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Field Chronobiology of a Molluscan Bivalve: How the Moon and Sun Cycles Interact to Drive Oyster Activity Rhythms

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The present study reports new insights into the complexity of environmental drivers in aquatic animals. The focus of this study was to determine the main forces that drive mollusc bivalve behavior *in situ*. To answer this question, the authors continuously studied the valve movements of permanently immersed oysters, *Crassostrea gigas*, during a 1-year-long *in situ* study. Valve behavior was monitored with a specially build valvometer, which allows continuously recording of up to 16 bivalves at high frequency (10 Hz). The results highlight a strong relationship between the rhythms of valve behavior and the complex association of the sun-earth-moon orbital positions. Permanently immersed *C. gigas* follows a robust and strong behavior primarily driven by the tidal cycle. The intensity of this tidal driving force is modulated by the neap-spring tides (i.e., synodic moon cycle), which themselves depend of the earth–moon distance (i.e., anomalistic moon cycle). Light is a significant driver of the oysters' biological rhythm, although its power is limited by the tides, which remain the predominant driver. More globally, depending where in the world the bivalves reside, the results suggest their biological rhythms should vary according to the relative importance of the solar cycle and different lunar cycles associated with tide generation. These results highlight the high plasticity of these oysters to adapt to their changing environment. (Author correspondence: d.tran@epoc.u-bordeaux1.fr)

Keywords: Anomalistic rhythm, *Crassostrea gigas*, Daily rhythm, Synodic rhythm, Tidal rhythm

INTRODUCTION

Biological rhythms are a fundamental property of life. All our metabolic, physiological, and behavioral activities are rhythmic and controlled in such a way as to assure an optimum synchrony between organisms and their environment (Emerson et al., 2008; Yerushalmi & Green, 2009). The organization of biological activities into different cycles (e.g., ultradian, circadian, infradian) is present in the phyla of all thus far investigated living organisms (Bell-Pedersen et al., 2005). These biological rhythms have two major components. The first one is endogenous and consists of a molecular clock (De Haro & Panda, 2006; Duguay & Cermakian, 2009), consisting of clock genes that give an autonomous and internal rhythmicity to living organisms, and the second is made up of the environmental factors (e.g., light, food, tidal action, etc.), called "zeitgebers," that synchronize rhythmicity.

Biological rhythms have been widely documented in marine organisms (Naylor, 2010; Palmer, 1995) and studied in the primary phyla (Bentley et al., 2001; Last et al., 2009; Naylor, 2001; Northcott, 1991; Saigusa et al., 2003; Takemura et al., 2004). These rhythms are adaptively important for aquatic organisms in anticipating future cyclic events. The marine environment is an exceedingly complex habitat and subject to solar cycles (daily rhythm) and lunar cycles (tidal, semi-lunar, lunidian rhythms, etc.), which constitute the main oscillators (Palmer, 1995). In all cases, rhythmic phenomena in intertidal and subtidal areas are driven by a complex association of the sun-earth-moon orbit, which brings about environmental changes, such as tides (a cycle every 12.4 h). These tides are not equally distributed, both spatially and temporally, and their amplitudes change cyclically following different lunar cycles. In particular, neap-spring tidal cycles explain the differences in tide amplitudes. Synodic moon cycles, which are the duration of time required for the moon to align with the sun and the earth in the same order (new or full moon), constitute the origin of neap-spring tides, which occur

with a period of 14.76 days (fortnight cycles called semi-lunar cycle). Moreover, other moon cycles can modulate and interact with the synodic cycle to modify tide intensity and periodicity, such as the anomalistic, sidereal, tropical, and draconic cycles (Kvale, 2006).

Since 1903, when the first discovery of a persistent tidal rhythm was made in the acoelomorph worm *Convoluta roscoffensis* (Bohn, 1903; Gamble & Keeble, 1903), the tidal driver of biological rhythms has been widely studied in different phyla (Gibson, 2003; Naylor, 2010; Palmer, 1995). In bivalves, tidal rhythms have been described in mussels: *Mytilus edulis* (Rao, 1954; Riisgard et al., 2006; Saurel et al., 2007), *M. californianus* (Gracey et al., 2008), *M. galloprovincialis* (Zaldibar et al., 2004), and *Perna viridis* (Wong & Cheung, 2001); in clams: *Venus mercenaria* (Brown et al., 1953), *Saxidomus purpurus* (Kim et al., 2003), and *Austrovenus stutchburyi* (Williams & Pilditch, 1997), and in pectens (Gompel, 1937). Circadian rhythms were also described in *M. edulis* and *Astarte borealis* (Wilson et al., 2005). The influence of the moon on bivalve cycles could also be direct, e.g., by moonlight (i.e., influence of sunlight reflected on earth by the moon's surface), as was shown in *Pinna nobilis*, which exhibits a circalunidian rhythm of valve activity, combined with a circadian rhythm, without any circatidal cycle (Garcia-March et al., 2008). However, all these rhythms seem to be characterized by a lability of the biological clocks probably due to interaction of these different solar and lunar oscillators (Palmer, 1995).

Although the existence of the tidal influence is well documented, in no case has the complexity and synergy of different solar and lunar cycle interactions, which are able to influence the behavior of animals living in the subtidal zone, been widely described. Our aim was to obtain more insight into the relative importance of the tidal and daily rhythms in shaping the behavior of *C. gigas* oysters all along the year. The present study took advantage of novel techniques that we developed allowing months of continuous recording of valve activity in a given group of animals without any human intervention (http://www.domino.u-bordeaux.fr/molluscan_eye). The analysis was performed during a full year under subtidal conditions, *in situ*, in the Bay of Arcachon, France. Our data demonstrate how closely an animal such as the oyster *C. gigas* can express a valve behavior modulated by different tidal variations due to lunar and solar cycle's interactions.

