

In situ biomonitoring shows seasonal patterns and environmentally mediated gaping activity in the bivalve, *Pinna nobilis*

Jose R. Garcia-March¹ · Santiago Jiménez² · Miguel A. Sanchis¹ · Sergio Monleon¹ · Jonathan Lees³ · Donna Surge³ · Jose Tena-Medialdea¹

Received: 15 September 2015 / Accepted: 4 January 2016 / Published online: 18 January 2016

Abstract The rhythms and responses of animals to environmental factors are important issues for their adaptation to natural cycles. These rhythms assure an optimum synchrony between organisms and their environment. Bio-logging enables monitoring these activity cycles remotely. To characterize rhythms and responses of fan mussels (*Pinna nobilis*) to environmental factors, six individuals were monitored from April 2009 to October 2011. The study was conducted at a station in the western Mediterranean at 11 m depth in Tabarca Island Marine Reserve (Alicante, Spain). Sensors at the station monitored dissolved oxygen (mg l^{-1}), turbidity (ntu), temperature ($^{\circ}\text{C}$), chlorophyll *a* concentration (chl *a*) (mg m^{-3}), current speed (cm s^{-1}), and direction ($^{\circ}$). One pattern of gaping activity (P1) occurred from mid-July–early August–early November, whereas another pattern (P2) occurred the rest of the time (i.e., from early November–mid-July–early August). The activity was synchronized among the fan mussels and showed autocorrelation peaks at a period of 21.9–24 h. In P1, the fan mussels

opened their valves according to the position and illumination of the sun and moon. In P2, however, individuals did not track sun and moonlight, although their gaping activity was regular and synchronized. Likewise, individuals were unaffected by high-frequency (daily) variation in dissolved oxygen and (chl *a*). Gaping activity was directly influenced by current intensity and direction. The shift between the two patterns and the presence of similar periods of autocorrelation in the activity time series indicate that *P. nobilis* has an internal clock.

Introduction

Gaping activity in bivalves reflects their biological processes and responses to environmental variation (Jou et al. 2013). The shell is the primary defense against predators and adverse conditions; thus, its closure efficiently isolates bivalves from external threats or water contaminants (Tran et al. 2007; Bae and Park 2014). This feature has been exploited to develop early warning systems of water pollution (Sluyts et al. 1996; Borcharding 2006; Liao et al. 2009; Sow et al. 2011; Bae and Park 2014). A few studies identified variations in gaping activity with changes in temperature that are probably related to biological oxygen demand (Rodland et al. 2009), physiological oxidative stress triggered by symbiotic zooxanthellae (Schwartzmann et al. 2011), or different algal concentrations (Ortmann and Grieshaber 2003; Riisgard et al. 2003). A plastic and dual circadian rhythm has been described in *Crassostrea gigas* (Mat et al. 2012). Gaping activity also reflects behavioral patterns entrained by extrinsic factors and regulated by internal biological clocks (Palmer 2000; Ortmann and Grieshaber 2003; Sobrino-Figueroa and Caceres-Martinez 2009; Robson et al. 2010). Biological activities are

Responsible Editor: J. Grassle.

Reviewed by A. A. Robson and D. Tran.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-016-2812-3) contains supplementary material, which is available to authorized users.

✉ Jose R. Garcia-March
jr.garcia@ucv.es

¹ Institute of Environment and Marine Science Research (IMEDMAR), Universidad Católica de Valencia SVM, C/Explanada del Puerto s/n, 03710 Calpe, Alicante, Spain

² Instituto de Ecología Litoral, El Campello, Alicante, Spain

³ Department of Geological Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

organized into different cycles, from sub-daily (ultradian) to daily (circadian) and monthly or seasonal (circalunidian, circannual). These cycles assure optimum synchrony between organisms and their environment (Rensing et al. 2001; Tran et al. 2011; Mat et al. 2012). Such shell closures produce circadian to ultradian microgrowth patterns, increments, and lines (Rhoads and Lutz 1980; Rodland et al. 2009). In fact, (Schwartzmann et al. 2011) demonstrated the close relationship between periodic gaping activity and regular growth line formation.

In the western Mediterranean, gaping activity of the fan mussel, *Pinna nobilis*, follows marked circadian and circalunidian rhythms from June to October with long periods of valve closure at night. Fan mussels from the same population monitored in situ followed the same behavioral pattern (García-March et al. 2008). According to de Gaulejac (1995), fan mussels are serial hermaphrodites with asynchronous gametogenesis and multiple spawnings between June and September. However, recent data from collectors (Cabanelas-Reboredo et al. 2009) show a distinct recruitment peak in late August–early September in the western Mediterranean, which may suggest a single spawning period.

Although suboptimal temperatures have been identified as the primary cause for prolonged valve closure, which affects shell growth for some bivalves (Rodland et al. 2009), circadian rhythms modulated by sun- and moonlight could be the primary trigger for valve closure in subtidal bivalves inhabiting non-tidal seas, such as the fan mussels. Based on previous knowledge, we tested the hypothesis that *P. nobilis* has a regular pattern of gaping activity mainly modulated by sun- and moonlight and position. Variations of this regular pattern, associated with strong storms and phytoplankton blooms, were expected. Also, it was hypothesized that hydrodynamic stress and light attenuation due to storms would affect gaping behavior by increasing the number of closures and reducing the maximum gape. To determine the influence of environmental factors other than tides and sun- and moonlight on the gaping activity of *P. nobilis*, an experiment was designed to monitor in situ the gaping activity of six individuals together with environmental data [sun and moon position, water temperature, dissolved oxygen (DO), current direction and intensity, chlorophyll *a* concentration (chl *a*) and turbidity]. Previous studies demonstrate that laboratory conditions alter the cycles and behavior in bivalves (Williams and Pilditch 1997; Kim et al. 2003; Robson et al. 2010). The effects of laboratory settings could, therefore, confound the interpretation of gaping activity data in relation to environmental variables. For this reason, in situ experiments were considered more appropriate than tank studies for the present study.

Materials and methods

Study area

The research was carried out in the Marine Reserve of Tabarca (southeast Spain, Mediterranean Sea), a small archipelago ~15 km southeast of Alicante and 9 km east of Santa Pola (Online Resource 1). Waters around Tabarca (1.754 ha) were declared a Marine Reserve in 1986, the first of its kind in Spain. This status was mainly granted due to the submarine *Posidonia oceanica* meadows that surround the island and extend to depths of ~30 m. The meadow is not continuous because of sandy patches and rocky bottoms. The sampling station was at the south end of Tabarca Island (N38°09'42.2", W0°28'51.9") at 11 m depth (Online Resource 1). The area is characterized by *P. oceanica* meadows with sandy patches and the seasonal (June–September) presence of the invasive macroalga, *Caulerpa racemosa*, which occasionally smothers small patches of *P. oceanica*.

The sample site was selected because of the abundance of adult fan mussels. The individuals were measured using a modified tree caliper (García-March et al. 2002), and shell length (SL) was estimated using the equation by García-March and Ferrer (1995). Six adult mussels (50–60 cm SL) were identified in a circular area 20 m in diameter. The position of each specimen was determined by their distance to the center of the circle and by the angle of the arc with respect to magnetic North. The center of the sample station was positioned with a GPS (EGNOS correction) (García-March and Vicente 2007). All selected individuals were marked with a float near them attached to the seabed with a camping peg. Specimens 1–5 had a marked N–S component in their shell orientation. Individual 6, however, had a clear E–W orientation of its valves (Online Resource 2).

