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Evidence for a Plastic Dual Circadian Rhythm in the Oyster *Crassostrea gigas*

Audrey M. Mat,[1] Jean-Charles Massabuau,[1,2] Pierre Ciret,[1,2] and Damien Tran[1,2]

[1]*Université Bordeaux and* [2]*Centre National de la Recherche Scientifique, Environnements et Paléoenvironnements Océaniques et Continentaux, Unité Mixte de Recherche, Arcachon, France*

Although a significant body of literature has been devoted to the chronobiology of aquatic animals, how biological rhythms function in molluscan bivalves has been poorly studied. The first objective of this study was to determine whether an endogenous circadian rhythm does exist in the oyster, *Crassostrea gigas*. The second objective was to characterize it in terms of robustness. To answer these questions, the valve activity of 15 oysters was continuously recorded for 2 mo in the laboratory under different entrainment and free-running regimes using a high-frequency noninvasive valvometer. The present work demonstrates the presence of a circadian rhythm in the oyster *Crassostrea gigas*. First, oysters were entrained by 12 L:12 D conditions. Then, free-running conditions (D:D and L:L) indicated that the most frequently observed period ranged from 20 to 28 h, the circadian range. That endogenous circadian rhythm was characterized as weak. Indeed, the period (τ) of the individual animals exhibited high plasticity in D:D and L:L, and the animals immediately followed a 4-h phase advance or delay. Additionally, *C. gigas* appeared as a dual organism: all oysters were nocturnal at the beginning of the laboratory experiment (January), whereas they were diurnal at the end (March). That shift was progressive. Comparison with a full-year in situ record showed the same behavioral duality as observed in the laboratory: the animals were nocturnal in autumn–winter and diurnal in spring–summer. The significant advantage of a plastic and dual circadian rhythm in terms of adaptability in a highly changing environment is discussed. (Author correspondence: d.tran@epoc.u-bordeaux1.fr)

Keywords: Biological rhythm, Bivalve, Clock, Diel, Dualism, Oyster, Weak oscillator

INTRODUCTION

Biological rhythms have been widely described in prokaryotes and eukaryotes, plants, and animals and seem to be ubiquitous across taxa. Indeed, living clocks constitute a fundamental property of life, from the gene to the ecosystem level, governing all behavioral, metabolic, and physiological functions of organisms (Bell-Pedersen et al., 2005; Dunlap, 1999; Hall, 1995; Panda et al., 2002). These rhythms, innate and endogenous, are entrained by external signals called zeitgebers (time givers). They are generally considered as adaptive, conferring to the organisms significant advantage, i.e., ability to anticipate changes in their environment and to adapt themselves to these oscillating variations (Ouyang et al., 1998; Pittendrigh, 1993; Yerushalmi & Green, 2009).

Although most chronobiology literature has considered terrestrial organisms, mainly governed by circadian rhythms, a significant amount of literature has been devoted to biological rhythms in marine organisms (for reviews see Naylor, 2010; Palmer, 1995). Indeed, a major interest in coastal zones is that they constitute highly complex biotopes, influenced by both solar and lunar cycles. Under the influence of various environmental oscillators, biological clocks in marine animals have often been reported as labile (Last et al., 2009; Naylor, 2010; Palmer, 1995). For many decades, different hypotheses have been put forward to explain the endogenous rhythms described in marine organisms (Kim et al., 2003). One hypothesis is the existence of two coupled, but separate, clocks—a circadian one and a circatidal one, each being unimodal (Naylor, 2010; Webb, 1976). The second hypothesis, given by Enright (1976), suggests that a single bimodal oscillator governs both circadian and circatidal rhythmicities. The third, and last, explanation envisions the presence of two unimodal clocks, both circalunidian, coupled in antiphase (Palmer, 1995, 1997).

The pacific oyster *Crassostrea gigas* is a marine bivalve of great economic importance that has successfully adapted to various biotopes around the world. Although

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Address correspondence to Damien Tran, Université Bordeaux, EPOC, UMR 5805, F-33120 Arcachon, France. Tel.: + 33(0)5 56 22 39 37; Email: d.tran@epoc.u-bordeaux1.fr

many aspects of its biology have been studied, its chronobiology remains largely unknown. A first insight was given by Tran et al. (2011), who indicated that oyster activity rhythms in situ are driven by a complex association of solar cycles and different lunar cycles. The behavior of permanently immersed oysters was shown to be primarily driven by the tidal cycle. Specifically, the tidal driver appeared modeled by synodic and anomalistic moon cycles: increased valve opening durations were associated with highest amplitude tides, whereas decreased valve opening durations were related with lowest ones. Importantly, under these natural in situ conditions, light appeared to be a significant zeitgeber of the oyster's biological rhythm, but its power was clearly weak in comparison with the tidal zeitgeber. This raised a series of questions that we address in the present work performed under simplified, but well-controlled, laboratory conditions, during which we specifically manipulated the light regime. In addition to an expected endogenous circatidal rhythm, does a circadian rhythm exist for the species *Crassostrea gigas*? If one does, how is it characterized? Is it a labile or robust clock? Are the animals diurnal or nocturnal? The study was carried out for 2 mo (January–March 2010) in the laboratory in Arcachon, France, by continuously monitoring oyster valve activity using high-frequency noninvasive techniques. Different entrained and free-running conditions were tested to characterize the putative existence of a potential circadian rhythm. The diurnal/nocturnal aspect of the behavior experimentally studied in the laboratory was then compared with a full-year record of field behavior.

MATERIALS AND METHODS

General and Experimental Conditions

All research detailed in this study complied with French law and was conducted in accordance with international ethical standards outlined in Portaluppi et al. (2010). The experiment was carried out in Arcachon, France (at the Marine Station) for 66 d, from January to March 2010, on 15 oysters (73 ± 1 mm shell length; 31 ± 1 g total fresh weight [shell + flesh]; 2 yrs old) *Crassostrea gigas* (Thunberg, 1793). The animals were collected at Le Grand Banc in the bay of Arcachon. The animals were acclimated for 1 mo in the laboratory, under 12 L:12 D, in the absence of tidal cycle, in running sea water.