MATERIALS AND METHODS

Field Study and General Conditions

The study was carried out on 14 oysters *Crassostrea gigas* (8.7 ± 0.3 cm length, 1.5 yrs old, diploids), which were collected in Bay of Arcachon (S.W. France), at a site located in front of the Marine Biological Station of Arcachon. Oysters were placed in a permanently immersed oyster bag (1×0.5 m) and secured to a concrete slab under Eyrac Pier, Bay of Arcachon (latitude 44.66°, longitude –1.16°). They were located in close proximity with savage oysters colonizing the pier (<0.5 m). The oysters were always in subtidal conditions, with at least 1.5 m of seawater above. The tides were semidiurnal (amplitude $\pm$ 2 m), and tidal flow at mid-tide reached 0.6 m·s^{-1} in the water column. The oysters were positioned in the field site on 6 December 2006, and the present study was performed from 1 January to 31 December 2007 (365 days of data). Note that initially 16 oysters were positioned in the bay and that 2 animals died during the 1-yr study. The data of water height at Eyrac Pier were provided from the SHOM (French Marine Service of Oceanography and Hydrography, www.shom.fr). The astronomical data related to the sun, earth, and moon positions were provided from the Web site http://www.imcce.fr. All experiments presented in this paper complied with the laws in effect in France, where they were performed, and they conformed to international ethical standards as outlined in Portaluppi et al. (2010).

HFNI Valvometer Device and Data Recording

To monitor valve movement behavior of bivalves directly in the field, we adapted for the field experiments a high-frequency (10 Hertz), non invasive (HFNI) valvometer technology, previously designed and built in our laboratory (Tran et al., 2003, 2007). Briefly, a pair of light electrodes was glued with cyanoacrylate glue (Cyanolit 241) on each half shell. The electrodes, designed to minimize disturbance to bivalve's behavior, were made up of two resin-coated electromagnets (50 mg each). The total weight of the electrode (resin + magnet) was approximately 0.5–1.0 g. Between the electrodes, an electromagnetic current was generated, which allowed measurement of the amount of valve opening and closing. Each pair of electrodes was connected by a floating cable to an analogical electronic card that managed the electrodes and was housed in a waterproof box next to the animals. The first electronic card was connected to a second located on the pier, which constituted the processor that saves and digitizes the data for transfer to the laboratory workstation. A Linux operating system drives the first card and processes the initial analysis of the data. The system is built to sample data at 10 Hz from 16 animals in a sequential order. Every 0.1 s, three packets of information are produced: distance between valves at the electrode level, sampling time, and animal number. Thus, as a whole, a total of $3 \times 864,000$ pieces of information/day describe the behavior of a whole group of 16 animals (2,592,000 data points). At the individual level, it means that the system performs a measurement of the opening status every 1.6 s, as there are 16 animals, and that a total of 54,000 data points characterize the gaping/closing behavior of any individual every day. The raw data were transferred from the field to the labotaory using a cellular telephone network (GPRS; General Packet Radio Service), with a mobile phone module embedded in the 2nd electronic

card, to a laboratory workstation (DELL precision 690 using both Bash Linux second mathematical codes written in R, http://CRAN.R-project.org/doc/FAQ.R-FAQ.html) each day at 00:00 h (GMT).

Data Treatment and Statistical Analysis

The raw, recorded data were treated in the following two ways. First, each day of the year, we reported the individual valve closure/opening periods of all oysters. Second, we calculated, on an hour-per-hour basis, the duration of time the valves of the oysters spent open, expressed as percentage of opening in the whole group. A value of 100% means that the valves of all animals were opened during the 1-h period and 0% that no animals were opened.

We used Laplace coefficients to measure the effect of tide amplitude on valve opening duration, a usual means of describing tide amplitude in a semi-diurnal tidal regime. For a given coefficient, in the same area, the tide amplitude is always the same. Arbitrarily, these coefficients range from 20 (lowest tide amplitude) to 120 (highest tide amplitude). In the Bay of Arcachon in 2007, these coefficients varied from 24 to 116. We organized the data into four quartile ranges (i.e., equal number of data per range) of coefficients (24–50, 51–69, 70–83, and 84–116), with the number/quartile being 169 tidal cycles.

Quality of Data

To ensure the quality of the data, we verified the absence of random distribution of data using the autocorrelation diagram (Box et al., 1994) and also the absence of stationary phenomenon by PACF (partial autocorrelation function) calculation (Box et al., 1994). Both verifications allow checking for the absence of a stochastic process, indicating a real biological or physiological phenomenon. The normal distribution of the data was assessed by the Kolmogorov-Smirnov test (K-S test).

Search for Periodicity

The recorded data were tested for periodicities by the spectral method of the Lomb and Scargle periodogram, which combines the principle of regression analysis and Fourier transformations (Scargle, 1982). It gives a threshold of probability ($p = .95$) defining the limit below which the signal can be regarded as "noise." We calculated the confidence interval of the period with the method of Halberg (1969).

Rhythmicity Modeling

Rhythmicity was modeled using the Cosinor model (Bingham et al., 1982; Nelson et al., 1979), which applies a cosine function calculated by regression. Prior to the use of this method, we checked the following criteria: average residue equal to zero (average test), normality of residues (K-S test), and homogeneity of variance of the residues (Bartlett test). Cosines curves of the following equation were fitted per physical parameters (water column height, sun height, tide coefficient) or a biological parameter (oyster opening time):

$$C(t) = M + A \cdot \cos((2\pi \cdot t)/\tau + \phi)$$

where $C(t)$ is an observation of mean valve opening duration at time t, M is the MESOR (midline-estimating statistic of rhythm, a time-series average), A is the amplitude (measure of the extent of rhythmic change), τ is the period of the rhythm, and ϕ is the acrophase (measure of the time when the fitted cosine approximation reaches its maximum value). To test the statistical existence of the model chosen, we applied the Ellipse test (Bingham et al., 1982). We calculated chronobiometric parameters, such as the "percent rhythm," which describes the percentage of rhythmic behavior explained by the model and the "percent error," which evaluates the percentage in the model that was not rhythmic. To validate the model, i.e., presence of absence of a rhythm, the probability for the assumption of a null amplitude (i.e., H0: amplitude = 0) was calculated (Nelson et al., 1979).