Oceanographic sensors

Ambient environmental conditions were characterized using a combination of in situ oceanographic measurements. A sensor rosette equipped with an Alec probe [DO, turbidity, temperature, and (chl *a*)], and an electromagnetic current meter (Infinity-EM AEM-USB) was installed in a position minimizing the distance to the monitored individuals. The sensors had a precision of 0.01 mg l⁻¹ for DO, 0.1 ntu for turbidity, 0.01 °C for temperature, 0.01 mg m⁻³ for (chl *a*), and 0.01 cm s⁻¹ and 0.1° for current speed and direction. Oceanographic data were collected every 10 min. The sensors were recovered once every 2 months for maintenance (battery replacement and sensor cleaning) and to download the data. Comparisons of monthly averages were

performed for only those months with at least 20 days of measurements.

Chlorophyll *a* concentration (chl *a*), measured by fluorescence, was monitored to evaluate low-frequency variations in primary productivity and to detect phytoplankton blooms. Turbidity, measured by nephelometry, was studied to evaluate the response of *P. nobilis* to changes in water transparency. These changes can be produced by different sources such as bottom sediment resuspension or phytoplankton blooms. Both (chl *a*) and turbidity sensors can produce sporadically anomalous outliers with points many times above the previous value. These are biased measurements usually due to debris affecting the sensors. To solve this problem, the data were replaced with the previous value when single observations were ten times the previous one. Current speed was measured to detect disturbance from hydrodynamics, usually produced during storms or days with strong swell. This single sensor was installed 1 m above the seabed, above the *Posidonia oceanica* leaves, to avoid their interference with the sensor; therefore, it was unaffected by the attenuating effect of the meadow (van Keulen and Borowitzka 2002; García-March et al. 2007). Current direction was measured to the nearest degree. The rest of the sensors were installed 50 cm above the seabed.

Gaping activity sensor

The biomonitoring system was installed in a small sandy patch within the meadow, attached to the seabed with ropes tied to stainless steel rods. Gaping activity was measured using magnetoresistive sensors (Honeywell[®]). These sensors were saturated in a magnetic field generated by a rare earth magnet, producing a precise reading for an opening of up to 25 mm (which is several millimeters > the maximum gape of live fan mussels). The sensor was encapsulated in a Plexiglas waterproof housing (40 × 30 × 5 mm) and calibrated in the laboratory. It was installed in situ according to laboratory specifications (García-March et al. 2008). To save energy, the system was dormant most of the time. Every second the system was active for a few milliseconds to record gape positions. To save memory, only when the control card sensed that at least one of the six fan mussels

had changed its gape by ≥ 1 mm, a new data line was recorded for all individuals. Data recording occurred during the few milliseconds that the system was active. To generate a continuous time series, the gape positions for every second were extrapolated from the recorded data (note that when a line was not recorded in a given second, its values were considered identical to the last recorded line). This procedure reduced energy consumption and maximized memory space. This system can be installed underwater without any links to land for up to 6 months powered by a 6-V 5-Ah lead battery and with a 1-Gb memory SD card for data storage. Although the possible measurement resolution was every second, the raw data were subsampled at 20-s intervals to facilitate the calculations given the vast amount of data after ~2 years of monitoring.

The selected specimens were monitored during five different time intervals (T): T1 April 8–May 20, 2009; T2 July 29–October 2, 2009; T3 February 5–July 6, 2010; T4 August 10–February 5, 2011; and T5 April 5–October 24, 2011, for a total recording duration of 639 days (Table 1 and Online Resource 3–6). For practical purposes, the dates will be expressed in Julian days (jd) in the graphs, with January 1, 2009 as jd 1. Each individual was fitted with a unique sensor (i.e., the same sensor was reused for the same individual). During T1, we performed a preliminary test with the new sensors and system, and not all sensors were installed. Only specimens 1, 3, and 4 were monitored at that time. During T2 (a total of 65 days), specimens 3 and 4 were monitored for 47 and 35 days, respectively. Their monitoring interval overlapped during the first 35 days of T2. The sensor attached to specimen 2 failed during T3 and only data for sensors 1, 3, 4, 5 and 6 were recorded. For the longest monitoring intervals (T4 and T5), all individuals were monitored.

Data treatment and statistical analyses

A data analysis program, GapeR, was developed using the open-source R platform to manage the vast amount of gaping activity data. GapeR enables the visualization of all data in intervals from 1 day to the length of the monitoring interval, including all oceanographic sensor data.

Table 1 Periods of operation of oceanographic and activity sensors

	From	To	Days	Individual	Observations
T1	April 8, 2009	May 20, 2009	42	1, 3, 4	Preliminary assays
T2	July 29, 2009	October 2, 2009	65	1, 2, 3, 4, 5, 6	Individual 3 July 29–September 14 and individual 4 July 29–September 2
T3	February 5, 2010	July 6, 2010	151	1, 3, 4, 5, 6	Individual 2 missing data
T4	August 10, 2010	February 5, 2011	179	1, 2, 3, 4, 5, 6	
T5	April 5, 2011	October 24, 2011	202	1, 2, 3, 4, 5, 6	

T1–T5, time intervals 1–5

Furthermore, GapeR enables the visualization of one individual or one sensor to all individuals and sensors, together with sun and moon position and illumination. This program was used to monitor activity trends, oceanographic sensor variation, detect outliers and describe the effects of light on gaping activity.

For the statistical treatment of the activity data, the main frequencies occurring in the time series were checked using fast Fourier transform (FFT). Then, the time series were filtered both with a second-order, low-pass Butterworth filter (pass frequency $< 48 \text{ h}^{-1}$) and a second-order, band-pass Butterworth filter (low frequency $= 48 \text{ h}^{-1}$, high frequency $= 15 \text{ h}^{-1}$). The Butterworth filter was chosen because it provides a monotonic response and the maximally flat pass-band response. To overcome the transient effect of the filter, both ends of all time series were expanded adding 200 values with their mean. Once the filter was applied, the 200 values in the tails were cut again to keep the original but filtered data. To test the synchronicity of gaping activity among individuals, the cross-correlations of the time series (raw data and low-pass- and band-pass-filtered data) were calculated in individuals monitored simultaneously. For this exercise, identical time windows were used, adjusting the length and the dates studied to the shorter of the two time series compared. The oceanographic data were also filtered with the same low-pass Butterworth filter, and raw and low frequencies of oceanographic data were compared to the activity data to check for correlations among them. The filters and statistics were applied using R code.

The program TSA-SerialCosinor 6.3 (Expert Soft Technologie) was also used to carry out a chronobiological analysis of the band-pass Butterworth filtered time series. For these analyses, the mean hourly opening was calculated (Mat et al. 2012). Then, the quality of the data was checked using exploratory data analysis (EDA). To control for the presence of a real biological or physical phenomenon and check for the absence of randomness, an autocorrelation diagram was performed. A partial autocorrelation function (PACF) was applied to check for the absence of stationarity (Mat et al. 2012). The main periods were determined using Fourier and Lomb and Scargle periodograms (Scargle 1982). Then, the entire time series was split into observed distinctive activity patterns, and the potential rhythm for each pattern was modeled by fitting a cosinor model. By calculating the parameters of the cosinor model, it will be possible to compare the activity data with those of other monitored bivalves.