Experimental Setup

During the entire experiment, the animals were isolated from external vibrations using an antivibrating bench and an isolated blind room to minimize any external influences on their spontaneous behavior. Experiments were performed in a 10.5-L tank (.35 × .25 × .12 m), continuously supplied with seawater (flow = 660 mL/min) of constant composition (17.0°C ± .2°C; chlorophyll a = .10 ± .07 μg/L, mean ± SD; pH = 8.0 ± .1, mean ± SE). Two tanks in series (45 and .2 m^3), with different retention times, were used to homogenize seawater pumped from the bay. Chlorophyll a and temperature were automatically measured every 5 min, with a 10 AU-005 Fluorimeter (Turner Designs, Sunnyvale, CA, USA) and electronic thermometer; pH value was measured daily with a R301 pH meter (Consort, Belgium).

Experimental Protocol

Seven series (lasting 8–10 d), based on different photoperiods, were performed: reference 12 L:12 D (10 d, light from 06:00 to 18:00 h Greenwich Mean Time [GMT]; series 1); D:D (10 d; series 2); reference 12 L:12D (10 d, light from 06:00 to 18:00 h GMT; series 3); 12 L:12 D with a phase advance (8 d, light from 02:00 to 14:00 h GMT; series 4); reference 12 L:12 D (9 d, light from 06:00 to 18:00 h GMT; series 5); L:L (10 d; series 6), ending with reference 12 L:12 D (9 d, light from 06:00 to 18:00 h GMT; series 7). L:D photoperiods in the laboratory matched those in the wild at the times of the experiments. Irradiance (photosynthetically active radiation, PAR) measured with the PAR (Biospherical Instruments, San Diego, CA, USA) was 19 $\mu E \cdot m^{-2} \cdot s^{-1}$ at water level during photophase (neon light MASTER TL-D Xtra 36W/865 1SL; Philips, Suresnes, France) and 1 $\mu E \cdot m^{-2} \cdot s^{-1}$ during scotophase.

Field Study

Field data were collected from 14 *C. gigas* oysters (87 ± 3 mm shell length) collected in the Bay of Arcachon and placed in an oyster bag attached to a concrete slab under Eyrac Pier (Bay of Arcachon). The oysters were permanently immersed. The animals were introduced in situ on December 6, 2006, and data were collected during the entire year of 2007 (n = 365 d). Experimental details are given in Tran et al. (2011).

Crassostrea gigas Behavior Measurements

The valve activity of the studied oysters was measured using a high-frequency noninvasive (HFNI) valvometer. It consists of lightweight electromagnets (.1 g) glued on both valves of each animal, with the electrodes linked by flexible wires to a laboratory or field valvometer. The measure is an application of Maxwell's law:

$$\varepsilon = -N.\frac{\partial \varphi_B}{\partial t}$$

where ε is the electromotive force (volts), N the number of turns in the coil, $\partial\varphi_B$ the magnetic flow (Webber), and t the time. For more details, see Tran et al. (2003) and Chambon et al. (2007). Sampling frequency for each individual was .2 Hz in the laboratory and .6 Hz in the field. Data were processed using Labview (National Instrument, Austin, TX, USA).

The laboratory study focused on the endpoints of mean hourly opening of each individual and of the group. Each hour, we considered the time oysters spent with their valves open. If an oyster was open for the

entire hour, the mean hourly opening was 100%. If the animal never opened its valves during an hour, it was 0%. All the intermediary stages in between did exist. To characterize the diurnal or nocturnal behavior in situ throughout the year, each day we calculated the difference in opening duration between the photophase and scotophase (www.imcce.fr).

Data Analysis

Double-plotted actograms (each line representing 2 d) were produced with Chronos-Fit 1.06 (Zuther et al., 2009). Activity levels >24-h average are represented by a black section, whereas levels <24-h average are represented by a white section. Chronobiological analyses were carried out using the software Time Series Analysis Serial Cosinor 6.3. Several steps were performed to first verify the quality of the data, then to determine the periodicity of the behavior of the oysters, if any, and finally model the potential rhythm. (Gouthière & Mauvieux, 2004; Gouthière et al., 2005a, 2005b).

Quality of the Data Set

First, we controlled for the absence of randomness in our data set using the autocorrelation diagram and then for the absence of a stationarity using a partial autocorrelation function (PACF) calculation (Box et al., 1994). These checks indicated a real biological or physical phenomenon.

Search for Periodicity

As suggested by Gouthière and Mauvieux (2004) and Gouthière et al. (2005a, 2005b), different methods were used to determine the period (τ) in our equally spaced data: Lomb and Scargle periodogram (Scargle, 1982), elliptic inverse spectral plot based on the surface of the confidence ellipse (Bingham et al., 1982; Nelson et al., 1979), autoperiodogram and autospectral plot of Jenkins and Watts (1968), and Fourier periodogram. Importantly, the different methods always gave identical periods to the first decimal. A period was accepted when shown to be significant by the Lomb and Scargle periodogram (spectral peak crossing the $p \geq .95$ line corresponding to $\alpha \leq .05$). The confidence interval of τ was determined by the method of Halberg (1969).

Modeling and Statistical Validation

Rhythmicity was then described and characterized with the cosinor model, which uses a cosine function calculated by regression to approximate the time-series data (Bingham et al., 1982; Nelson et al., 1979). The model for a given period is written as: $Y(t) = A \cdot \cos(2\pi t/\tau + \phi) + M + \varepsilon(t)$, where A is the amplitude (one-half the peak-trough variation), ϕ the acrophase (peak time), τ the period, M the MESOR (time series mean), and ε the relative error (Gouthière & Mauvieux, 2004; Gouthière et al., 2005a). It is necessary to check that the amplitude and phase remain constant over time, which was done using complex demodulation amplitude and phase plots (Granger & Hatanaka, 1964). Most of the time, the amplitude remained constant over time, but the phase did not, indicating complex phenomena that were not controlled. This has previously been mentioned for biological data (Gouthière & Mauvieux, 2004; Gouthière et al., 2005a, 2005b). Two tests were absolutely essential to validate the calculated model and existence of a rhythm: the elliptic test (Bingham et al., 1982) and probability of the null amplitude ($A = 0$) hypothesis must be rejected at $p < .05$. These tests were always validated when we mention a rhythm. Two chronobiometric parameters were evaluated: the percent rhythm, i.e., percentage of the cyclic behavior explained by the model, and percent error. For one given period (τ) and at a chosen probability ($p = .05$), secondary periodicities were investigated by replacing the current data set by the residuals of the cosinor calculated. Results are presented as mean ± 1 SEM. For all statistical tests, significance was considered to be reached at $p = .05$.