Results are expressed as mean ± 1 SEM. Treatment differences were determined using one-way analysis of variance (ANOVA) after checking assumptions (normality and homoscedasticity of the error term). When assumptions were not met, the nonparametric Kruskall-Wallis test was used. If the null hypothesis was rejected, the Tukey test was applied to determine significant differences between conditions. For all statistical results, a probability of $p < .05$ was considered significant. Statistical analyses were performed using Sigma Stat software (Version 3.1; Systat, Chicago, IL, USA).

RESULTS

Figure 1A shows the global pattern of valve activity of the 14 *C. gigas* oysters studied *in situ* in the Bay of Arcachon in the year 2007. The *C. gigas* were permanently immersed (i.e., always in subtidal condition) in a semi-diurnal tide ecosystem (i.e., two tidal cycles of 12.4 h/lunar day). Figure 1B, an enlargement of 2 consecutive days, illustrates how the timing of each individual behavior was characterized daily by alternation of open and closed status. Thus, each line in Figure 1A represents the activity of a single individual and the whole Figure 1A is composed of 5110 lines (365 days × 14 oysters). The distribution of valve opening was clearly not random; on the contrary, it followed a robust pattern throughout the year. The main behavior pattern follows a tidal cycle, with two main periods of valve closure/day and synchronized to low tide.

Description of the Local Tidal Cycles

Figure 2A shows the water column height at Eyrac Pier during the same time period. The tidal movements correspond to neap-spring cycles, which occur two times/synodic month ($\tau = 29.53$ days): the highest tides (spring tides) occur during syzygy (full or new moon), whereas the lowest tides (neap tides) occur at first- and
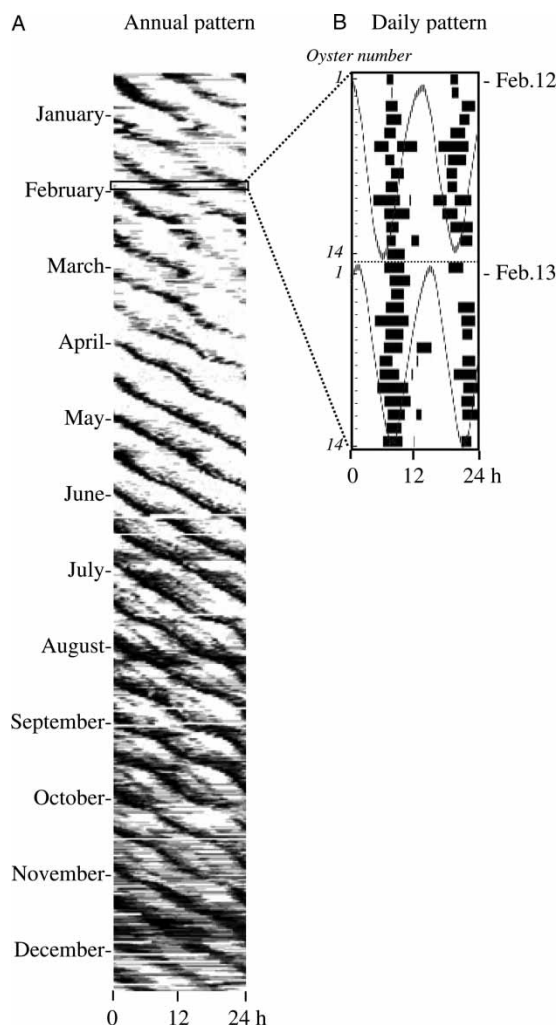
A   Annual pattern

B   Daily pattern

FIGURE 1.   (A) Raw record of *C. gigas* valve behavior (n = 14 oysters) during the whole year 2007 (ordinate), under Eyrac Pier, Bay of Arcachon, France (latitude 44.66°, longitude −1.16°) and hours of the day (abscissa). (B) Enlarged image of 2 successive days in the year. Each day of the year and oyster number (ordinate) and hours of the day (abscissa) of valve closure/opening periods of each oyster. Valve closure period is shown with a black line and valve opening period with a white line, successively, for oyster 1 (line 1), then oyster 2 (line 2 below), ..., oyster 14 (line 14), and then oyster 1, oyster 2, ..., on day 2, and so on, for the 365 days of 2007. The circatidal cycle of water column height for these days is shown in parallel. This representation in successive layers suggests a robust cyclic behavior pattern based on a tidal cycle of valve activity throughout the year.

third-quarter phases of the moon. The superimposition of the moon anomalistic cycle ($\tau = 27.55$ days) on the synodic cycle modifies the neap-spring tides' amplitude during the year. Coincidence of the perigee (closest distance between moon and earth) and spring tides gives a "perigean spring" tide, which amplifies the spring tide effect normally observed. Alternately, coincidence of the apogee (farthest distance between moon and earth) and spring tides gives an "apogean spring" tide, which reduces the effect of the normal spring tide. The consequence is the difference of spring tidal heights called "fortnightly inequality."

