values < 0.05 were considered significant [10]. A chronobiometric parameter was calculated; the percent rhythm (PR,

%) is the percentage of cyclic behavior explained by the model. For a set of data, several significant periodicities could occur. To identify significant secondary periodicities, we reinjected the previously calculated residues of the Cosinor model to remove the trend related to the first statistical period and then repeated the entire procedure (1-4 steps). This entire procedure was necessary to validate secondary periodicities.

Gene expression quantification

For the field study, total RNA from the gills was extracted from samples using SV Total RNA Isolation System kits (Promega). Total RNA quantity and quality were assessed by spectrophotometry and reverse transcribed using GoScript^{TM} Reverse Transcription System kits (Promega). Real-time qPCR reactions were performed on individual samples using the GoTaq^{R} qPCR Master Mix kit (Promega). For experiments in controlled environments (DD and LD), methods for total RNA extraction from the gills and gene expression quantification are explained in [3] and [4], respectively.

Primer sets of circadian clock genes, clock-associated genes and housekeeping genes applied in this study are listed Table S3. Elongation factor 1 was selected as the housekeeping gene using geNorm, BestKeeper and Normfinder [11]. The comparative Ct method $2^{-\Delta\Delta Ct}$ method [12] was used to determine the relative transcript levels of candidate genes, where ΔCt = Ct (gene) – Ct (housekeeping gene). The data were then normalized by the lowest expression value of each gene.

Gene expression statistical analysis

Datasets of gene expressions were investigated for tidal and circadian periodicities in R [13] (32-bit, version 3.2.2) using the RAIN package [10]. The RAIN algorithm is a robust nonparametric method for the detection of rhythms of specified periods in biological data that can detect arbitrary wave forms. Each time series (field study, LD laboratory experiment, and DD laboratory experiment) was analyzed separately as one dataset. The field study and LD laboratory experiment time series were analyzed as regular time series, while the DD laboratory experiment was analyzed as an irregular time series. All the datasets were treated with an independent method as time points were sampled independently from different biological specimens. Different peak shapes were tested for each dataset, and the most significant model was selected for each gene. Circatidal/tidal periodicities were defined by a significant period range of 12.4 ± 3.1 h, and circadian/daily periodicities were defined by a significant period range of 24 ± 4 h. To account for multiple testing of genes (a total of 56 tests of gene expression), Benjamini-Hochberg adjusted *p*-values < 0.05 were considered significant [10].

CODE AVAILABILITY

For the RAIN rhythm analysis script, the algorithm could be find using the RAIN package [10].