RESULTS

Existence of a Circadian Rhythm in *Crassostrea gigas*

Figure 1 presents the full data set obtained for each oyster (15 oysters) during the seven experimental conditions. The records are organized by eye. The record in the upper left part of the figure exhibits highest apparent order, and records shown at the bottom are less ordered. Such a presentation underlines the fact that many animals exhibited clearly cyclic valve activity under entrained 12 L:12 D conditions. Under free-running conditions of L:L and D:D (series 2 and 6, respectively), a pattern appeared, more obviously for some oysters (e.g., oysters 1 to 5) than others (e.g., oysters 11 to 15). This analysis is the first sign suggesting the existence of an underlying circadian clock. Furthermore, residual rhythms appears even under the L:D regime. These actograms also reveal strong variability among individual oysters, which appears more important than the variability within individuals.

Tracking down the potential existence of a circadian clock requires a deeper analysis, as depicted in Figure 2, which presents calculations conducted for the whole oyster pool. From left to right, it shows the mean hourly behavior as a function of experimental day (d 1 to d 8–10; Figure 2A); mean daily behavior for the corresponding experimental series and its cosinor model curve (Figure 2B); corresponding Lomb and Scargle periodogram (Figure 2C); and distributions of the first significant period for the individuals (Figure 2D). The spectral analysis shown in Figure 2C reveals a circadian τ of between 23.6 and 24.1 h for the group as a whole during the L:D series. Chronobiological models (Figure 2B) indicate that this rhythm explained from 49% to 63% of the pattern of the studied group under 12 L:12 D conditions. The same analysis was also conducted for each individual. For all series, it is worth noting all animals showed a significant period: 100% of the animals were rhythmic under L:D conditions. As

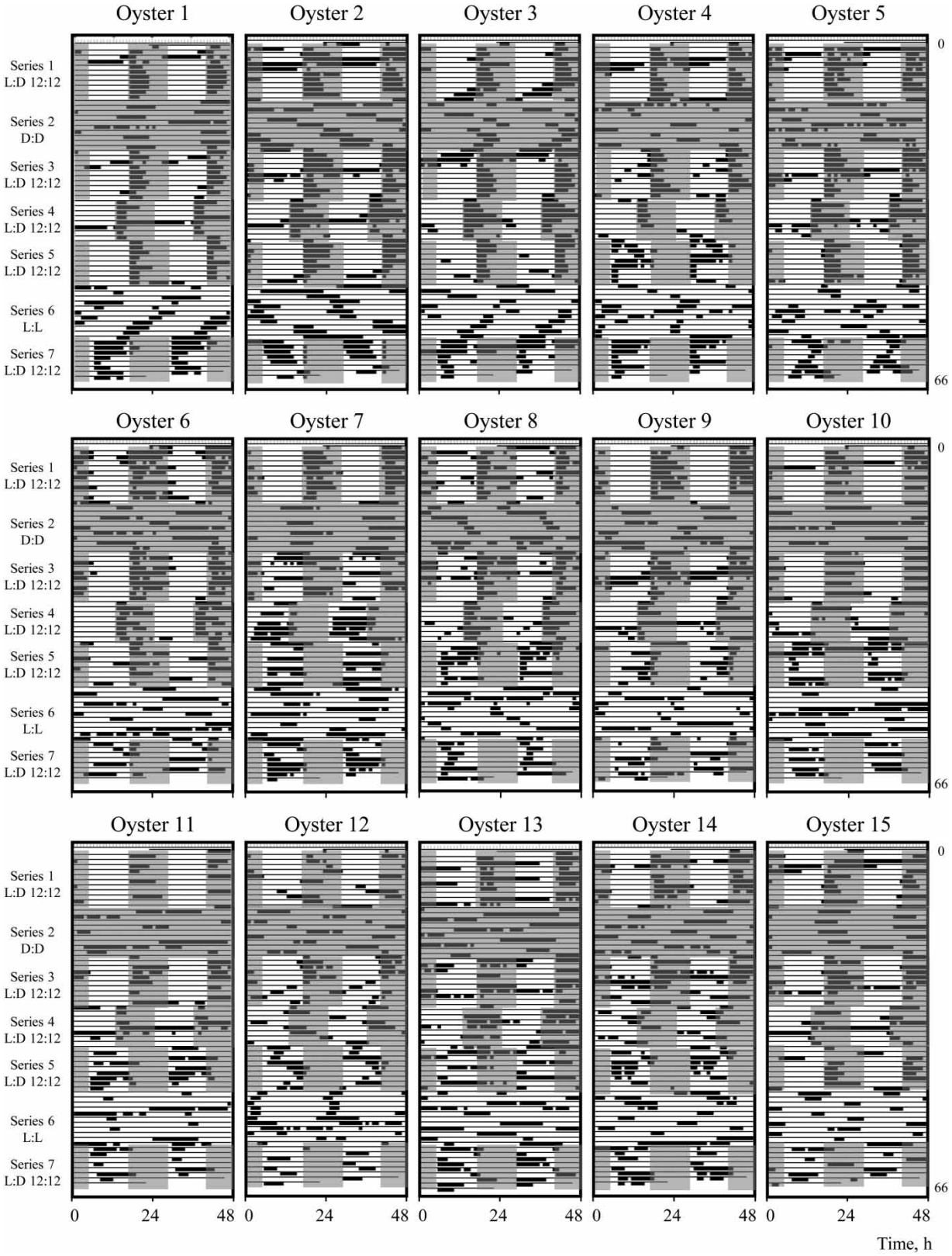

FIGURE 1. Individual actograms of valve activity in the oyster *Crassostrea gigas* based on the mean hourly opening (15 oysters, 7 temporal series, 66 d); 48 h on each line, starting at 02:00 h. Scotophase is indicated by gray rectangles. Organization of records was fitted by eye. The record exhibiting the highest apparent order was placed in the upper left, and the apparently less ordered records placed on the bottom.