Relationship Between Tidal Amplitude and Valve Activity

This analysis was done with the whole data set of 2007 (365 days, Figures 1A and 2A). To illustrate the tide amplitude effect on the tidal rhythm, Figures 2B and 2C present two extreme and different behaviors (2 × 3 days), which are characteristic of two opposite conditions in terms of neap-spring tides (see inserts in Figure 2A). Figure 2B illustrates a typical rhythmic behavior of *C. gigas* in conditions of high amplitude tides during 3 days (19–21 March), which correspond to a "perigean spring" tide. The tide coefficients were between 108 and 116, which correspond to a variation of ≈4.5 m of the water column height. The results show the oysters were opened most of the time (88.5% ± 2.2% during the 3 days). They closed their shells during the low-tide slack water, with a maximum of closure during the flow tide, and were opened at maximum during the ebb tide and at high-tide slack water. Figure 2C shows the opposite condition in terms of the tide amplitude effect on the oyster rhythm. It illustrates a typical rhythmic behavior of *C. gigas* during 3 days (20–22 September) at low tide amplitudes, which correspond to neap tides due to the third-quarter phases of the moon. The tide coefficients were between 27 and 37, which correspond to a variation of ≈1.5 m of the water column height. Typically, the oyster tidal rhythm is the same with high-amplitude tides, but the time of closure is increased. The results show the oysters were opened 56.4% ± 3.3% during the 3 days, i. e., ≈30% less than in the previous condition. We also observed that the time window in which closures happened was increased. A maximum of oysters were open only during the beginning of the ebb tide. To quantify and characterize the valve behavior for the whole year 2007, Figure 3 shows the mean tide amplitude effect on the mean percentage of opening duration/tidal cycle. It significantly increased ($p < .05$) with the increase of tide coefficients, varying from 74.7% ± 0.6% in neap tides (tide coefficients from 24 to 50) to 82.8% ± 0.5% in spring tides (tide coefficients from 84 to 116). To further explore the basic drives determining the above-described gaping behavior in *C. gigas*, we then turned to a more detailed chronobiological analysis.

Chronobiological Treatments

Figure 4A–D show the spectral analysis by Lomb and Scargle periodograms, allowing characterization of different periodicities in valve behavior throughout the year, and Table 1 shows the chronobiometric values of the Cosinor model determined for each corresponding period. Figure 4A1 and A2 show a period of 12.40 ± 0.04 h for water column height at Eyrac and 12.40 ± 0.16 h for *C. gigas* opening time, respectively. The percent rhythm (PR) shown in Table 1 was 98.17% and 45.31% for water column height and *C. gigas* opening time, respectively, demonstrating a significant relationship between valve behavior and the tidal cycle. Figure 4B1 and B2 show a significant period of 24.00 ± 0.01 h for sun altitude at Eyrac and 24.00 ± 0.29 h for *C.*
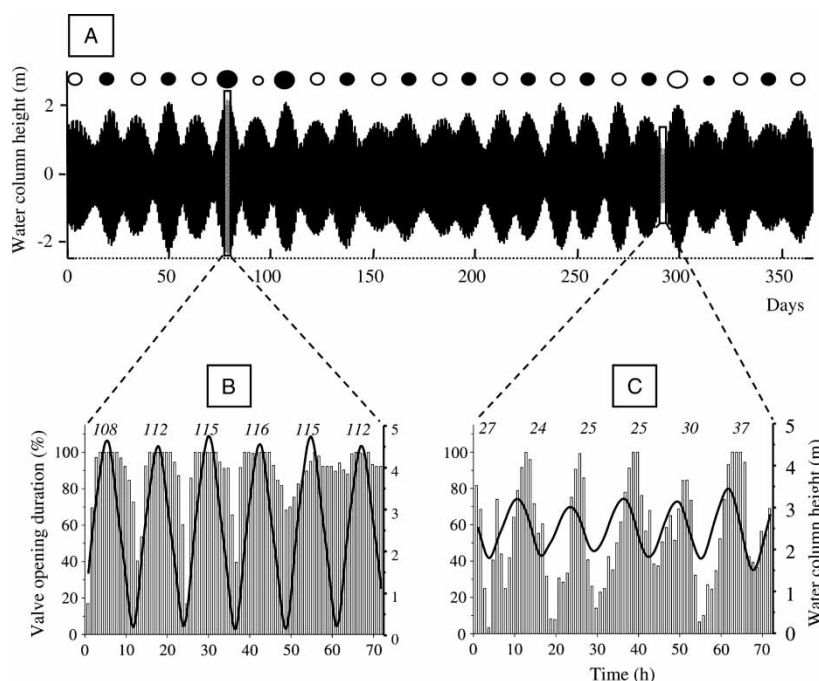
FIGURE 2.   Tidal cycles during the 365 days of the year 2007 at Eyrac Pier, Bay of Arcachon, France. (A) Water column height data provided by the SHOM (French Marine Service of Oceanography and Hydrography, www.shom.fr). In the top plot, circles correspond to the lunar phases related to the synodic month (29.53 days) and lunar perigee (closest distance to the earth) and apogee (farthest distance from the earth) related to the anomalistic month (27.55 days). Black circles: new moon; white circles: full moon; large circles: perigee; small circles: apogee. (B and C) Characteristic illustrations of the influence of tide amplitude on the tidal oyster rhythm. Superimposition of valve opening duration (left abscissa, box plot of mean opening duration/h in %, n = 14 oysters) and water column height (right abscissa) according to time. In italics, the tide coefficient of each tidal cycle. B, Enlarged image of characteristic *C. gigas* valve movement cycles during high amplitude of a tide that occurs during spring tide. Example of 3 days (19–21 March). C, Enlarged image of characteristic *C. gigas* valve movement cycles during a low tide amplitude that occurs during neap tide. Example of 3 days (20–22 September).
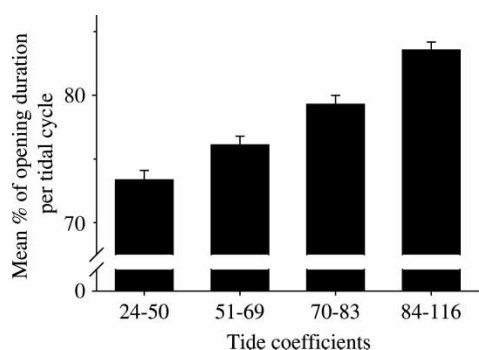

FIGURE 3.   Mean percentage of valve opening duration/tidal cycle as a function of tide coefficient distributed in quartiles (n = 169 per quartile). Mean ± SEM. $p < .05$. *Significantly different from the range 24–50. **Significantly different from the range 51–69. ***Significantly different from the range 70–83.