To analyze current data, water directions were grouped in 16 intervals of 22.5° each. To select the current events for statistical analysis, low-frequency curves of current velocity and gaping activity averaged among all individuals for each monitored time interval (i.e., red and blue lines,

respectively, in Online Resource 3–6) were compared in three steps. In the first step, the largest peaks in current velocity were chosen to determine whether they coincided with valleys in the gaping activity curve (i.e., low gape angle and/or prolonged closures). This selection process allowed testing the hypothesis that current velocity has some effect on gaping activity. Because not all the valleys in the gaping activity curve coincided with the largest peaks in current velocity, in the second step the remaining clear valleys in gaping activity coinciding with smaller peaks in current velocity were chosen. A priori, if the valley in gaping activity did not correspond with a peak in current velocity, it was discarded because the individuals likely changed their gaping activity and closed their valves due to conditions other than the hydrodynamics. Then, the remaining peaks in current velocity that were of similar magnitude to those selected in the previous step, but that did not coincide with a valley in gaping activity, were chosen. To further test the hypothesis that only specific combinations of current velocity and direction affected gaping activity, clear valleys in gaping activity corresponding to smaller, less conspicuous peaks in current velocity, and the peaks of similar magnitude which did not coincide with a valley in the gaping activity curve were also examined. According to this hypothesis, most of the smaller peaks coinciding with valleys in gaping activity should show the expected combination of orientation and current velocity affecting *P. nobilis*, whereas those peaks not coinciding with valleys in activity should deviate from the expected combination. Once the events were selected, all individual responses of the six monitored fan mussels were explored using graphics generated in GapeR (see below). The intervals of the time series corresponding to the selected events were plotted, the time of the day when they occurred (day or night), and the position of the moon and the season checked, and the individual responses classified into one of four responses to hydrodynamics (*yes/no/little/undetectable*). *Yes* means a clear closing of the valves; *no* means that there was no response. *Little* indicated an uncertain response because the change was small and dubious. In this case the change was retained as a possible closing. *Undetectable* was noted when the closing coincided with a night during summer when all individuals were naturally closed due to the absence of moonlight. Sun and moon positions were computed according to latitude and longitude of the study site and time of year (Duffett-Smith 1988).

Principal component analysis (PCA) was used to evaluate whether there was any preferential combination of current orientation and velocity that affected valve closure for each individual. Two PCAs for each individual were performed: The first PCA included only the current orientations centered by mean subtraction, and the second PCA included the current orientations and the mean and

maximum current velocity normalized by mean subtraction and standardized by dividing by the variance.

Results

Environmental data are summarized in Online Resource 7

Gaping activity

The variation of low frequency in the curves was clearly observed after applying the low-pass Butterworth filter. Furthermore, specimens opened and closed their valves at nearly the same time and showed a synchronized gaping activity (period of ~23.4 h) (Fig. 1; Online Resource 7). Additionally, the fan mussels showed marked seasonality in gaping activity. The seasonal behavior in gaping activity followed two patterns (Online Resource 7). The first pattern (P1) tracked sun- and moonlight, with maximum opening during daylight or during nights that were sufficiently illuminated by a full moon. In contrast, gaping activity in the second pattern (P2) did not track sun- and moonlight. For example, the valves were opened for longer periods, eventually closed during the day, and they were open at night most of the time despite moon position and illumination. P1 occurred from mid-July–early August–early November (i.e., ~4 months), whereas P2 occurred the rest of the time, from early November–mid-July–early August depending on the individual (i.e., ~8 months). The change between these seasonal behaviors occurred within a few days; however, some individuals seem to have changed the behavior several weeks earlier than the others (Online Resource 8 and 9). After observing the broad behavioral change between the two patterns, P1 and P2 curves were analyzed separately. The day when the shift in the gaping pattern was evident for each individual

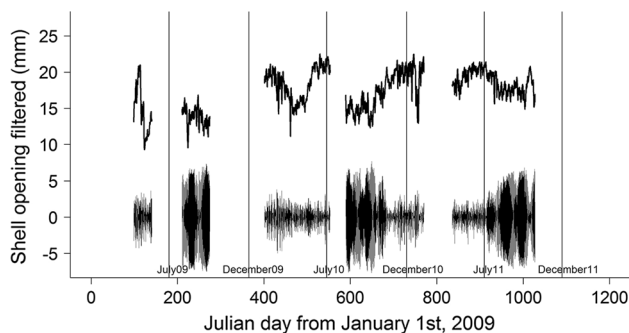


Fig. 1 Time series of low-frequency (*top panel*) and high-frequency (*bottom*) *Pinna nobilis* shell openings averaged for all individuals ($n = 6$)

was manually selected using GapeR graphics, and the T4 and T5 time series were split into the two activity patterns observed for all specimens. Cross-correlation coefficients confirmed that gaping activity was synchronized among all except one individual for most of the time [in P1, correlations above 0.9 were common, whereas in P2 correlations between 0.6 and 0.9 were more typical (Online Resource 10)]. The exception was the low correlation of specimen 5 in winter of T4 (from August 10, 2010–February 5, 2011, Online Resource 10). Specimen 5 maintained P1 for several weeks longer than the rest in T4. In P1, the maximum cross-correlation occurred when the low-frequency variation was filtered. In contrast, the correlation was higher in P2 when the high frequency was filtered. In most time series, the filtered data showed higher correlation than the raw data either for low or high frequency. Chronobiometric parameters from the cosinor model were significant in all but two cases: T1 and winter of T4 (Online Resource 11).

Spectral analyses (FFT and Lomb and Scargle) revealed that under natural conditions the fan mussels had a distinct circadian activity with cycles of 21.9–24 h. The cycles were very clear in P1. In P2, gaping activity followed the same periodicity, but unlike in P1, the signals were noisier (Fig. 2; Online Resource 11).

Relation between environmental parameters and gaping activity

Water temperature

The most remarkable changes in water temperature were the sudden decreases in spring and summer. The fan mussels did not alter their behavior during these events, despite temperatures that could quickly drop 10 °C (e.g., jd 919 and 980 in Online Resource 6).

Dissolved oxygen

Despite a daily sinusoidal pattern in oxygen concentration during spring and summer, similar to that of gaping activity, comparison with activity curves showed that the mussels did not respond directly to high-frequency variation in DO concentration (Online Resource 7 and 12). There was no alteration of gaping activity in P1 during nights with high DO concentration nor during times of the day with low DO concentration (Online Resource 12). Instead, the individuals usually tracked the sun and moon position with their gape (Online Resource 7). In spring, oxygen showed the daily cyclic pattern, but the individuals did not show P1, and hence, valve closures did not coincide with low oxygen concentrations (Online Resource 7).

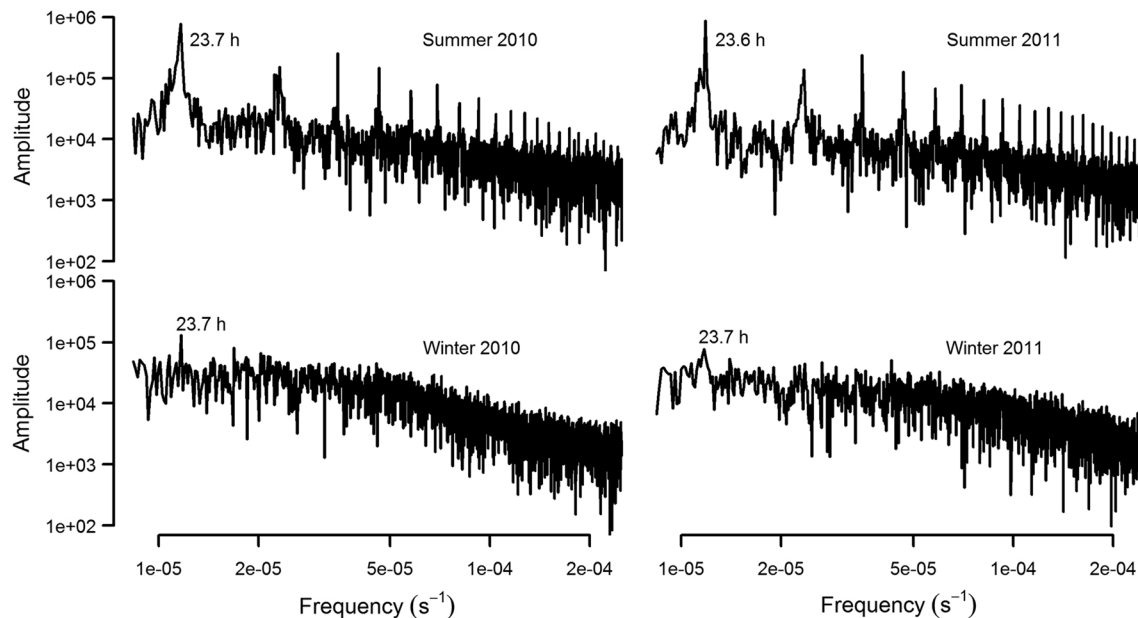


Fig. 2 Fast Fourier transforms of averaged raw data for all *Pinna nobilis* ($n = 6$) in Pattern 1 (*top*) and Pattern 2 (*bottom*) in intervals T4 and T5. Periods corresponding to maximum amplitude indicated