indicated in Figure 2D, 100% of the animals presented a circadian rhythm in series 1. In series 3, 73% were circadian and 27% infradian (6.7% having a τ of 48.1 h and 6.7% presenting a τ of 70.3 h, periods close to harmonics of 24 h). In series 4, 73% were again circadian (6.7% with a τ of 49.2 h). In series 5, 93% of the oysters showed a

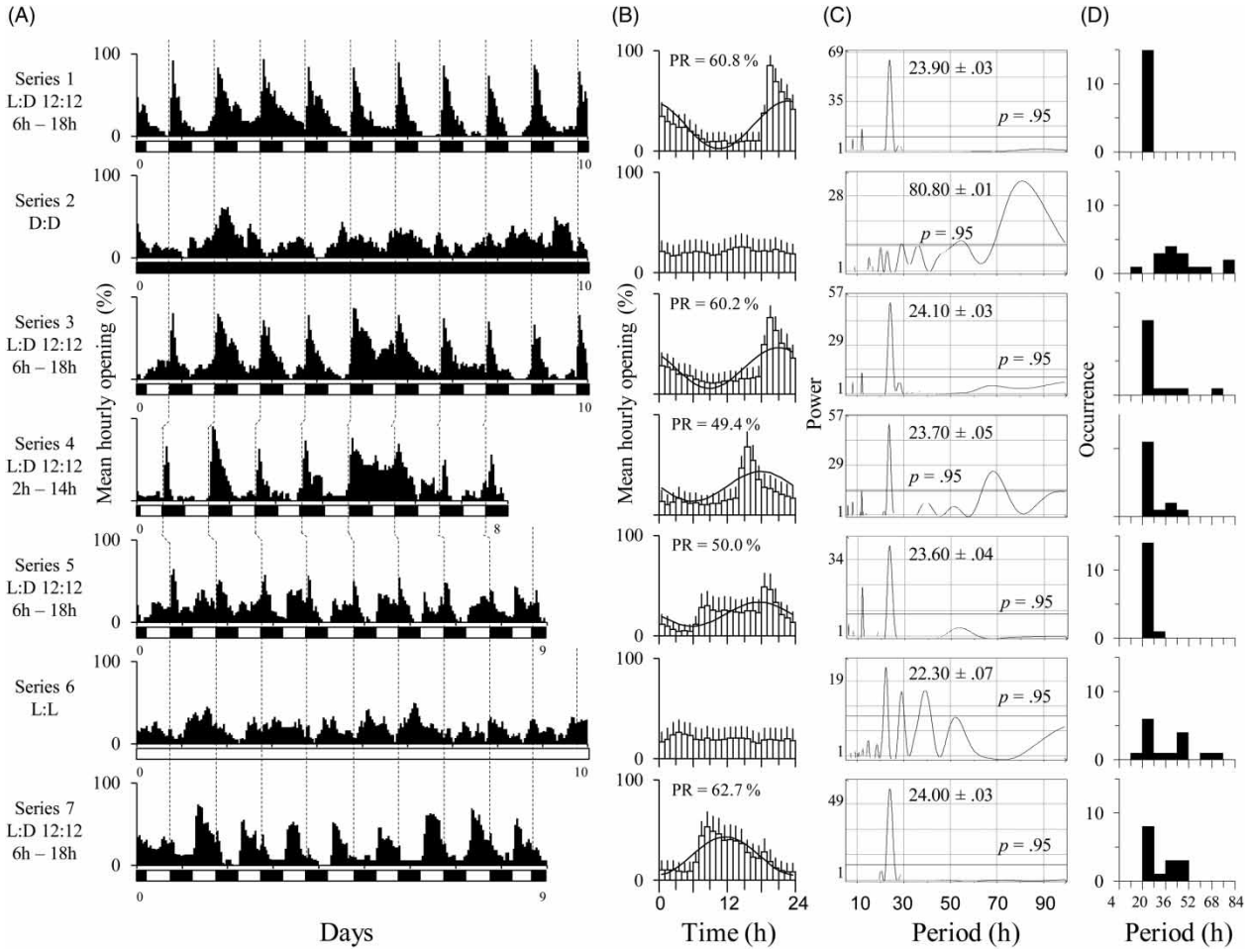

FIGURE 2. Chronobiological analysis in the oyster *C. gigas* at the group level. (A) Mean hourly opening (%) of the group (n = 15 oysters) for each series. (B) Mean daily behavior for each series (mean hourly opening ± SEM) and its cosinor model curve (and percent rhythm [PR]). (C) The period of the population (± SD) is determined by spectral analysis for each series (Lomb and Scargle periodogram). (D) Distribution of each first period for the individuals (n = 15 oysters).

circadian rhythm (7% being infradian), and in series 7, 53% of the individuals displayed a circadian rhythm and 47% an infradian one (with 20% being close to a τ of 48 h, their periods being 44.1, 46.0, and 50.2 h, respectively). A phase advance was applied between series 3 and 4 (+4 h) and a phase delay between series 4 and 5 (–4 h). As shown at the individual and group levels in Figures 1 and 2A and B, oysters rapidly shifted to the new scheduled photoperiod, indicating that the observable rhythm of circadian activity could easily be manipulated.