gigas opening time. PR (Table 1) was 99.47% and 6.29% for sun altitude and *C. gigas* opening time, respectively, revealing a significant relationship between valve behavior and the daily cycle. Figure 4C1 and C2 show a significant period of 14.70 ± 0.03 days for tide coefficients at Eyrac and 14.50 ± 0.38 days for *C. gigas* opening time. PR (Table 1) was 97.86% and 8.98% for tide coefficients

and *C. gigas* opening time, respectively, indicating a significant relationship between valve behavior and the semi-lunar synodic month period (14.76 days), which correspond to neap-spring tides. Figure 4D1 and D2 show a significant period of 13.70 ± 0.11 days for tide coefficients at Eyrac and 13.80 ± 0.01 days for *C. gigas* opening time. PR (Table 1) was 97.25% and 1.11% for tide coefficients and *C. gigas* opening time, respectively. These results show a weak, but highly ($p = .00001$) significant, relationship between valve behavior and the semi-lunar anomalistic month period (13.78 days), which corresponds to the alternation of perigee and apogee.

Finally, we looked for effects of other moon cycle periods, such as the tropical ($\tau = 27.32$ days), sidereal ($\tau = 27.32$ days), and draconic ($\tau = 27.21$ days) cycles, on valve activity data or physical parameters of the tides. We did not observe any significant periods. We also researched the existence of a significant ≈ 24.8 h period in the data (Figure 4B2), which could correspond to a complete lunar-day cycle, but we did not find any relationship. This means that a moonlight cycle, based on a period of 24.8 h, does not seem to influence *C. gigas* rhythmicity in the Bay of Arcachon, France.
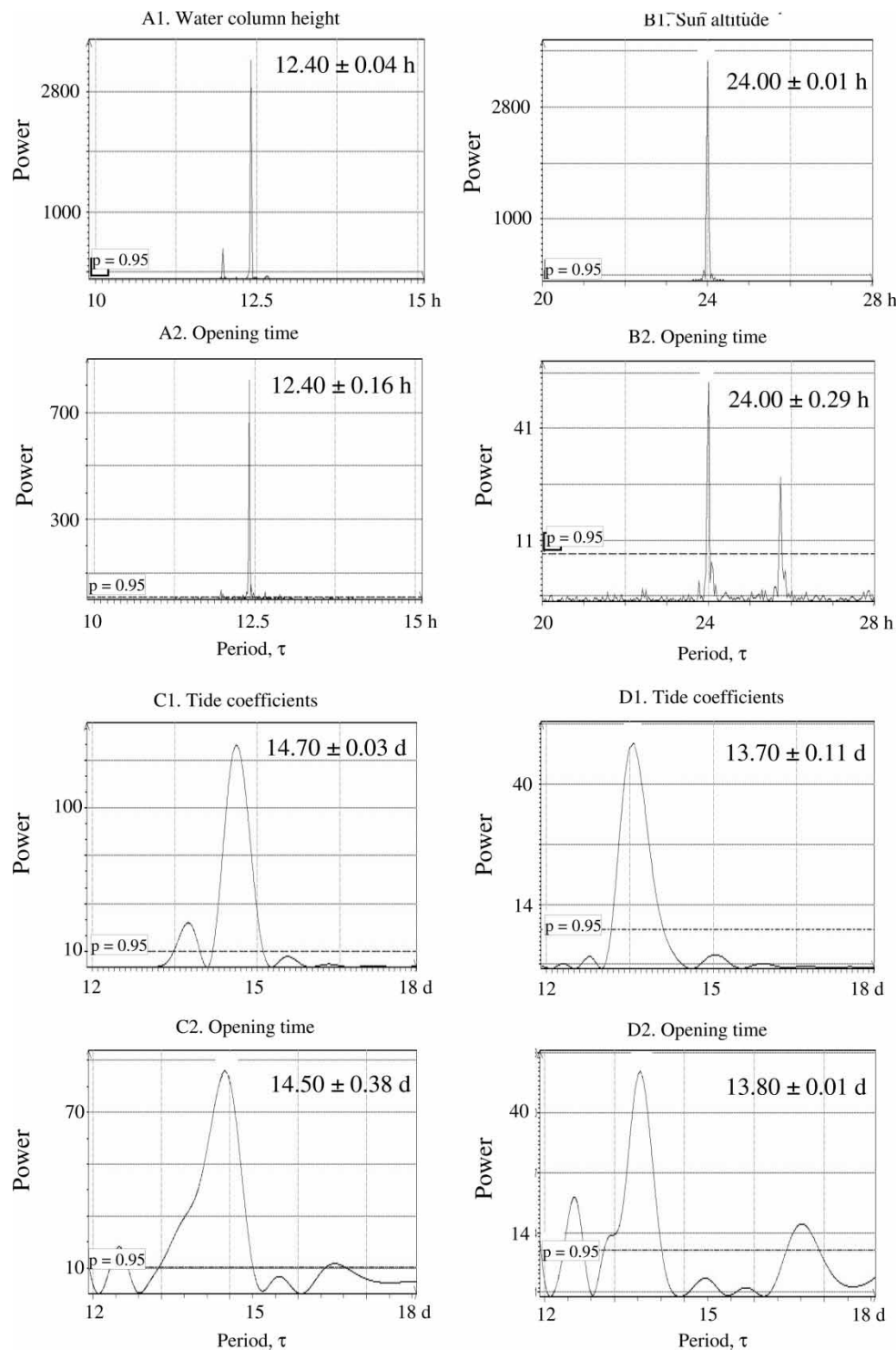
FIGURE 4.   Rhythmic patterns of *C. gigas* valve behavior correlated with parameters associated with moon and sun cycles. The periods of each cycle studied were calculated by spectral analysis (1-yr data) of the Lomb and Scargle periodograms. Dashed line corresponds to threshold of probability ($p = .95$) defining the limit below which the signal can be regarded as insignificant. Study parameters (measured at Eyrac Pier) were (A1) water column height; (A2) valve opening time (1st rhythm); (B1) sun altitude; (B2) valve opening time (2nd rhythm); (C1) tide coefficients (1st cycle); (C2) valve opening time (3rd rhythm); (D1) tide coefficients (2nd cycle); (D2) valve opening time (4th rhythm).