Chlorophyll *a*

Contrary to our predictions, a clear relationship between peaks in (chl *a*) and degree of gape in either high- or low-frequency variation was not observed (Online Resource 3–6 and 13). Gaping behavior did not coincide with the highest peaks in (chl *a*) nor with the variation in (chl *a*) levels.

Sun, moon, and turbidity

Gaping activity of the monitored individuals clearly responded to sun and moon position during P1. High turbidity affected this response such that the individuals behaved as if the night was in a new moon phase by closing their valves (Online Resource 13). When the individuals were in P2, neither the sun, nor moon nor turbidity affected the gaping behavior.

There were 22 complete full moon events in the monitored months. Full moon events that occurred during P2 did not affect the gaping activity of the individuals. The rest of the full moon events usually affected gaping activity, where the individuals kept their valves open during nights when the moon was visible in the sky. However, there were some nights with full moon and low turbidity during P1 in which no change in gaping activity of some individuals was observed. Individuals 5 and 6 were the least sensitive to moonlight, with three and four cases, respectively, when they did not respond to it (Online Resource 14).

Hydrodynamics

When currents were strong, the individuals commonly responded by increasing the number of movements, reducing the maximum gape, and closing their valves for variable lengths of time. This response could be prolonged for several days as observed during storm events between January 20 and February 6, 2011 (jd 750 and 767 in Online Resource 5). The minimum values of gape opening for the entire time interval were indeed observed during some storms. Not all the peaks in current velocity, however, had the same effect on gaping behavior among all specimens.

Of 86 selected events (gray vertical lines in Online Resource 3–6), the analysis showed: (1) 34 events that resulted in a clear change in the gaping activity of at least one individual; (2) 17 events that resulted in a possible but not clear response in one or more individuals; (3) 31 events that resulted in no response in any individual; or (4) four events where the effect was undetectable because it coincided with a new moon during P1 and, hence, all individuals were naturally closed (these *undetectable* events were not used in the analyses).

In the first PCA analysis performed individually for all specimens, including only the current orientations, the first two eigenvalues explained 59.7–72.6 % of the variance (Online Resource 15 and 16 and Fig. 3). PCA1 and PCA2 accounted for 41.2–53.1 and 17.6–19.5 % of the variance, respectively (Online Resource 15). The loadings showed that a combination of ESE and WNW orientations were influencing PCA1, whereas NW, WNW and SE, ESE

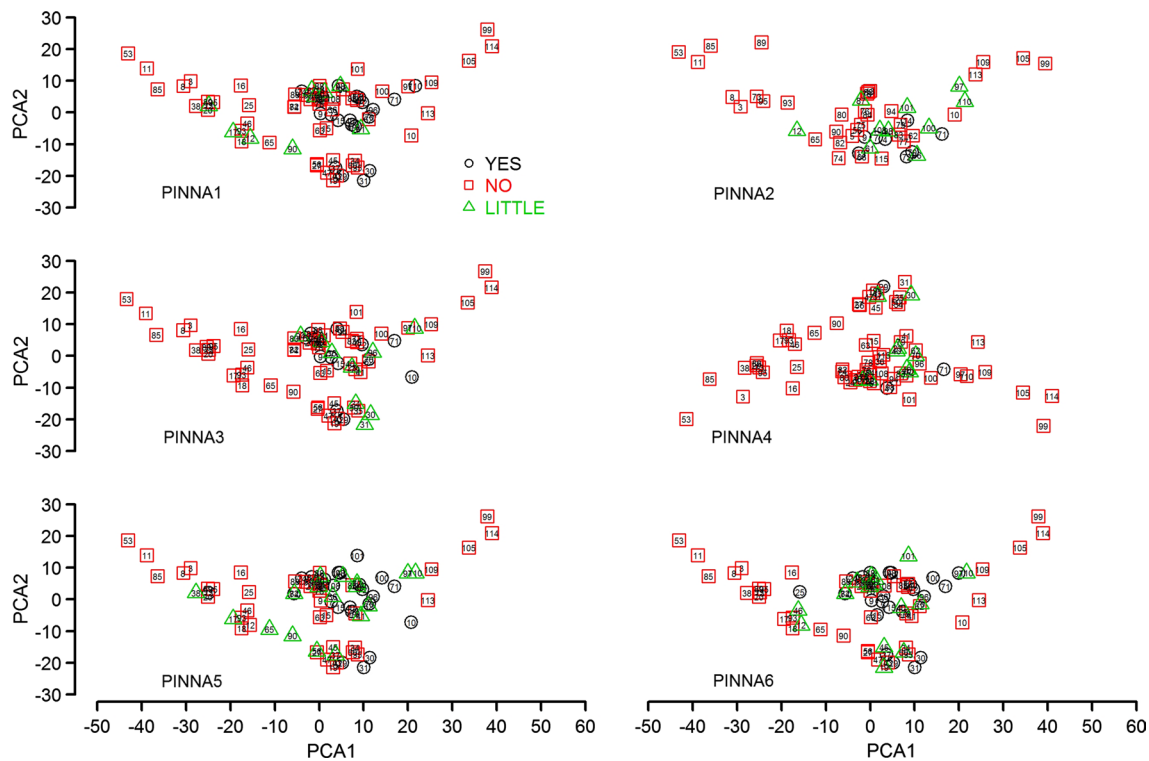


Fig. 3 Results of first PCA (only current direction). Events are identified by ID number. Centers of *graphs* represent bimodal current events; unimodal current events are on either side

were the dominant orientations of PCA2 depending on the individuals (Online Resource 16). The events were spread along the horizontal axis, but the valve closures (considered as *yes* in the graphs) were concentrated in the center of the graph (Fig. 3). The events with *little* or dubious closings were also concentrated in the center of the graph, but showed more dispersion. In the second PCA (including mean and maximum current intensities together with the current orientations), the first two eigenvalues explained 42.1–51.4 % of the variance (22.8–31.0 % for PCA1; 19.3–20.4 % for PCA2) (Online Resource 15 and 16; Fig. 4). Therefore, they did not separate groups appropriately. The mean intensity showed high loadings except for specimen 2, indicating that the current velocity may have had some influence on the distribution of the events along the x-axis.