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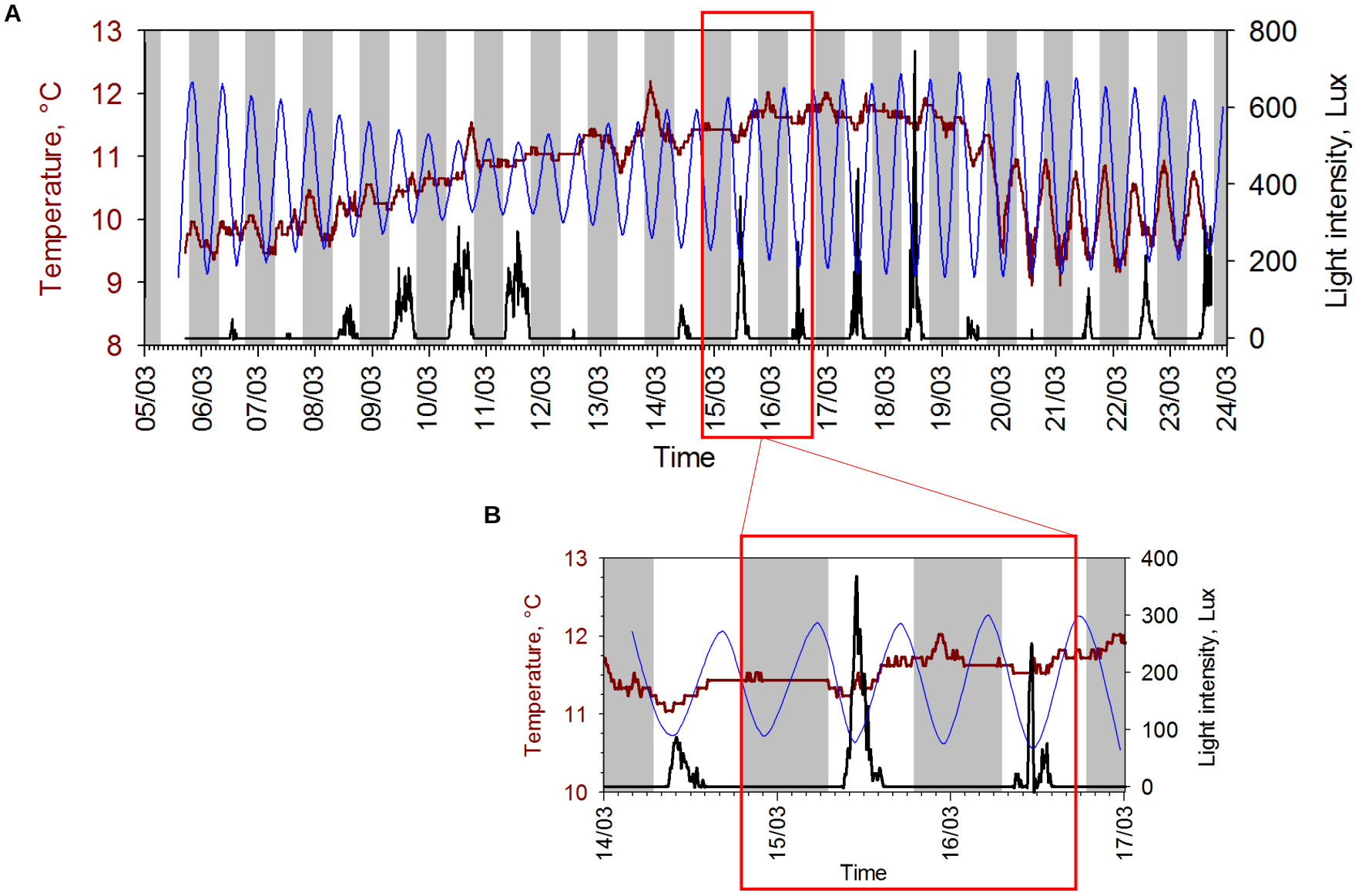
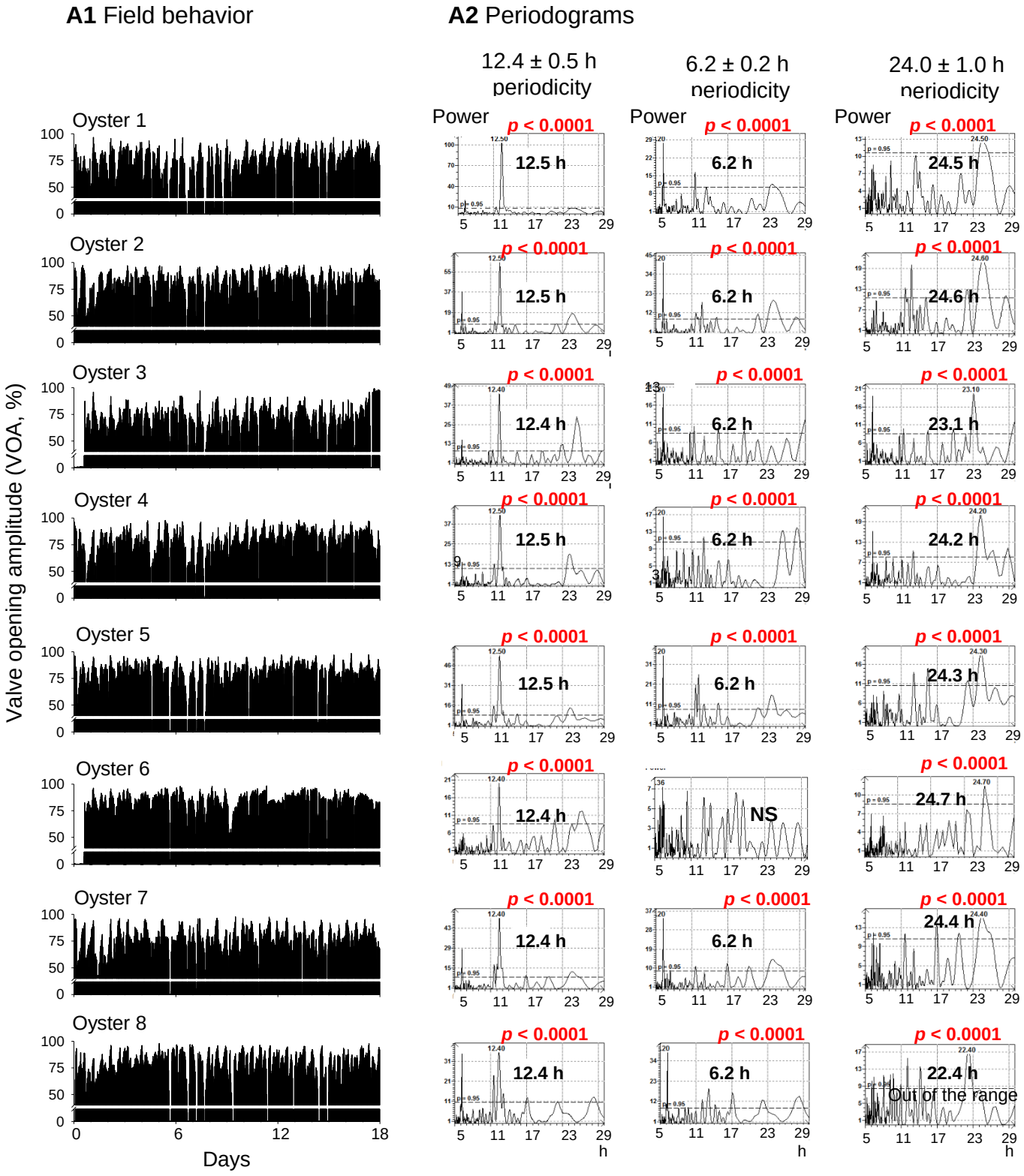