DISCUSSION

The aim of this work was to examine the main forces that drive mollusc bivalve behavior *in situ*. To address this question, we studied valve movement of the oyster *Crassostrea gigas* while permanently immersed during a

1-yr-long *in situ* study. The present work highlights a strong relationship between the rhythms of valve behavior in the oyster *C. gigas* in subtidal conditions and the complex association with the sun-earth-moon positions. We showed that the *C. gigas* rhythm was

TABLE 1. Cosinor model characteristics describing oyster rhythms.

Tested parameters	Chronobiometric values of the model				Chronobiometric parameters		
	Period (τ)	Amplitude	Acrophase (radians)	Mesor	Percent rhythm (PR)	Percent error (PE)	p value (H0: amplitude = 0)
Water column height	12.40 ± 0.04 h	0.179 m [0.049 to 0.309 m]	−1.86 [−2.64 to −1.08]	2.520 m [2.519 to 2.521 m]	98.17%	1.83%	0.0046
Opening time (1st rhythm)	12.40 ± 0.16 h	3.43% [3.31% to 4.78%]	−0.589 [−1.210 to 0.322]	74.9% [73.1% to 76.6%]	45.31%	54.69%	0.0001
Sun amplitude	24.00 ± 0.01 h	43.0° [42.9° to 43.0°]	−3.42 [−3.43 to −3.42]	0.320° [−0.839° to 1.480°]	99.47%	0.53%	0.00001
Opening time (2nd rhythm)	24.00 ± 0.29 h	4.17% [3.46% to 4.87%]	−4.74 [−4.95 to −4.54]	74.9% [73.1% to 76.6%]	6.29%	93.71%	0.00001
Tide coefficients (1st cycle)	14.70 ± 0.03 d	26.6 [23.1 to 30.2]	−2.38 [−2.54 to −2.22]	67.3 [65.8 to 68.8]	97.86%	2.14%	0.00001
Opening time (3rd rhythm)	14.50 ± 0.38 d	67.0% [58.9% to 76.0%]	−3.21 [−3.72 to −2.70]	44.2 [−98.5 to 101.0]	8.98%	91.02%	0.00001
Tide coefficients (2nd cycle)	13.70 ± 0.11 d	11.6 [12.4 to 16.6]	−4.07 [−4.77 to −3.37]	67.3 [65.8 to 68.8]	97.25%	2.25%	0.0069
Opening time (4th rhythm)	13.80 ± 0.01 d	4.44 [3.53 to 5.27]	−1.79 [−1.99 to −1.60]	−0.05 [−0.66 to −0.56]	1.11%	98.89%	0.00001

The rhythmicity of each parameter under investigation was calculated with a cosinor model and the chronobiometric parameters stated. the period (τ) was calculated with a standard deviation, whereas amplitude, acrophase, and MESOR were calculated with a confidence interval. Chronobiometric parameters done by the model allow determining the % of rhythmicity explained by the model (percent rhythm) and the "noise" associated (percent error). The model was statistically significant with p value = 0.05.

primarily driven by the tidal cycle ($\tau = 12.4$ h), but this driver was in turn modeled by other lunar cycles. Indeed, we observed that the tide amplitude cycle influences the circatidal driver force. *C. gigas* behavior follows the neap-spring cycle, which occurs two times/synodic month ($\tau = 29.53$ days). During highest tides (spring tides), occurring during syzygy (i.e., earth, moon, and sun are nearly aligned at new or full moon), the percent of valve closure decreased and the time windows in which it happened were reduced. Alternately, during the lowest tides (neap tides), which occur when the sun and moon are aligned at right angles to the earth during the first- and third-quarter phases of the moon, the percent of valve closure increased. Moreover, the *C. gigas* opening time followed a slight, but significant, rhythm equal to the anomalistic moon cycle ($\tau = 27.55$ days). In the latitudes of the North Atlantic, the semi-diurnal tide cycle (two tides/day) is modeled mainly by the neap-spring tides cycle, itself subject to the moon's anomalistic cycle (arising from the moon's orbit being slightly elliptical), the period of time for the moon to move from perigee (closest approach to the earth) to perigee (Archer, 1996). At Eyrac, the tides truly exhibit this anomalistic rhythmicity, superimposed on the neap-spring tides rhythmicity. This is at the origin of the difference of spring tidal heights called "fortnightly inequality" (Kvale, 2006) due to the slight difference of period between the neap-spring tides ($\tau = 14.76$ days) and the perigee-apogee cycle ($\tau = 13.78$ days).

The present results show the neap-spring tide rhythmicity of the oysters was modified every fortnight by the anomalistic cycle, which induced a change in the response to the tidal driving force. However, in the dominant diurnal tide cycle (one tide/day), which occurs in other latitudes (typical in large tracts of the Pacific and Indian Oceans), the neap-spring tide cycle could be essentially synchronized by the tropical moon cycle ($\tau = 27.32$ days), i.e., the time it takes the moon to complete one orbit moving from its maximum northerly declination to its maximum southerly declination and returning (Archer, 1996). Although we did not observe any period of tide components or oyster rhythm close to the tropical cycle at Bay of Arcachon (France), the present work highlights the possibility that in other parts of the world, where the tropical cycle is dominant, oyster rhythms should follow a tropical cycle rather than a synodic-anomalistic one. Finally, in this study, we did not observe any influence of other moon cycles (<1 yr, in our experimental time), such as the sidereal ($\tau = 27.32$ days) and draconic ($\tau = 27.21$ days) moon cycles, on oyster rhythms.