Discussion

The data showed that there were predictable cycles in gaping activity of the fan mussels that were related to environmental variables. For some responses in gaping activity to environmental variation, such as the effect of light during P1, the relationship was very clear: the individuals detected the ambient light and opened and closed their valves following the position and illumination of the sun

and moon. In combination with sun and moon positions, high turbidity most likely explains the dim light arriving at the seabed during full moon nights. During nights with a full moon and high turbidity, individuals did not open their valves. The high turbidity likely reduced ambient light to levels that are undetectable by the fan mussels. The presence of clouds hiding the moon could explain some of the summer nights with full moon when several individuals kept their valves closed. However, it was rarely cloudy during the summer in the study area, so this cannot explain the apparently random closures by some individuals on certain summer, full moon nights. On the other hand, the response during P1 to light shows that the individuals opened the valves at dawn, as soon as there was some light in the sky. Considering this light sensitivity of fan mussels, and their binary (open–close) response to light–no light during P1, it seems that clouds present during day hours would not reduce light intensity sufficiently as to affect gaping activity.

Conversely, spectral analyses showed that a periodic 21.9- to 24-h cycle of gaping activity occurred in all individuals over the whole year. This circadian periodicity indicates that the mussels have an internal clock. Otherwise, the periods would run at their own speed and would differ among individuals. The occurrence of a shift in the timing of valve closure between behavioral patterns (i.e.,

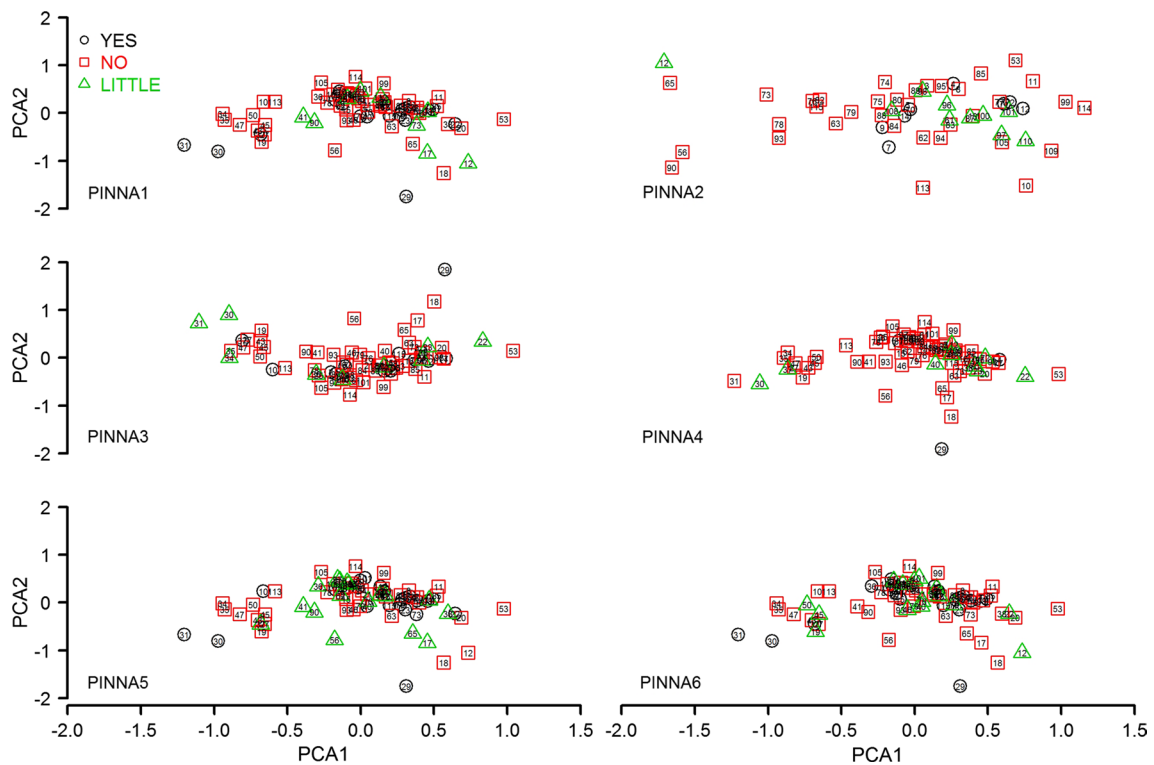


Fig. 4 Results of second PCA (current direction, mean and maximum current velocity). Events identified by ID number

in mid-July–early August and in early November) suggests the presence of a circannual rhythm. In many species these rhythms are entrained by changes in day length (Foster and Kreitzman 2004). However, the timing of the shift between the two behaviors seems to be unrelated to long days, since the activity pattern changed to synchronized day/night gaping activity by mid-July, a month past the longest day of the year. The incremental reduction in the photoperiod, however, is an entraining trigger for other invertebrates such as sea urchins (McClintock and Watts 1990), and similarly this may be the entraining factor for the fan mussel. Gaping activity also seems to be unrelated to (chl *a*) concentration, but the shift occurs with rising temperatures and diminishing DO concentrations in early summer and with dropping temperatures and rising DO concentrations in early autumn. Based on these data, an alternative hypothesis is that temperature or DO concentration, or a combination of both, may be responsible for modulating the seasonal behavior changes in mid-July–early August and in early November. In P1, the individuals spent more time with their shell valves open during daylight hours with higher DO concentration, whereas the fan mussels may be saving energy when the valves were closed during the night (Ortmann and Grieshaber 2003). Furthermore, P1 in *P. nobilis* is likely similar to that of the tropical bivalve, *Hippopus hippopus* (Schwartzmann et al. 2011), which reinforces the idea that

water temperature is relevant in the activity change of *P. nobilis*. However, disturbances in gaping activity due to heat stress, as occurs with *H. hippopus*, were not observed in *P. nobilis*. When temperature decreases and DO levels increase, P1 ceases and a freer gaping activity pattern occurs where they gape for longer periods of time. In any case, the relationship between these environmental variables (water temperature and DO concentration) and gaping activity was not linear (Bae and Park 2014). In *P. nobilis*, temperature was higher and DO concentration lower when shifting from P2 to P1 and vice versa. Other environmental or biological variables not measured in the present study may also modulate the timing of change between activity patterns. For example, internal needs related to gametogenesis and reproduction, which occurs June–September in the fan mussel (Basso et al. 2015), could modulate the timing of change between activity patterns.

Monitoring the difference between juvenile and adult fan mussels may provide a better understanding of this change. It cannot be ruled out that fan mussels show variable sensitivity to environmental factors with age, since growth slows during ontogeny in this and other bivalve species (Kennedy et al. 2001; Katsanevakis 2007). Other bivalves, such as *Crassostrea gigas*, also show an annual shift in activity patterns analogous to that observed in *P. nobilis*. *Crassostrea gigas* is nocturnal in autumn/winter and diurnal in spring/

summer (Mat et al. 2012). These authors asked the following question in their study regarding the shift in activity patterns: “Is it only typical of *C. gigas* or is it part of a more general pattern?” The present findings indicate that the shift in the behavioral patterns, or the “dual circadian rhythm,” is a more general pattern. However, there are differences in the shift between the two species because *P. nobilis* changes from one pattern to the other more abruptly and at different times than *C. gigas*.

The two observed patterns of gaping activity have implications in the design of future laboratory experiments with subtidal bivalves living in non-tidal seas. Depending on the season of collection, the fan mussels have a different natural behavior. This may affect the conclusions obtained from laboratory experiments because they may produce different results. If the behavior is characterized in situ prior to laboratory experiments, as in the present study, it is possible to obtain much more precise information and better interpretations of the results. Additionally, if environmental parameters trigger the change between behavioral patterns in the fan mussel, the species can be used as an environmental biomonitor in the context of climate change. The combination of gaping activity biomonitoring, with shell sclerochronology may also be used to extend the time series into the past, prior to the recording of gape angle and environmental data (García-March et al. 2011; Schwartzmann et al. 2011; Tran et al. 2011). Combining these methods makes it possible to monitor changes in environmental parameters and reconstruct long-term patterns of variation. The correlation of oxygen and stable carbon isotope ratios (proxies for ambient water temperature and primary productivity, respectively) and microgrowth increment width with environmental variables will potentially enable discrimination between physiological and environmental effects on sclerochronology (Schöne 2008; Schöne and Surge 2012). This has direct implications for studies of climate change and paleoclimate in the fossil record.