At the group level under free-running conditions (D:D and L:L; series 2 and 6, respectively), no circadian rhythm was observed, neither in the raw data (Figure 2A) nor in the mean daily behavior and chronobiological model (Figure 2B). Even if a circadian period was detected by spectral analysis (Figure 2C, series 6), under L:L conditions no significant rhythm was detected. There was variability, as expected, in the distribution of the first significant period among the individual animals (Figure 2D). Notwithstanding, 100% of the studied oysters remained rhythmic under L:L and D:D conditions. Under L:L conditions, three modes appeared more frequently, around 24, 48, and 72 h. Moreover, no circatidal period was observed in any free-running oyster. The mean hourly openings were 20.3% ± 9.8% in D:D and 18.8% ± 9.4% in L:L. There was no statistical difference between these two conditions ($p = .309$, t test).

Does a circadian rhythm exist then? To address this question, it was necessary to delve deeper into the details of the data. The first four significant periods for each individual were consequently taken into account in the free-running conditions of both D:D and L:L. The distribution of these periods is displayed in Figure 3, which extends the results presented in Figure 2. Both graphs clearly indicate that in D:D, as in L:L, the most frequently observed τ fell between 20 and 28 h, the circadian range. Furthermore, this figure indicates that there was similar variability in the expressed τ under both D:D and L:L conditions. Note that among these four first significant periods, we observed in our whole data set (107 significant periods) only two periods in the circatidal range (10.4–14.4 h) in D:D and only one in L:L. Based on the above data, one must, therefore, conclude that a

circadian rhythm of valve activity does exist in the oyster *C. gigas*. Further, the fact that this rhythm immediately follows a phase shift suggests the existence of a weak circadian oscillator.

Existence of a Dual Circadian Rhythm

Importantly, comparison of series 1 with 7 in Figure 2 shows that oysters under 12 L:12 D conditions were nocturnal in series 1 and diurnal in series 7, 2 mo later. This raised the question about the progressive or abrupt character of that change. To obtain more insight into this, we examined the transitory events. In series 5 (12 L:12 D), although the circadian profile shown in Figure 2A looks less obvious in comparison with all reference series (1, 3, and 7), chronobiological analyses showed a statistically significant circadian τ of 23.60 ± .04 h (Figure 2C), with high percent rhythm of the cosinor model. We extended the analysis in Figure 4. Two subgroups were distinguished according to the time the animals spent open during the day and night. An oyster was considered diurnal if mean hourly opening$_{day}$ > mean hourly opening$_{night}$ + 10%. The opposite calculation was used to determine which animals were nocturnal. For series 5, 8 oysters out of 15 presented diurnal behavior, whereas 6 oysters showed nocturnal activity and 1 oyster mixed behavior. The analysis shown in Figure 2 was then applied to these three subgroups. Clearly, the mean hourly opening (%) of the whole group, characterized by a circadian τ of 23.6 h (Figure 4A), consisted in fact of two superimposed circadian rhythms (compare Figure 4B and C, both with τ = 23.8 h). In Figure 4B, oysters typically exhibit a diurnal pattern (compare with Figure 2, series 1), whereas in Figure 4C the pattern is nocturnal (compare with Figure 2, series 7). Finally, note the mean opening time did not differ among the subgroups: 22.6% ± 6.5% for diurnal, 19.4% ± 6.6% for nocturnal, and 18.7% ± 2.9% for mixed oysters.

To go further, Figure 5A presents the progressive change in the mean hourly opening during photophase

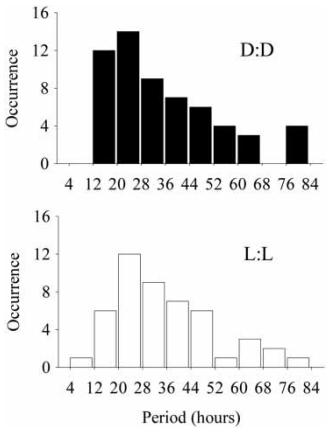

FIGURE 3. Free-running studies in the oyster *C. gigas* (same data as in Figure 2). Cumulative distribution of the first four significant periods in D:D and L:L (n = 15 oysters). The most frequently observed period (τ) in both conditions lies in the circadian range of 20–28 h.