Circatidal rhythmicity in bivalve behavior has been previously described (Gracey et al., 2008; Kim et al., 2003; Saurel et al., 2007; Williams & Pilditch, 1997; Zaldibar et al., 2004), but the modulation of the tidal effect by other moon cycles has remained open to investigation. Lunar/semi-lunar cycles ($\tau = 29.53/14.76$ days), in particular, have been known to drive different

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physiological processes in marine organisms (Reid & Naylor, 1993; Segusa, 1980; Takemura et al., 2004; Zeng et al., 1999), but not in bivalves. We found only one study related to the difference between neap and spring tides on bivalve physiology. Wong and Cheung (2001) showed the filtration rate of the mussel *Perna viridis* underwent temporal variation with tides and was positively correlated to water organic content. Pseudo-feces were only produced during spring tides and not during neap tides, which further illustrates the effect of these rhythms on bivalve physiology. Finally, to our knowledge the effects of anomalistic or tropical cycles on marine animals remain totally new.

In addition to moon cycles related to the tides, *C. gigas* exhibited a weak, but significant, daily component in valve behavior. The driving effect of light on the diurnal rhythm of valve activity has been shown in other bivalves, such as *Mytilus edulis* and *Astarte borealis* (Wilson et al., 2005). Kim et al. (2003) presented evidence for two endogenous rhythms of oxygen consumption in the clam *Saxidomus purpuratus* under free-running conditions in the laboratory: a bimodal rhythm during 7–9 days, i.e., circalunidian cycle of $\tau = 24.8$ h, or unimodal rhythm, i.e., circatidal cycle of $\tau = 12.4$ h, and then a unimodal and circadian one of $\tau = 24$ h. However, under the present field conditions, the light as zeitgeber was clearly minor in comparison with the impact of the tides as zeitgeber. Indeed, the PR explained by the model was 45.31% with the circatidal cycle, against only 6.29% with the circadian cycle. Similarly, Last et al. (2009) showed the rhythm in the polychaete worm *Nereis virens* follows tidal cycles modulated by circadian and lunar-month cycles. They argued the tidal rhythm is able to modulate the circadian clock or its output and lengthen the behavioral circadian periods as much as necessary to match a double-tidal or lunar-day periodicity. Others studies have shown the superimposition of tidal and diurnal rhythms in gastropods (Sandeen et al., 1954), crustaceans (Naylor, 1996; Saigusa et al., 2003), or polychaetes (Bentley et al., 2001).

We also showed that moonlight as zeitgeber in *C. gigas* was insignificant under the present conditions. In the same way, Skov et al. (2005) showed that intertidal crabs were more synchronized by the tidal than moonlight zeitgeber. On the contrary, the marine bivalve *P. nobilis* showed a circadian and circalunar rhythm in its shell gaping activity (Garcia-March et al., 2008). Its activity was essentially diurnal but increased when moonlight intensity increased. This suggests that the use of moonlight as a zeitgeber by marine organisms (Hauenschild, 1960; Franke, 1986; Naylor, 2001) could be an efficient method for bivalves to entrain valve activity when tidal signals are weak (i.e., tidal amplitude close to zero), which is the case in the Mediterranean Sea, where Garcia-March et al. (2008) conducted their experiments. In the same way, the freshwater oyster *Etheria elliptica* showed a moonlight cycle of shell growth (Abell et al., 1996).

If the tidal cycle is the main driving force in *C. gigas*, the discussion remains open as to what are the direct or indirect components associated with the tide, physical, chemical, or trophic, driving the oyster rhythm. Having a biological rhythm that matches the oscillating environment should be adaptive (Yerushalmi & Green, 2009). The cyclic occurrence of various concentrations of phytoplankton, a source of food for the bivalve, could be a zeitgeber (Stephan, 2002). Williams and Pilditch (1997) experimentally demonstrated a tidal rhythm in the bivalve *Austrovenus stutchburyi* that could be entrained by cyclic availability of food. They suggested that a two-component system (two circalunar day clocks coupled in 180° antiphase) may operate in this bivalve, one component entrained by food and the second one by physical tidal factors. Saurel et al. (2007) studied valve activity by video analysis, directly *in situ* on a mussel bed of *M. edulis* in subtidal conditions, during two tidal cycles. They associated valve aperture behavior with [Chlorophylle a], which was synchronized by the tidal cycle. In the same way, Riisgard et al. (2006) correlated *M. edulis* valve activity to phytoplankton. Valve activity increased when phytoplankton concentration increased, directly synchronized to tidal cycles.

The tidal zeitgeber could also be a physical parameter, such as hydrostatic pressure or water velocity, which would not be directly important to bivalve physiology, but instead associated with an essential component for the bivalve, such as phytoplankton for nutrition or dissolved oxygen for respiration. Rao (1954) showed, as we do here, the tidal cycle drives *M. edulis* in subtidal conditions, with a greater valve gape activity at high tide than at low tide. This rhythm was found in mussels on the underside of floats (i.e., no variation of hydrostatic pressure), only subject to water current change as a parameter of the tide. Interestingly, Hutchinson (1988) showed the tidal clock system of the bivalve *Austrovenus stutchburyi* could be entrained by a hydrostatic pressure cycle (±1.6 m). In other phyla, Northcott (1991) showed intertidal teleosts *Gobius paganellus* and *Lipophrys pholis* have circatidal swimming activity, the first one a circalunidian oscillator and the second one a circatidal oscillator. Both oscillators were entrained by hydrostatic pressure cycles that acted as zeitgeber. Akiyama (1997) showed the zooplanktonic crustacean *Dimorphostylis asiatica* exhibited a circatidal swimming rhythm. The tidal cycle was experimentally entrained by a hydrostatic pressure change stimulus and the circadian one by a light pulse.