The effects of currents on gaping activity indicate that similar velocities have different effects on the same individual. This implies that current velocity is not the only factor influencing gaping activity. To assess the effects of current direction on gaping activity, we determined whether they flowed in two directions (bimodal) or one direction (unimodal). The bimodal currents observed would be produced by waves approaching the shore that are in contact with the seabed. When this occurs, the orbital water movement produced by waves becomes more elliptical, until becoming a net forward-backward movement with shallower depths. Unimodal currents could be produced by several phenomena such as wind-driven coastal upwelling and shelf waves. The first PCA (only current direction) showed that valve closure events were concentrated in the center of the graphs (black circles in Fig. 3). Moreover, if the orientations are

observed in detail, this zone corresponds to events with bimodal currents. Additionally, there was no event with unimodal current that affected gaping activity (Online Resource 17 for some examples). Event 73 was selected in the time series to compare with the previous and following events (72 and 74), all occurring during the same storm (jds 758, 760 and 763). Events 72 and 74 corresponded with abrupt valleys in the smoothed curve of gaping activity (i.e., valve closures). Their average and maximum current velocities were 10.3 ± 6.5 and 33.1 cm s^{-1} for event 72 ($n = 133$) and 8.7 ± 5.3 and 31.3 cm s^{-1} ($n = 232$) for event 74, respectively. Event 73, on the other hand, coincided with a clear positive slope in the gaping activity curve that occurred despite the high average and maximum current velocities (9.5 ± 4.4 and 25.9 cm s^{-1} ($n = 149$), respectively). What is more significant is that events 72 and 74 were bimodal, whereas event 73 was unimodal (Fig. 5). This observation is not the only example, but is the most clear. Other similar situations demonstrating that unimodal currents did not affect gaping activity despite their velocity whereas bimodal do, were events 7, 8, and 9 (Fig. 5) and events 4 and 53, and 88 and 89 (Online Resource 17). The bimodal event affecting gaping activity with lower current velocity was event 14 (jd 270). It showed an average and maximum current velocity of 3.9 ± 2.5 and 12.1 cm s^{-1} ($n = 118$), respectively. As a consequence, average current velocity affecting gaping activity would be low, around 3.9 cm s^{-1} , and above this threshold the orientation of the current would be what determines the effect of currents on gaping activity. This implies that considering the current velocity, most events have the capacity to affect gaping activity of the fan mussel. This influence is probably one of the reasons why the inclusion of this variable in the second PCA introduced little additional information, which does not compensate for the information lost by normalizing variables having different units.

Considering the low current velocities required to affect gaping activity, most waves may have an effect on *P. nobilis*. However, not all current events falling in the middle of the first PCA (i.e., not all bimodal events) affect gaping activity. Other factor(s) likely determine whether the valves close once the requirements of current velocity and direction are met. Since there is no clear tendency among individuals and events, biological factors may play a role (e.g., an individual needs to keep filter feeding or it is too exhausted to adduct its shell valves, perhaps after being attacked by a predator). Future research is required to better understand the role of such biological factors.

Unimodal currents did not affect gaping activity even in the strongest events, when mean and maximum velocities were as high as 13.3 ± 4.2 and 25.2 cm s^{-1} ($n = 117$), respectively (e.g., event 53 in jd 668). Suzuki et al. (2007) observed responses to storms in *Atrina pectinata*. The

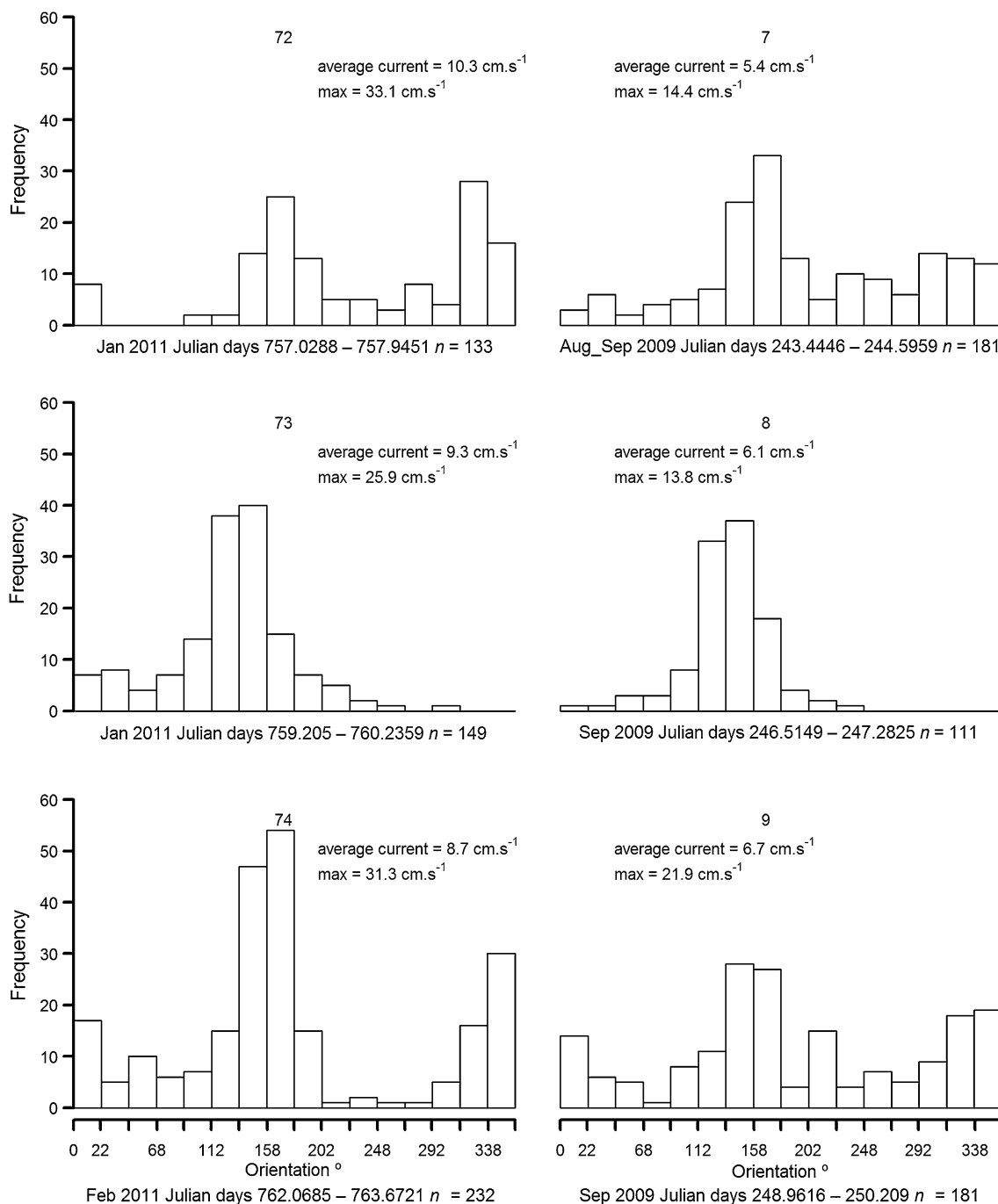


Fig. 5 Histograms of current direction in two groups of three consecutive events (72, 73 and 74 and 7, 8 and 9) when *Pinna nobilis* behaved differently. Events 72, 74, 7, and 9 (all with bimodal cur-

rents) coincided with clear dips in gaping activity; events 73 and 8 (with unimodal currents) coincided with a positive slope or peak in gaping activity, i.e., the currents did not affect gaping activity

behavior was described as “single close–open actions” and was interpreted as “a reaction of the pen shells to high turbidity, since they were covered with sand from heavy waves.” The possible negative effects of strong storms and water movement on *P. nobilis* were previously reported (García-March et al. 2007; Hendriks et al. 2011). In the present study, evidence is provided that slow, bimodal

currents may affect gaping activity of this species, reducing the gape angle and increasing the number of movements. Both reactions are usually defensive, probably to reduce the entry of undesirable particles into the palial cavity as observed in *A. pectinata* (Suzuki et al. 2007). Some water movement is beneficial to the fan mussel, as detritus resuspended from the seabed is part of the diet of this species