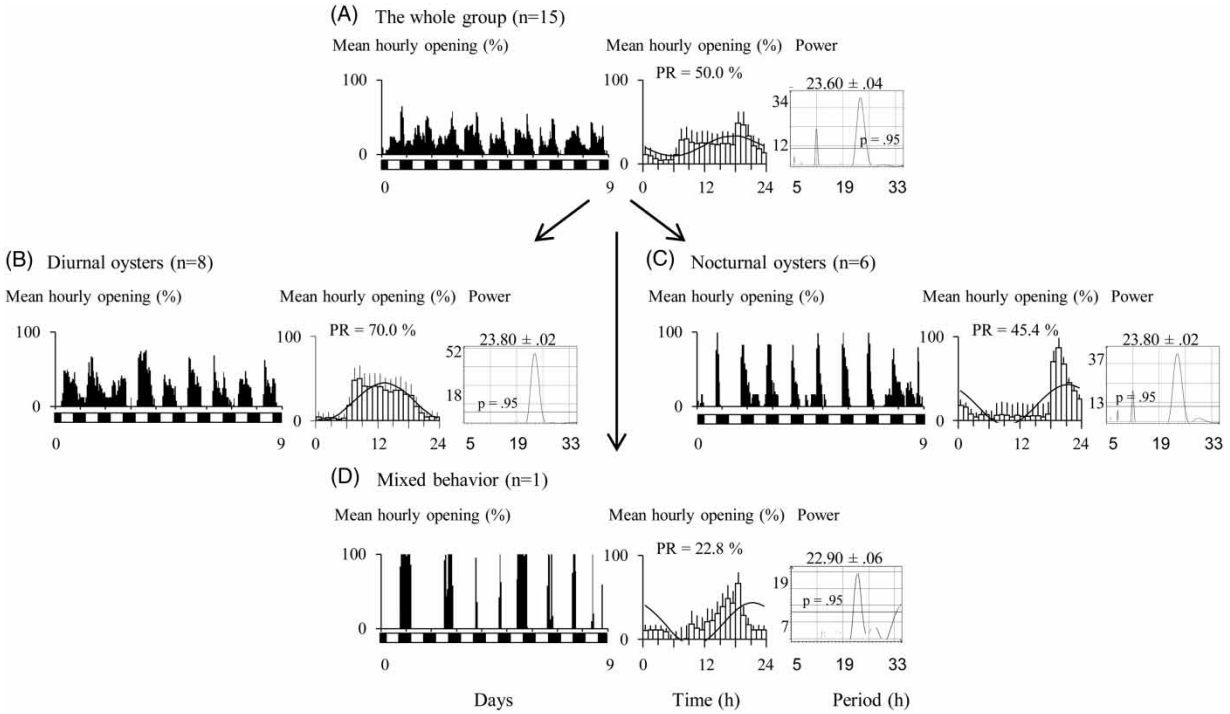

FIGURE 4. Subanalysis of series 5 (12 L:12 D) from Figure 2, late February to early March. (A) Whole group, (B) diurnal oysters, (C) nocturnal oysters, and (D) single oyster with mixed behavior. In each panel, from left to right: mean hourly opening (%) of the group; mean daily behavior (mean hourly opening ± SEM) with its cosinor model curve (and percent rhythm [PR]); period (± SD) determined by spectral analysis (Lomb and Scargle periodogram).

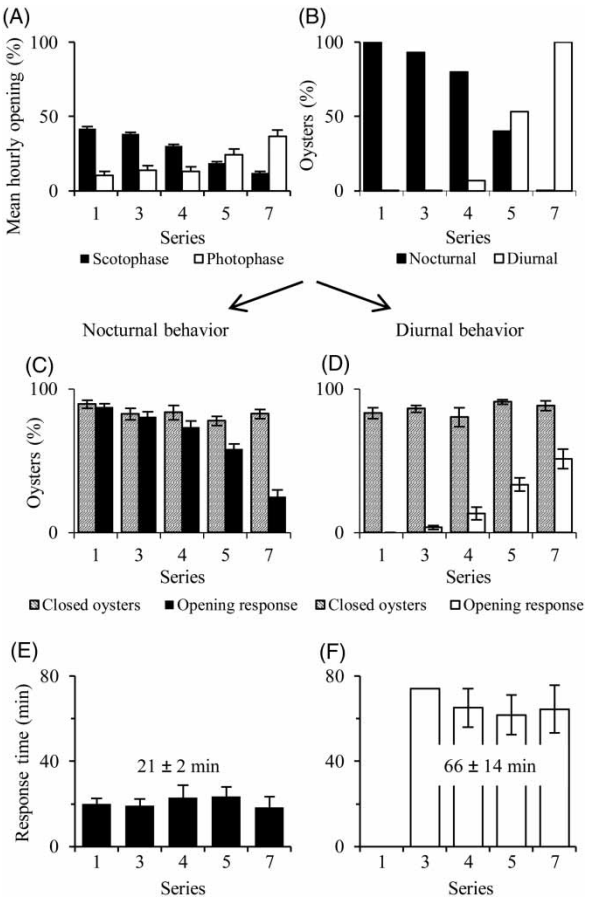

FIGURE 5. Progressive switch from nocturnal to diurnal circadian rhythm. The analysis focuses on L:D series exposed in Figure 2. (A) Mean hourly opening (%) of the group (n = 15) during photophase and scotophase for each 12 L:12 D series. (B) Number of oysters presenting a diurnal or a nocturnal behavior for each series; an oyster was considered as diurnal if mean hourly $\text{opening}_{\text{day}}$ > mean hourly $\text{opening}_{\text{night}}$ + 10 %. Oysters (%) opening their valves at (C) lights-off and (D) at lights-on. Mean reaction time (min) per series: Inserts in (E) and (F): mean time responses. For series 1, 3, 4, 5, and 7, respectively, n = 10, 10, 8, 9, and 9 d.

and scotophase throughout the five different 12 L:12 D series exposed in Figure 2. Although the total opening duration remained stable for all L:D series (21–26%), the majority of the opening activity moved progressively from scotophase to photophase. Figure 5B indicates that 100% of the oysters were nocturnal in series 1, but became 100% diurnal in series 7. This change of pattern is illustrated again by the progressive change of reactivity to lights-off and lights-on exhibited by all animals. Indeed, Figure 5C and D show that, independent of the series, the percentage of closed animals before the light switch remained constant, but the percentage of responses to lights-off decreased with time (from 88% to 25%), whereas the percentage response to lights-on increased (from 0% to 51%). Moreover, it is worth noting that the response time remained constant throughout the experimental series. It was 21 ± 2 min after lights-off (Figure 5E) and 66 ± 14 min after lights-on (Figure 5F).

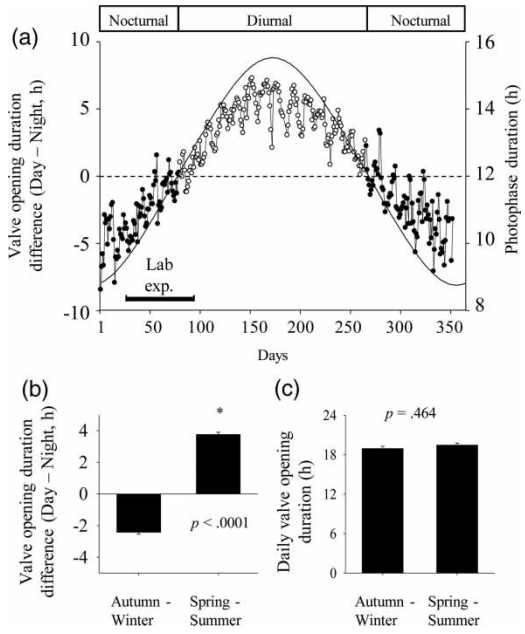

FIGURE 6. Day and night field behavior of valve opening in the oyster *Crassostrea gigas*. (A) Change of valve opening duration difference (day – night, h) in the Bay of Arcachon (France) during the entire year of 2007. Lab exp. = laboratory experiment. (B) Valve opening duration difference for the contrasted periods autumn–winter and spring–summer. (C) Comparison of the daily valve opening duration between autumn–winter and spring–summer. *C. gigas* is dual in the Bay of Arcachon.