Figure S1. Temperature and light intensity during the field experiment at 4 m depths (± tides) (Eyrac, Arcachon Bay, France, 44,66°N). Related to Figure 2 and Figure 3**.** Temperature (°C, dark red line) and light intensity (Lux, dark line) were measured every 10 minutes by a data logger (HOBO Pendant® Temperature/Light Data Logger) fixed to the bag with oysters. Gray areas indicate night phases and blue line indicate the tide wave. (A) Data over the 18 days of behavioral analysis (March 5, 2018, 17 h to March 23, 2018, 17h (local time, UTC +1)). (B) Focus on the days of sampling for molecular analysis. The red box indicates the exact time of molecular sampling (from March 14, 2018, at 18 h 48 to March 16, 2018, at 17 h 18 (local time, UTC +1)). Each date is indicated at midnight (local time UTC +1).


A1 Field behavior
A2 Periodograms
12.4 ± 0.5 h periodicity
6.2 ± 0.2 h periodicity
24.0 ± 1.0 h periodicity
Power
Oyster 1
Oyster 2
Oyster 3
Oyster 4
Oyster 5
Oyster 6
Oyster 7
Oyster 8
Valve opening amplitude (VOA, %)
Days
p < 0.0001
12.5 h
6.2 h
24.5 h
12.5 h
6.2 h
24.6 h
12.4 h
6.2 h
23.1 h
12.5 h
6.2 h
24.2 h
12.5 h
6.2 h
24.3 h
12.4 h
NS
24.7 h
12.4 h
6.2 h
24.4 h
12.4 h
6.2 h
22.4 h
Out of the range
h