(Cabanelas-Reboredo et al. 2010; Davenport et al. 2011; Najdek et al. 2013; Trigos et al. 2014). This behavior may explain why the individuals continue to gape when unimodal currents occur. Unimodal currents may also be more predictable and less stressful for the byssus attaching the mussel to the seabed (Basso et al., 2015).

In conclusion, the gaping activity of the fan mussel had six main features: (1) Gaping was synchronous most of the time, although occasionally an individual mussel delayed changing its behavior before eventually becoming synchronous with the other mussels. (2) The mussels showed two distinct seasonal activity patterns. One from mid-July–early August–early November (P1) lasting for ~4 months and the other from early November–mid-July–early August (P2) lasting for ~8 months. (3) Individuals showed strong correlation with sun and moon positions and illumination during P1. (4) Specimens showed ~21.9–24 h periods of gaping activity during the entire year, although the timing of valve closure varied between the two behavioral patterns. This observation suggests the existence of one internal clock activated by a Zeitgeber that is entrained on a daily basis. (5) Gaping activity was insensitive to the natural high-frequency variation in dissolved oxygen concentration, (chl *a*) concentration, and water temperature. (6) Individuals exhibited sensitivity to bimodal current direction, such as those from waves, at average velocities of $\geq 3.9 \pm 2.5 \text{ cm s}^{-1}$. They were insensitive to unimodal current directions despite their high velocity. The fact that not all bimodal current directions affected gaping activity suggests that other factor(s) modulate the responses of individuals to waves once the thresholds of current velocity and direction are met.

Acknowledgments The project was funded by “Obra Social Caja Madrid” of “Caja Madrid” Bank. We are grateful to Felio Lozano, the coordinator of Tabarca Island Marine Reserve, the guards of the Reserve, Silvia Revenga and the RMIP—SGM—MARM, for their permissions, collaboration, and inestimable help with the work in the MPA. We are also grateful to the anonymous reviewers and the editors for their comments that have strongly improved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

Bae MJ, Park YS (2014) Biological early warning system based on the responses of aquatic organisms to disturbances: a review. *Sci Total Environ* 466:635–649. doi:10.1016/j.scitotenv.2013.07.075

Basso L, Vázquez-Luis M, García-March JR, Deudero S, Alvarez E, Vicente N, Duarte CM, Hendriks IE (2015) The Pen Shell, *Pinna nobilis*: a review of population status and recommended research priorities in the Mediterranean Sea. In: Curry B (ed) *Advances in marine biology*, vol 71. Academic Press, London, pp 109–160. doi:10.1016/bs.amb.2015.06.002

Borcherding J (2006) Ten years of practical experience with the Dreissena-Monitor, a biological early warning system for continuous water quality monitoring. *Hydrobiologia* 556:417–426. doi:10.1007/s10750-005-1203-4

Cabanelas-Reboredo M, Deudero S, Alos J, Valencia JM, March D, Hendriks IE, Alvarez E (2009) Recruitment of *Pinna nobilis* (Mollusca: Bivalvia) on artificial structures. *Mar Biodivers Rec* 2:1–5. doi:10.1017/S1755267209001274

Cabanelas-Reboredo M, Blanco A, Deudero S, Tejada S (2010) Effects of the invasive macroalga *Lophocladia lallemandii* on the diet and trophism of *Pinna nobilis* (Mollusca: Bivalvia) and its guests *Pontonia pinnophylax* and *Nepinnotheres pinnotheres* (Crustacea: Decapoda). *Sci Mar* 74:101–110. doi:10.3989/scimar.2010.74n1101

Davenport J, Ezgeta-Balic D, Peharda M, Skejic S, Nincevia-Gladan Z, Matijevec S (2011) Size-differential feeding in *Pinna nobilis* L. (Mollusca: Bivalvia): exploitation of detritus, phytoplankton and zooplankton. *Estuar Coast Shelf Sci* 92:246–254. doi:10.1016/j.ecss.2010.12.033

de Gaulejac B (1995) Mise en évidence de l’hermafroditisme successif à maturation asynchrone de *Pinna nobilis* (L.) (Bivalvia: Pterioidea). *C R Acad Sci III Sci Vie* 318:99–103

Duffet-Smith P (1988) *Practical astronomy with your calculator*. Cambridge University Press, Cambridge

Foster RG, Kreitzman L (2004) *Rhythms of life. The biological clocks that control the daily lives of every living thing*. Yale University Press, New Haven

García-March JR, Ferrer JF (1995) Biometría de *Pinna nobilis* L., 1758: una revisión de la ecuación de De Gaulejac y Vicente (1990). *Bol Inst Esp Oceanogr* 11:175–181

García-March JR, Vicente N (2007) Protocol to study and monitor *Pinna nobilis* populations within marine protected areas. MEPA, La Valette

García-March JR, García-Carrascosa AM, Pena AL (2002) In situ measurement of *Pinna nobilis* shells for age and growth studies: a new device. *Mar Ecol PSZN I* 23(3):207–217. doi:10.1046/j.1439-0485.2002.02781.x

García-March JR, Perez-Rojas L, García-Carrascosa AM (2007) Influence of hydrodynamic forces on population structure of *Pinna nobilis* L., 1758 (Mollusca : Bivalvia): The critical combination of drag force, water depth, shell size and orientation. *J Exp Mar Biol Ecol* 342:202–212. doi:10.1016/j.jembe.2006.09.007

García-March JR, Sanchis-Solsona MA, García-Carrascosa AM (2008) Shell gaping behaviour of *Pinna nobilis* L., 1758: circadian and circalunar rhythms revealed by in situ monitoring. *Mar Biol* 153:689–698. doi:10.1007/s00227-007-0842-6

García-March JR, Surge D, Lees JM, Kersting DK (2011) Ecological information and water mass properties in the Mediterranean recorded by stable isotope ratios in *Pinna nobilis* shells. *J Geophys Res Biogeosci* 116:1–14. doi:10.1029/2010JG001461

Hendriks IE, Cabanelas-Reboredo M, Bouma TJ, Deudero S, Duarte CM (2011) Seagrass meadows modify drag forces on the shell of the fan mussel *Pinna nobilis*. *Estuar Coasts* 34:60–67. doi:10.1007/s12237-010-9309-y

Jou LJ, Lin SC, Chen BC, Chen WY, Liao CM (2013) Synthesis and measurement of valve activities by an improved online clam-based behavioral monitoring system. *Comput Electron Agric* 90:106–118. doi:10.1016/j.compag.2012.09.008

Katsanevakis S (2007) Growth and mortality rates of the fan mussel *Pinna nobilis* in Lake Vouliagmeni (Korinthiakos Gulf, Greece):