Is this dual behavior an artifact of our experimental protocol or is it a natural feature? To test this hypothesis, we searched for the same behavior in a full-year record of oysters from the Bay of Arcachon, France (2007; Tran et al., 2011). As shown in Figure 6A, a similar dual behavior, in relation to the photoperiod, was exhibited by oysters in their natural environment. Although the daily valve opening duration did not differ between autumn–winter (scotophase > photophase) and spring–summer (photophase > scotophase; Figure 6B), oysters presented diurnal behavior in spring and summer and nocturnal behavior in autumn and winter (Figure 6C). The behavioral change was clearly progressive, and the distribution of data points compared with the photophase duration shows that the switches in the Bay of Arcachon were close to the spring and autumn equinoxes. Consequently, both the data obtained in the laboratory and in the field demonstrate the dual behavior of the circadian rhythm in the oyster *C. gigas*.

DISCUSSION

Existence of a Circadian Rhythm in *Crassostrea gigas*

The objective of this study was to determine if a circadian rhythm exists in the oyster *Crassostrea gigas* and to derive more insight into its characteristics. Indeed, Tran et al. (2011), following a 1-yr continuous valve-activity record in the Bay of Arcachon (North Atlantic Ocean, Western Europe), reported weak in situ expression of a daily rhythm compared with a strong circatidal component

in oysters. Here, we searched for the existence of a circadian clock in *C. gigas* under simplified, but well-controlled, laboratory conditions of different L:D regimes and without a tidal component (no current, no hydrostatic change). Our work clearly shows the existence of an endogenous, weak and dual, circadian clock in *C. gigas*. We did not observe any experimental support for the existence of an endogenous circatidal rhythm. These conclusions are based on four observations. First, under the 12 L:12 D entrainment regimes, both the group and the individuals exhibited clear cyclic valve activity, presenting a daily pattern (τ between 23.6 and 24.1 h). Second, the behavior under free-running conditions, considering the first four significant periods of individuals, showed that most periods were circadian, demonstrating that this characteristic is endogenous. Third, high variability among the period of the individual oysters, similar in D:D and in L:L, was observed. Fourth, the circadian rhythm was easily entrained: the animals immediately followed a 4-h phase advance and delay (Figures 1 and 2, series 3, 4, and 5).

Even if tidal rhythms have been widely reported as the major rhythm, circadian rhythms have also been mentioned in marine organisms. The reported observations are listed and summarized below in this paragraph. The worm *Nereis virens* expressed burrow emergence activity that could be either circatidal, circadian, or circalunidian, depending on entrainment conditions (Last et al., 2009). Together with a circatidal one, circadian rhythmicity has been described in the locomotor activity of the crab *Carcinus meanas* (Naylor, 2010; Reid & Naylor, 1989). The tropical labrid fish *Halichoeres chrysus* showed a daily locomotor activity rhythm in the laboratory, both under entrainment and free-running conditions (Gerkema et al., 2000). The fish *Takifugu obscurus* (8 to 12 mo of age) exhibited a circadian rhythm of instantaneous rise of oxygen consumption in constant darkness (Kim et al., 1997).

Although the above literature is scarce, the chronobiological literature for bivalves is even more scarce. It is mostly descriptive and/or based on a relatively small number of observations in terms of time series or number of individuals and, to our knowledge, no chronobiological study has been previously published on *C. gigas*. Bivalve reports include the following. The gaping activity of the Mediterranean molluscan bivalve *Pinna nibilis* exhibited both apparent circadian and circalunar cycles in situ, under permanent subtidal conditions (Garcia-March et al., 2008). Under laboratory conditions, valve activity of unfed immersed *Mytilus edulis* was found to display a weak circadian pattern in L:L (Ameyaw-Akumfi & Naylor, 1987). Robson et al. (2010) did refer to a daily cycle in animals exposed to natural light without tides. Wilson et al. (2005) reported nocturnal activity in gaping frequency in mussels transferred from laboratory to field conditions. Thus, to our knowledge, the present work is the first to demonstrate the existence of a circadian rhythm of valve activity in a molluscan bivalve.

A weak circadian oscillator. Gwinner and Brandstätter (2001), working on bird rhythms, summarized the general principles of oscillatory theory for a weak oscillator. Specifically, reduced degree of self-sustainment of an oscillator implies (i) increased range of entrainment in the synchronized state, which also presents decreased resynchronization time after a zeitgeber phase shift; and (ii) damped or less stable rhythmicity under constant conditions (Aschoff, 1981; Klotter, 1960). Moreover, applying a phase shift in the L:D cycle and counting the number of transient cycles before resynchronization is currently used to evaluate the strength of the endogenous control of circadian rhythms (Aschoff, 1960). An example of the link between the ability of an organism to shift its phase and strong endogenous control of a rhythm is well known by travelers who cross several time zones rapidly. They experience a transient desynchrony between their endogenous circadian rhythm and their new environment. On average, in humans the resynchronization process requires ~1 d per time zone traversed (Haimov & Arendt, 1999; Kunz & Herrmann, 2000). Again, in aquatic animals, relatively few data are available, but the situation appears quite different. The only available data were obtained in fish. Specifically, it is known that the sea bass is able to shift immediately after a 12-h reversal of the LD cycle, indicating a rather weak circadian oscillator (Sánchez-Vázquez et al., 1995a). In the present work on oysters, a forward or backward shift of 4 h was immediately followed by the animals, without any transient desynchrony (Figure 2, series 3, 4, 5). We suggest that this constitutes major support for the hypothesis of a weak endogenous clock in *C. gigas*, as the number of transient cycles before resynchronization is zero. Is the absence of transient cycles before resynchronization a masking effect? This is unlikely, as there is no statistical difference in valve opening duration in D:D and L:L, whereas positive and negative masking effects are known to respectively enhance and decrease activity (Mrosovsky, 1999). A second indication favoring the weak oscillator hypothesis in *C. gigas* is the large distribution of τ under free-running conditions. As stated by Aschoff (1960), the accuracy of a circadian clock is measured by how precisely the clock keeps a circadian period under free-running conditions. The data shown in Figure 3 indicate that although a circadian range dominates, the distribution spread in τ ranges from 4 to 84 h in free-running conditions (D:D and L:L). Finally, actograms also point out the strong variability among individual oysters, which is another argument favoring the existence of a weak oscillator in *C. gigas*. Some individuals display strong rhythmic behavior, whereas others present very weak or even absent patterns of rhythmic behavior. For the latter, the oscillator may be exceptionally weak and their behavior might be mainly exogenously driven.