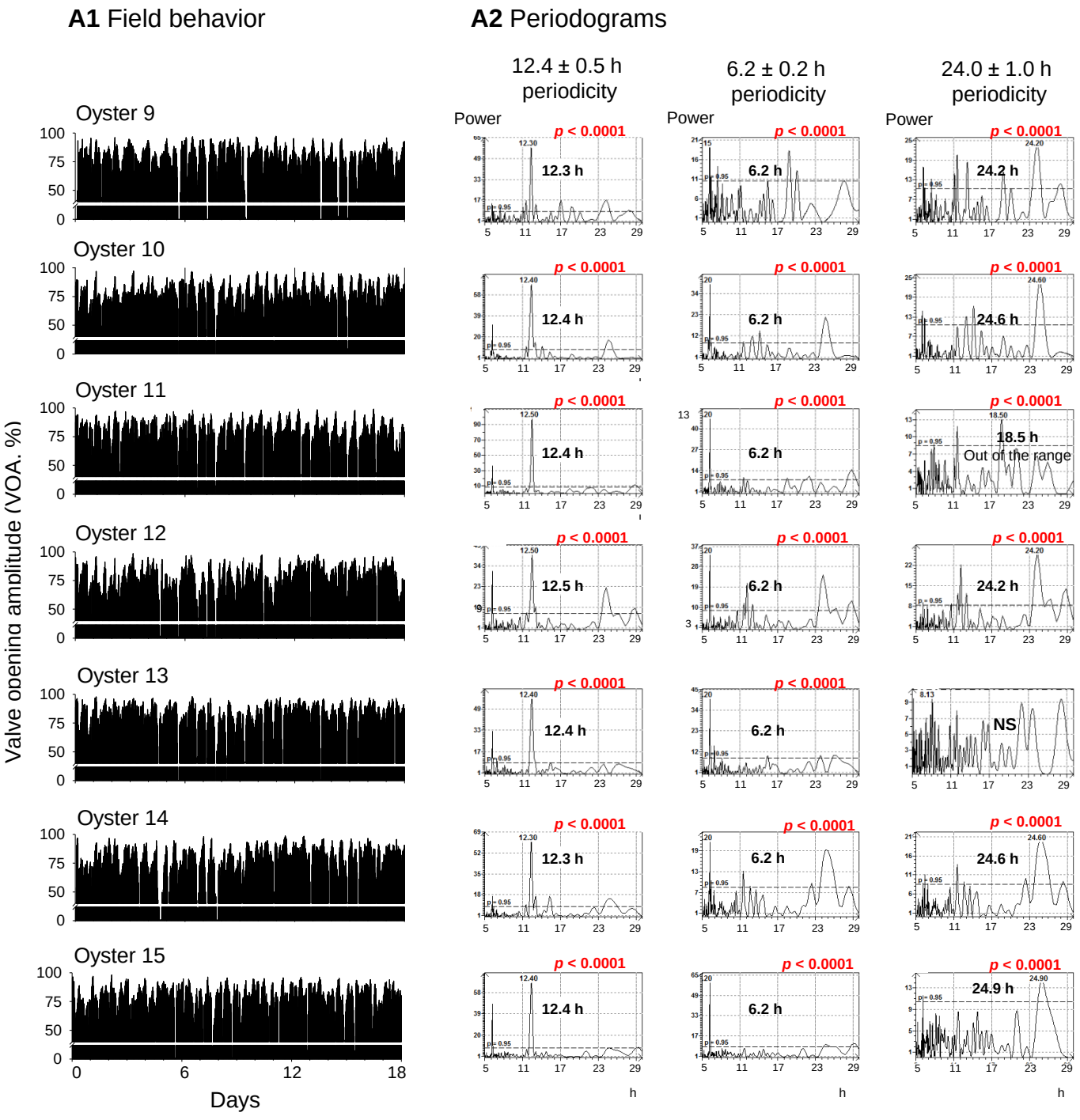
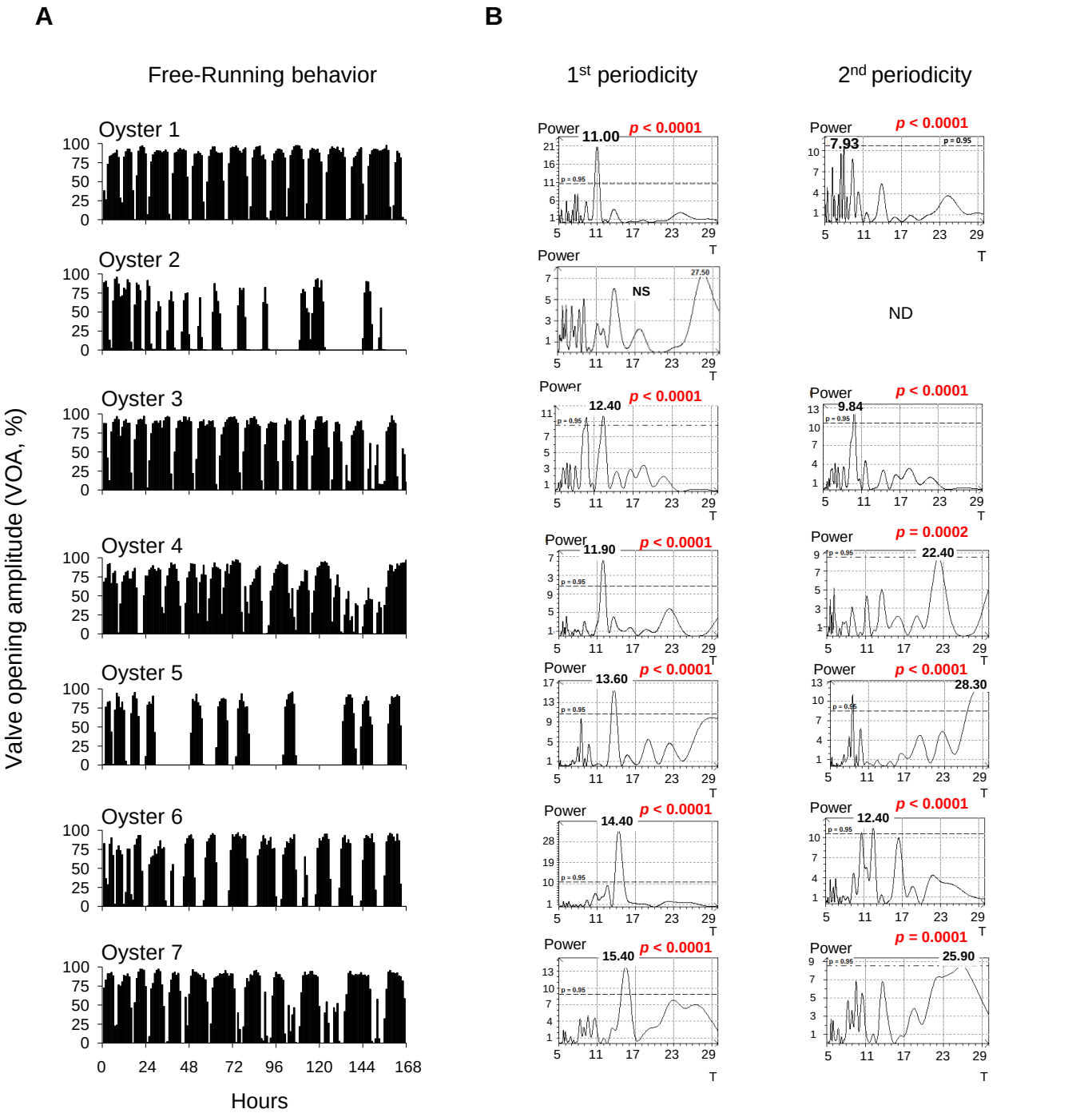


Figure S2. Individuals valve behavior in the field. Related to Figure 2B. (A1) Individuals hourly valve opening amplitudes (VOA, %) during the 18 days of field experiment (05/03/2018 – 23/03/2018, Eyrac, Arcachon Bay, France at 44.66°N). (A2) Individuals chronobiological analyses: search of tidal (12.4 ± 0.5 h and 6.2 ± 0.2 h) and daily (24 ± 1 h) periods determined by spectral analysis (Lomb and Scargle periodogram; dotted line for *p*-value > 0.95) and adjusted *p*-value (in red) of the associated Cosinor model. 2nd significant periodicities were found after residues injection. NS means "non significant".