The *C. gigas* rhythm exhibits two closure periods correlated with the two tidal cycles/day. To explain how tides could entrain tidal rhythms in marine organisms, there exist at least three hypotheses, which have been debated since the 1960s (Kim et al., 2003). One hypothesis envisages two separate and unimodal circalunidian oscillators ($\tau = 24.8$ h), coupled together in 180° antiphase, able to produce two tidal components in each day's pattern. The coupling of these two oscillators
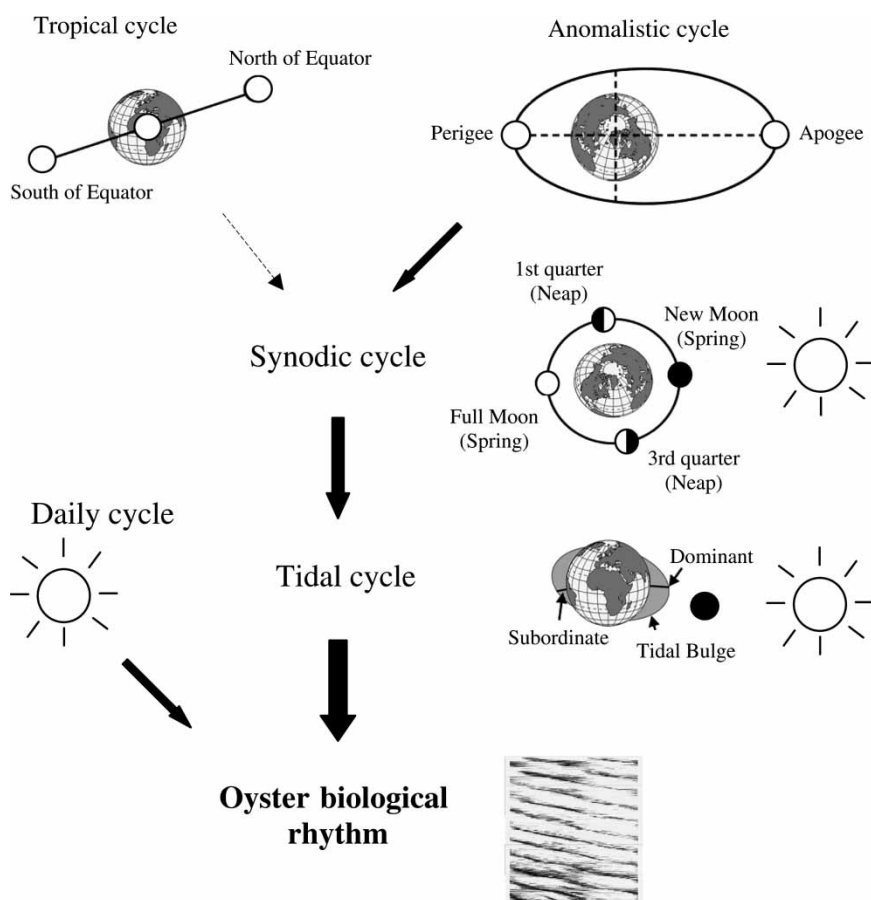
FIGURE 5.   A hypothesis to explain how moon and sun cycle interactions drive oyster behavior in subtidal conditions. Daily cycle as tropical, anomalistic, synodic, and tidal cycles refer to the different respective positions and cycles of the earth, moon, and sun.

could break apart and each could act separately, for example, in free-running conditions (Palmer, 1995, 1997; Williams, 1998). This hypothesis also suggests the lack of a circadian component in marine organism rhythms, as shown by the clam *Austrovenus stutchburyi* and the crab *Macrophthalmus hirtipes* (Williams, 1998). A second hypothesis to explain the same rhythmicity phenomenon is the coupling of two separate unimodal oscillators (Webb, 1976), a circatidal oscillator ($\tau = 12.4$ h) and a circadian oscillator ($\tau = 24$ h), where the latter influences the former. This hypothesis is based on studies of rhythms in the crab, *Carcinus maenas* (Naylor, 1958, 1996). A third hypothesis, developed by Enright (1976), combines circadian and circatidal rhythmicity into a single bimodal circadian oscillator. Supporting this hypothesis, Akiyama (1997) showed the zooplanktonic crustacean *Dimorphostylis asiatica* exhibited a circatidal swimming rhythm, which under free-running condition split into a circadian rhythm. The study strongly suggested that these two rhythms were governed by (an) identical pacemaker(s). Whatever the hypothesis, (an) internal biological clock(s) should exist that controls the behavior of bivalves, as this has been shown in all other phyla studied (De Haro & Panda, 2006). Note, however, that Gusev and Golubev (2001) did not see any clear evidence of the endogenous origin of tidal rhythmicity in the bivalve *Mya arenaria* or the amphipod *Gammarus finmarchicus*.

CONCLUSIONS

The present study constitutes the first reported example of how moon and sun cycles participate in the normal physiological repertoire of an oyster life cycle during a 1-year study *in situ*. Furthermore, the present study gives new insight into the complexity of environmental drivers in influencing marine animal ecology and ecophysiology in the field of marine ethology. Figure 5 presents a scheme showing the different components, moon cycles and daily cycles, which together explain the main behavior rhythmicity of the oyster *C. gigas* in subtidal conditions. Permanently immersed *C. gigas* exhibits a robust and strong behavior primarily driven by the tidal one. The intensity of this tidal driving force is modulated by neap-spring tides (i.e., synodic moon cycle), which themselves depend of the earth–moon distance (anomalistic moon cycle). We suggest that under other latitudes subject to diurnal tides (i.e., one tide/lunar day), it would probably be the tropical moon cycle that would interact with the synodic cycle on oyster behavior. Moreover, the light as zeitgeber is a significant component in *C. gigas* rhythms, although it

masked the driving force associated with the tides, which remain the predominant zeitgeber. More globally, the results suggest that, depending on where in the world the bivalves reside, their biological rhythms will be driven by the solar cycle and different lunar cycles associated with tide generation, and the relative weight of these rhythms will be different. This should lead to a multiplicity of ecological and ecophysiological consequences for the species, giving rise to an exciting guide for future research.

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