In terms of the ecologically relevant advantages of a weak oscillator, it is worth noting that the oyster *C. gigas* is a species found worldwide. Initially present in the Okhotsk sea, in Japan and Korea, it has been introduced to the North American Pacific coast from South Alaska to California, to Europe, and to Australia (Ruesink et al., 2005; Troost, 2010). A weak endogenous oscillator would certainly give plasticity to the oyster *C. gigas*, partly explaining its worldwide success and adaptability to various environments. Finally, this whole set of conclusions about the weakness of the oscillator is in agreement with Palmer (1995) and Naylor (2010), who considered tides to be less regular than day/night alternations in terms of period, tide amplitude, geographical location, and weather situations. Palmer (1995) proposed the selection pressure on the clock of marine organisms might, therefore, have been much less towards the acquisition of an accurate clock.

Existence of a Dual Circadian Rhythm

In the present work, we surprisingly observed that oysters were all nocturnal at the beginning of the laboratory experiment in January, but diurnal by the end of it in March, with a different activity pattern during each period. The shift was not abrupt, but progressive from series 1 to 7 (Figures 2, 4, and 5). A comparison with field data (Figure 6) revealed that the phenomenon does exist in situ, the animals being nocturnal in autumn and winter and diurnal in spring and summer. This, again, is a new and original observation in mollusk bivalves.

Is it only typical of C. gigas or is it part of a more general pattern? To our knowledge, only a few examples of either diurnal or nocturnal bivalves are alluded to in the literature, but without clear-cut evidence. *Pinna nobilis* has been reported to be diurnal, closing its valves at night (Garcia-March et al., 2008). This is also the case for the giant clam *Hippopus hippopus* (Schwartzmann et al., 2011). The blue mussel *Mytilus edulis* is the opposite; it has been reported to be nocturnal (Robson et al., 2010; Wilson et al., 2005). The situation is better documented in fishes. Activity patterns include either strict diurnality or nocturnality or switches between these two behaviors, and the behaviors are often qualified as plastic (Reebs, 2002). Specifically, Sánchez-Vázquez et al. (1995a) reported a dual pattern of feeding activity in sea bass in the laboratory, with coexisting diurnal and nocturnal fish. The authors mentioned that flexibility of the circadian system is required to have this plasticity in phasing. This plasticity is even stronger in the goldfish *Carassius auratus*, whereas some fish appeared strictly diurnal or nocturnal in the laboratory, some night-active animals displayed day feeding and vice versa (Sánchez-Vázquez et al., 1996).

In fish, the main reasons for a switch are explained in terms of food availability, photoperiod, temperature, predation risk, and light intensity (Bolliet et al., 2001; Eriksson, 1973, 1978; Fraser et al., 1995; Reebs, 2002; Sánchez-Vázquez et al., 1995b, 1997). In oysters, only a series of hypotheses can be proposed to explain the presently observed dual behavior. The Atlantic Ocean is characterized by semidiurnal tides, which means that two tides occur per day. Furthermore, variations in tidal height follow an annual cycle: in spring and summer daytime high tides are higher than nighttime ones, whereas in autumn and winter the opposite is true. However, in Arcachon, (i) differences in water height between the two high tides are small, ranging from 0% to 10% at Eyrac Pier; and (ii) the tidal pattern reverses on a regular basis. Thus, it seems very unlikely that this cycle could explain the statistical behavior patterns exhibited by oysters in the present work. An alternative explanation is that oysters are sessile filter-feeders that feed mainly on phytoplankton. In temperate regions, phytoplankton exhibit a seasonal cycle. After the winter period of lowest plankton abundance, spring is generally characterized by high abundance and by algal blooms. Blooms occur in summer and autumn as well (Cloern, 1996; Glé et al., 2010). Spring is also the beginning of the multiplication and maturation of gametes in oysters (Enriquez-Diaz et al., 2009; Marteil, 1976), and these annual changes are associated with annual change in metabolic demand (Tran et al., 2008). A strict maintenance of nocturnality, for example, would be counterproductive in terms of feeding purposes during the warm season. So, we suggest a link between dualism in *C. gigas*, food availability, gametogenesis, and metabolic demand. In oysters, the adaptive value of dualism could be a response to changes in energy needs at the circannual level.

Figure 6 shows that in *C. gigas* in the Bay of Arcachon, switches happened at the time of the spring and autumn equinoxes, when day and night are nearly the same length. It is remarkable to note that, both in the laboratory and in the field, the switches were nearly synchronous, even though in the laboratory the animals were disconnected from field conditions for 2 mo, exposed to a consistent 12 L:12 D regimen, except under free-running conditions. This suggests potential internal origin for this annual rhythm, as under laboratory conditions an ecologically relevant zeitgeber, if any, was unavailable. Thus, we may have observed the circannual clock operating in free-running conditions.

CONCLUSION

The present work demonstrates that a circadian rhythm exists in the oyster *C. gigas,* although an apparent circatidal rhythm dominates in the field (Tran et al., 2011). The rhythm is driven by a weak oscillator, allowing plastic behavior and dualism. Specifically, a circatidal rhythm in free-running conditions was not observed. This raises the question about the existence of a circatidal clock and gives new insights into the hypotheses previously proposed to account for biological rhythms in marine organisms (Enright, 1976; Naylor, 2010; Palmer, 1995, 2000; Webb, 1976; Williams, 1998).

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