A
B
Free-Running behavior
1st periodicity
2nd periodicity
Valve opening amplitude (VOA, %)
Oyster 1
Oyster 2
Oyster 3
Oyster 4
Oyster 5
Oyster 6
Oyster 7
Hours
Power
11.00
p < 0.0001
7.93
p < 0.0001
NS
ND
12.40
p < 0.0001
9.84
p < 0.0001
11.90
p < 0.0001
22.40
p = 0.0002
13.60
p < 0.0001
28.30
p < 0.0001
14.40
p < 0.0001
12.40
p < 0.0001
15.40
p < 0.0001
25.90
p = 0.0001
T

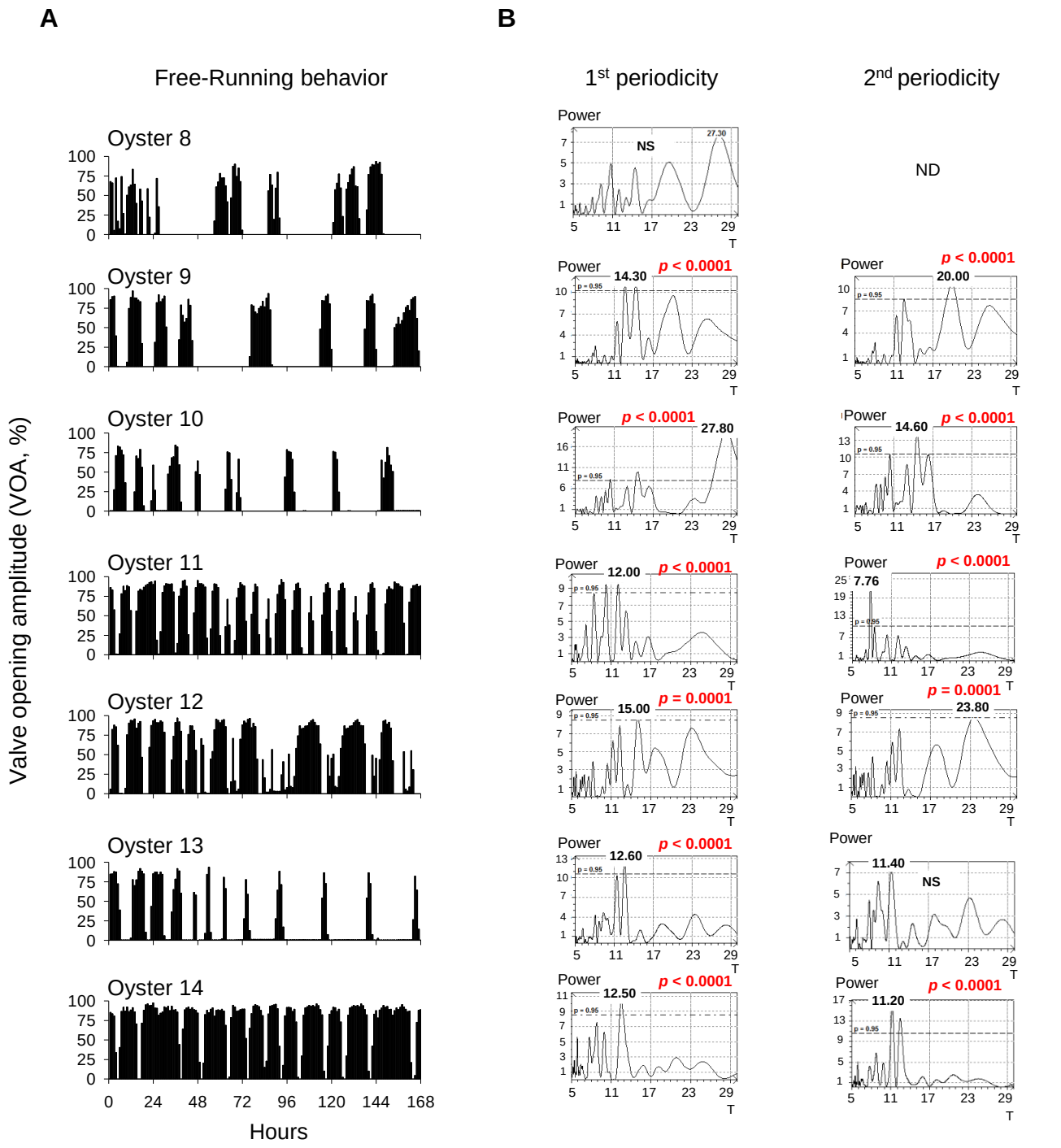


Figure S3. Individual valve behavior under constant darkness in laboratory. Related to Figure 2C. (A) Individual hourly valve opening amplitudes (VOA, %) during the 7 days of free-running experiment under constant darkness (DD, no food supply, 13°C, 18/03/2014, 00h00 – 24/03/2014, 00h00) after 3.5 weeks on the field (20/02/2014 – 17/03/2014, Eyrac, Arcachon Bay, France at 44.66°N). (B) Individual chronobiological analyses: search of circatidal (12.4 ± 3.1 h) and circadian (24 ± 4 h) periods determined by spectral analysis (Lomb and Scargle periodogram; dotted line for *p*-value > 0.95) and adjusted *p*-value (in red) of the associated Cosinor model. 2nd periodicities correspond to the second significant periodicities after residue injection. NS means "non significant", ND means "not determined" because of no first significant period found.

Table S1A. Experimental supplementary information's of behavorial and molecular analyses in the field (Eyrac, Arcachon, FRANCE, lat. 44.66°N) in March 2018. Hours are expressed in local time (UTC +1). Related to Figure 2; Figure 3. Moon, Sun and water temperature data.

Date	Moon phases	Moonrise	Moonset	Moonrise	Meridian passing	Angle of the moon at the meridian	Moon illumination	Sunrise	Sunset	Daylength, h	Solar noon, h and angle	Water temperature (°C)
05/03/2018		--	09 h 37	23 h 00	03 h 50	(39,8°)	88,4%	07 h 35	18 h 57	11:21:58	13 h 16 (39,4°)	9.7
06/03/2018		--	10 h 06	--	04 h 37	(35,4°)	80,6%	07 h 33	18 h 58	11:25:04	13 h 15 (39,8°)	9.8
07/03/2018		00 h 04	10 h 38	--	05 h 24	(31,6°)	71,7%	07 h 31	19 h 00	11:28:09	13 h 15 (40,2°)	9.9