- a generalized additive modelling approach. *Mar Biol* 152:1319–1331. doi:[10.1007/s00227-007-0781-2](https://doi.org/10.1007/s00227-007-0781-2)
- Kennedy H, Richardson CA, Duarte CM, Kennedy DP (2001) Oxygen and carbon stable isotopic profiles of the fan mussel, *Pinna nobilis*, and reconstruction of sea surface temperatures in the Mediterranean. *Mar Biol* 139:1115–1124
- Kim WS, Huh HT, Je JG, Han KN (2003) Evidence of two-clock control of endogenous rhythm in the Washington clam, *Saxidomus purpuratus*. *Mar Biol* 142:305–309. doi:[10.1007/s00227-002-0952-0](https://doi.org/10.1007/s00227-002-0952-0)
- Liao CM, Jau SF, Lin CM, Jou LJ, Liu CW, Liao VHC, Chang FJ (2009) Valve movement response of the freshwater clam *Corbicula fluminea* following exposure to waterborne arsenic. *Ecotoxicology* 18:567–576. doi:[10.1007/s10646-009-0314-5](https://doi.org/10.1007/s10646-009-0314-5)
- Mat AM, Massabuau JC, Ciret P, Tran D (2012) Evidence for a plastic dual circadian rhythm in the oyster *Crassostrea gigas*. *Chronobiol Int* 29:857–867. doi:[10.3109/07420528.2012.699126](https://doi.org/10.3109/07420528.2012.699126)
- McClintock JB, Watts SA (1990) The effects of photoperiod on gametogenesis in the tropical sea-urchin *Eucidaris tribuloides* (Lamarck) (Echinodermata, Echinoidea). *J Exp Mar Biol Ecol* 139:175–184. doi:[10.1016/0022-0981\(90\)90145-3](https://doi.org/10.1016/0022-0981(90)90145-3)
- Najdek M, Blazina M, Ezgeta-Balic D, Peharda M (2013) Diets of fan shells (*Pinna nobilis*) of different sizes: fatty acid profiling of digestive gland and adductor muscle. *Mar Biol* 160:921–930. doi:[10.1007/s00227-012-2144-x](https://doi.org/10.1007/s00227-012-2144-x)
- Ortmann C, Grieshaber MK (2003) Energy metabolism and valve closure behaviour in the Asian clam *Corbicula fluminea*. *J Exp Biol* 206:4167–4178. doi:[10.1242/jeb.00656](https://doi.org/10.1242/jeb.00656)
- Palmer JD (2000) The clocks controlling the tide-associated rhythms of intertidal animals. *Bioessays* 22:32–37
- Rensing L, Meyer-Grahe U, Ruoff P (2001) Biological timing and the clock metaphor: oscillatory and hourglass mechanisms. *Chronobiol Int* 18:329–369. doi:[10.1081/cbi-100103961](https://doi.org/10.1081/cbi-100103961)
- Rhoads DC, Lutz RA (1980) Skeletal growth of aquatic organisms. Plenum Publishing Corporation, New York
- Riisgard HU, Kittner C, Seerup DF (2003) Regulation of opening state and filtration rate in filter-feeding bivalves (*Cardium edule*, *Mytilus edulis*, *Mya arenaria*) in response to low algal concentration. *J Exp Mar Biol Ecol* 284:105–127. doi:[10.1016/S0022-0981\(02\)00496-3](https://doi.org/10.1016/S0022-0981(02)00496-3)
- Robson AA, De Leaniz CG, Wilson RP, Halsey LG (2010) Behavioural adaptations of mussels to varying levels of food availability and predation risk. *J Moll Stud* 76:348–353. doi:[10.1093/mollus/eyq025](https://doi.org/10.1093/mollus/eyq025)
- Rodland DL, Schöne BR, Baier S, Zhang ZJ, Dreyer W, Page NA (2009) Changes in gape frequency, siphon activity and thermal response in the freshwater bivalves *Anodonta cygnea* and *Margaritifera falcata*. *J Moll Stud* 75:51–57. doi:[10.1093/mollus/eyn038](https://doi.org/10.1093/mollus/eyn038)
- Scargle JD (1982) Studies in astronomical time-series analysis. II. Statistical aspects of spectral-analysis of unevenly spaced data. *Astrophys J* 263:835–853. doi:[10.1086/160554](https://doi.org/10.1086/160554)
- Schöne BR (2008) The curse of physiology—challenges and opportunities in the interpretation of geochemical data from mollusk shells. *Geo-Mar Lett* 28:269–285. doi:[10.1007/s00367-008-0114-6](https://doi.org/10.1007/s00367-008-0114-6)
- Schöne BR, Surge D (2012) Chapter 14. Bivalve sclerochronology and geochemistry. In: Seldon P, Hardesty J (eds) *Treatise online* 46: Part N, revised, vol 1. The University of Kansas, Paleontological Institute, Lawrence, pp 1–24
- Schwartzmann C, Durrieu G, Sow M, Ciret P, Lazareth CE, Massabuau J (2011) In situ giant clam growth rate behavior in relation to temperature: a one-year coupled study of high-frequency noninvasive valvometry and sclerochronology. *Limnol Oceanogr* 56:1940–1951. doi:[10.4319/lo.2011.56.5.1940](https://doi.org/10.4319/lo.2011.56.5.1940)
- Sluyts H, VanHoof F, Cornet A, Paulussen J (1996) A dynamic new alarm system for use in biological early warning systems. *Environ Toxicol Chem* 15:1317–1323
- Sobrino-Figueroa A, Caceres-Martinez C (2009) Alterations of valve closing behavior in juvenile Catarina scallops (*Argopecten ventricosus* Sowerby, 1842) exposed to toxic metals. *Ecotoxicology* 18:983–987. doi:[10.1007/s10646-009-0358-6](https://doi.org/10.1007/s10646-009-0358-6)
- Sow M, Durrieu G, Briollais L, Ciret P, Massabuau JC (2011) Water quality assessment by means of HFNI valvometry and high-frequency data modeling. *Environ Monit Assess* 182:155–170. doi:[10.1007/s10661-010-1866-9](https://doi.org/10.1007/s10661-010-1866-9)
- Suzuki K, Kiyomoto S, Koshiishi Y (2007) Observation on behavior of the large suspension feeding bivalve *Atrina pectinata lischkeana* under natural conditions. *Bull Fish Res Agency* 19:17–25
- Tran D, Fournier E, Durrieu G, Massabuau JC (2007) Inorganic mercury detection by valve closure response in the freshwater clam *Corbicula fluminea*: Integration of time and water metal concentration changes. *Environ Toxicol Chem* 26:1545–1551. doi:[10.1897/06-390R1.1](https://doi.org/10.1897/06-390R1.1)
- Tran D, Nadau A, Durrieu G, Ciret P, Parisot JP, Massabuau JC (2011) Field chronobiology of a molluscan bivalve: how the moon and sun cycles interact to drive oyster activity rhythms. *Chronobiol Int* 28:307–317. doi:[10.3109/07420528.2011.565897](https://doi.org/10.3109/07420528.2011.565897)
- Trigos S, García-March JR, Vicente N, Tena J, Torres J (2014) Utilization of muddy detritus as organic matter source by the fan mussel *Pinna nobilis*. *Med Mar Sci* 15:667–674. doi:[10.12681/mms.836](https://doi.org/10.12681/mms.836)
- van Keulen M, Borowitzka MA (2002) Comparison of water velocity profiles through morphologically dissimilar seagrasses measured with a simple and inexpensive current meter. *Bull Mar Sci* 71:1257–1267
- Williams BG, Pilditch CA (1997) The entrainment of persistent tidal rhythmicity in a filter-feeding bivalve using cycles of food availability. *J Biol Rhythm* 12:173–181. doi:[10.1177/074873049701200208](https://doi.org/10.1177/074873049701200208)