08/03/2018		01 h 06	11 h 12	--	06 h 11	(28,5°)	62,1%	07 h 30	19 h 01	11:31:15	13 h 15 (40,6°)	10.1
09/03/2018	Third Quarter	02 h 04	11 h 49	--	06 h 58	(26,3°)	52,3%	07 h 28	19 h 02	11:34:22	13 h 15 (41,0°)	10.4
10/03/2018		02 h 59	12 h 31	--	07 h 46	(24,9°)	42,5%	07 h 26	19 h 04	11:37:28	13 h 14 (41,3°)	10.8
11/03/2018		03 h 50	13 h 18	--	08 h 34	(24,4°)	33,0%	07 h 24	19 h 05	11:40:35	13 h 14 (41,7°)	11.0
12/03/2018		04 h 37	14 h 09	--	09 h 22	(25,0°)	24,2%	07 h 22	19 h 06	11:43:42	13 h 14 (42,1°)	11.2
13/03/2018		05 h 18	15 h 05	--	10 h 10	(26,4°)	16,3%	07 h 21	19 h 07	11:46:49	13 h 14 (42,5°)	11.4
14/03/2018		05 h 55	16 h 04	--	10 h 57	(28,8°)	9,7%	07 h 19	19 h 09	11:49:56	13 h 13 (42,9°)	11.4
15/03/2018		06 h 29	17 h 05	--	11 h 44	(31,9°)	4,5%	07 h 17	19 h 10	11:53:03	13 h 13 (43,3°)	11.6
16/03/2018		07 h 00	18 h 08	--	12 h 30	(35,7°)	1,3%	07 h 15	19 h 11	11:56:10	13 h 13 (43,7°)	11.7
17/03/2018	New Moon	07 h 29	19 h 13	--	13 h 16	(40,0°)	0,1%	07 h 13	19 h 13	11:59:18	13 h 13 (44,1°)	11.7
18/03/2018		07 h 57	20 h 19	--	14 h 03	(44,7°)	1,2%	07 h 11	19 h 14	12:02:25	13 h 12 (44,5°)	11.7
19/03/2018		08 h 25	21 h 26	--	14 h 51	(49,4°)	4,8%	07 h 10	19 h 15	12:05:33	13 h 12 (44,9°)	11.3
20/03/2018		08 h 55	22 h 35	--	15 h 40	(54,0°)	10,7%	07 h 08	19 h 16	12:08:40	13 h 12 (45,3°)	10.2
21/03/2018		09 h 28	23 h 44	--	16 h 31	(58,2°)	18,7%	07 h 06	19 h 18	12:11:47	13 h 11 (45,7°)	10.0
22/03/2018		10 h 05	--	--	17 h 25	(61,6°)	28,5%	07 h 04	19 h 19	12:14:54	13 h 11 (46,1°)	10.0
23/03/2018		--	00 h 53	10 h 48	18 h 21	(64,0°)	39,6%	07 h 02	19 h 20	12:18:02	13 h 11 (46,5°)	10.1
24/03/2018	First Quarter	--	02 h 00	11 h 38	19 h 19	(65,1°)	51,4%	07 h 00	19 h 21	12:21:09	13 h 10 (46,9°)	10.3

Table S1B. Experimental supplementary information's of behavioral and molecular analyses in the field (Eyrac, Arcachon, FRANCE, lat. 44.66°N) in March 2018. Hours are expressed in local time (UTC +1). Related to Figure 2; Figure 3. Tides data.

Date	Hours	Sea level, m	Tide coefficients
05/03/2018	01h32	0.29	
	07h43	4.45	100
	13h53	0.4	
	20h00	4.2	94
06/03/2018	02h05	0.46	
	08h18	4.2	88
	14h25	0.63	
	20h34	3.97	81
07/03/2018	02h38	0.69	
	08h52	3.9	74
	14h57	0.9	
	21h10	3.71	66
08/03/2018	03h13	0.96	
	09h33	3.59	58
	15h33	1.19	
	21h55	3.45	51
09/03/2018	03h53	1.25	
	10h26	3.29	44
	16h17	1.47	
	22h58	3.22	38
10/03/2018	04h48	1.52	
	11h38	3.09	33
	17h31	1.68	
11/03/2018	00h16	3.13	30
	06h25	1.63	
	13h03	3.06	30
	19h12	1.64	
12/03/2018	01h39	3.21	33
	07h48	1.47	
	14h27	3.21	37
	20h18	1.42	
13/03/2018	02h48	3.43	43
	08h46	1.22	
	15h20	3.45	49
	21h09	1.17	
14/03/2018	03h35	3.69	55
	09h33	0.98	
	16h01	3.7	62
	21h54	0.93	
15/03/2018	04h15	3.93	68
	10h16	0.77	
	16h37	3.91	74
	22h36	0.73	
16/03/2018	04h52	4.13	79
	10h57	0.6	
	17h13	4.09	84
	23h15	0.57	
17/03/2018	05h28	4.29	88
	11h37	0.48	
	17h47	4.21	92
	23h54	0.46	
18/03/2018	06h03	4.39	94
	12h14	0.41	
	18h21	4.28	96
19/03/2018	00h31	0.4	
	06h37	4.43	98
	12h50	0.4	
	18h53	4.3	98
20/03/2018	01h05	0.41	
	07h11	4.41	97
	13h23	0.45	
	19h28	4.26	95
21/03/2018	01h38	0.46	
	07h49	4.32	92
	13h56	0.55	
	20h06	4.15	88
22/03/2018	02h13	0.57	
	08h30	4.13	84
	14h33	0.71	
	20h50	3.96	78
23/03/2018	02h54	0.73	
	09h18	3.89	72
	15h16	0.92	
	21h41	3.74	66
24/03/2018	03h44	0.95	
	10h16	3.63	59
	16h11	1.16	
	22h46	3.55	53

Table S1C. Experimental supplementary information's of behavorial and molecular analyses in the field (Eyrac, Arcachon, FRANCE, lat. 44.66°N) in March 2018. Hours are expressed in local time (UTC +1). Related to Figure 2; Figure 3. Details of sampling times for the molecular analysis.

Sampling time	Date	Hour
1	14/03/2018	18h48
2	14/03/2018	21h54
3	15/03/2018	1h00
4	15/03/2018	4h06
5	15/03/2017	7h12
6	15/03/2018	10h18
7	15/03/2018	13h24
8	15/03/2018	16h30
9	15/03/2018	19h36
10	15/03/2018	22h42
11	16/03/2018	1h48
12	16/03/2018	4h54
13	16/03/2018	8h00
14	16/03/2018	11h06
15	16/03/2018	14h12
16	16/03/2018	17h18

Table S2. Field chronobiological analysis of individual oyster behavior. Related to Figure 2. Valve opening amplitudes (VOA) were recorded at 4 m depth (± tides) over 18 days of field experiment (05/03/2018 – 23/03/2018, Eyrac, Arcachon Bay, France at 44.66°N). TSA Cosinor analysis was used to detect tidal periodicities (12.4h and 6.2 h periods) and daily ones (24 h). For each oyster (n = 15), significant rhythms were determined by the periods found with Lomb and Scargle periodograms and by the adjusted *p*-value of the Cosinor model. Value of the percent rhythm (%) of the model is indicated in parenthesis 2nd periodicities correspond to the second significant periodicities after residue injections. ****p*-value < 0.0001; *****p*-value < 0.00001.

TSA Cosinor analysis – Field Individual analysis of VOA

Individual	12.4 ± 0.5 h range	6.2 ± 0.2 h range	24 ± 1 h range
Oyster 1	12.4 ± 0.5**** (24.0%)	6.2 ± 0.8**** (6.2%)	24.5 ± 0.2**** (2.9%)
Oyster 2	12.5 ± 0.2**** (15.1%)	6.2 ± 0.7**** (10.1%)	24.6 ± 0.2**** (5.5%)
Oyster 3	12.4 ± 0.2**** (10.6%)	6.2 ± 1.0*** (21.9%)	23.1 ± 0.2**** (4.7%)
Oyster 4	12.5 ± 0.2**** (20.5%)	6.2 ± 1.1**** (3.9%)	24.1 ± 0.1**** (4.9%)
Oyster 5	12.5 ± 0.2**** (12.8%)	6.2 ± 0.7**** (8.3%)	24.3 ± 0.2**** (4.2%)
Oyster 6	12.4 ± 0.3**** (4.8%)	*NS*	*NS*
Oyster 7	12.4 ± 0.2**** (12.1%)	6.2 ± 0.8**** (7.6%)	24.5 ± 0.2**** (3.4%)
Oyster 8	12.4 ± 0.3**** (8.4%)	6.2 ± 0.7**** (8.6%)	*NS*
Oyster 9	12.3 ± 0.2**** (13.8%)	6.2 ± 0.8**** (2.4%)	24.2 ± 0.1**** (4.8%)
Oyster 10	12.4 ± 0.2**** (16.1%)	6.2 ± 0.7**** (8.6%)	24.6 ± 0.1**** (5.5%)
Oyster 11	12.5 ± 0.1**** (23.3%)	6.2 ± 0.7*** (10.8%)	*NS*
Oyster 12	12.5 ± 0.2**** (9.5%)	6.2 ± 0.8**** (7.9%)	24.1 ± 0.1**** (6.1%)
Oyster 13	12.5 ± 0.2**** (13.5%)	6.2 ± 0.7**** (8.6%)	23.8 ± 0.2*** (2.0%)
Oyster 14	12.3 ± 0.2**** (14.7%)	6.2 ± 1.0**** (4.9%)	24.7 ± 0.1**** (4.9%)
Oyster 15	12.4 ± 0.1**** (16.0%)	6.2 ± 0.6**** (13.4%)	24.4 ± 0.2**** (2.6%)
Total	100% (15/15)	93.3% (14/15)	80% (12/15)

Table S3. Forward, reverse primer sequences of Real-Time PCR analyzed genes.
Related to figure 3; figure 4.

GeneBank access	Gene name	Forward primer	Reverse primer
Crassostrea gigas core clock genes			
KX371073	*Cgclock* (Clock)	5'-TGGGAATGATGTCCAACAGAG-3'	5'-GGTCCATCAATGACAGGAAGT-3'
KT991835	*Cgcryptochrome 1* (*Cry1*)	5'-TCATGAAGCAGCTCAGATACG-3'	5'-ACCTCCCAGTTCAACCAAAG-3'
KX371074	*Cgcryptochrome 2* (*Cry2*)	5'-AACCTTACAGCAAGCACGAA-3'	5'-TGACATCTGGCTGTGGTTTC-3'
KX371075	*Cgbmal1* (*Bmal*)	5'-CACAAGTTCAGGTCAGAGTGTAG-3'	5'-TCACCTGAGGTAGACTGGTTAT-3'
KX371076	*Cgperiod* (*Per*)	5'-CCGATGACAGAAATCCCAGTAG-3'	5'-CCATCCTATTCTCCTGCTCTTG-3'
KJ188106	*Cgrev-erb* (*Rev-erb*)	5'-GACTTTGCTGATCGCTTCAAC-3'	5'-CTTTCCAACTGCTCCACATTTC-3'
EKC18621	*Cgror* (*Ror*)	5'-CTACGTGAGCAGGTGTTTGA-3'	5'-CGTCCGCTATGTCCTTCAAT-3'
KX371077	*Cgtimless 1* (*Tim1*)	5'-AAAGATCCCGGACACAGTATG-3'	5'-TGGAACTCGTTCCTGACTTG-3'
Clock-associated genes			
EKC41768	*Hiomt-like*	5'-CGGGTGGATCAGTGTTAGTAATG-3'	5'-TCTCTTGGCCCTGTGATAGA-3'
XM_011433587	*Rhodopsin-like 1*	5'-TAGTTCGGCGTCGGAATTTATC-3'	5'-CTGTTCGAATCTCTGCTCTCAC-3'
XM_011448766	*Rhodopsin-like 2*	5'-CCCTGAGTCATCCCAAATTCA-3'	5'-GATGTTCTCGGCGTAGCTTTA-3'
XM_020065754	*Rhodopsin-like 3*	5'-TGACTTTGACGGCGATACTG-3'	5'-ATAGATCCGCCACCGAAATG-3'
Housekeeping genes			
AB122066	*CgElongation Factor 1* (*Ef1*)	5'-ACCACCCTGGTGAGATCAAG-3'	5'-ACGACGATCGCATTTCTCTT-3'
CAD67717	*CgGadph*	5'-CGTACCAGTTCCAGATGTTTCC-3'	5'-GCCTTGATGGCTGCCTTAATA-3'