Science of the Total Environment 768 (2021) 145085

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

How to cope in heterogeneous coastal environments: Spatio-temporally endogenous circadian rhythm of valve gaping by mussels

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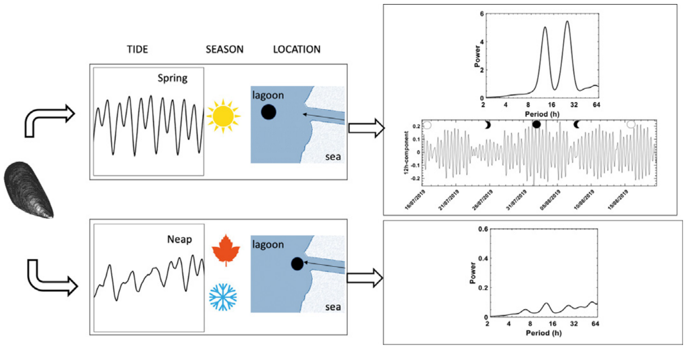
HIGHLIGHTS

- It is necessary to understand how organisms respond to environmental changes.
- Transitional coastal areas are great model systems.
- Mussels behaviour was monitored long-term and in continuous in three sites.
- It followed the tidal rhythm particularly in more internal site and in summer.
- Responses to small scale changes are important for management and for predictions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 September 2020
Received in revised form 4 January 2021
Accepted 5 January 2021
Available online 11 January 2021

Editor: Daniel A. Wunderlin

Keywords:
Circadian rhythms
Fluctuating environment
Mytilus galloprovincialis
Transitional coastal zones
Venice lagoon

ABSTRACT

Transitional coastal zones are subject to high degrees of temporal fluctuation in environmental conditions, with these patterns varying in space. Gaining an in depth understanding of how sessile organisms cope with and respond to such environmental changes at multiple scales is needed to *i)* advance fundamental knowledge, *ii)* predict how organisms may react to stressors and *iii)* support the management of halieutic resources in transitional coastal areas. We addressed this question using mussels (*Mytilus galloprovincialis*) as model system. Valve-gaping sensor were deployed at multiple sites within the southern Venice Lagoon over a period of 6 months, to investigate the existence of periodicity in valve-gaping and its relationship with environmental variables, such as temperature and chlorophyll-a.

Gaping behaviour was found to have periodic rhythms, of ~12 h and ~ 24 h, which were most pronounced in the inner part of lagoon part and were strongest during summer months. In autumn, the dual periodicity became weaker and mostly the 12 h remained. Gaping was closely linked with tide, but the relationship in terms of phasing varied upon location. Surprisingly, no clear direct relationships were found with chlorophyll-a, but food delivery may be mediated by tide itself. The results highlight the heterogeneity of behaviour and the endogenic nature of circadian rhythms in space and time. These findings have important implications for management of transitional areas where tidal alteration may have impacts on key behaviours, and emphasize the importance of characterizing their rhythms before using these as stress indicator. Moreover, the described tidal relationships should be included in growth models of bivalves in these systems.

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https://doi.org/10.1016/j.scitotenv.2021.145085
0048-9697/

1. Introduction

Coastal habitats such as deltas and lagoons, are critical transition zones at the interface between terrestrial, freshwater and marine habitats. The interaction of temporal highly variable rain fall and river discharges with the much more regular tides, drives a high degree of variability in both abiotic (temperature, salinity, dissolved oxygen, speed of currents, Falcao, 2003) and biotic factors (e.g. plankton composition, Bianchi et al., 2003). In river deltas and coastal lagoons, the extent of temporal fluctuations in environmental conditions may also be spatially variable. For example, lower temperature fluctuations are expected in sites closer to lagoon inlets, relative to those located near the lagoon edges, which are often shallower and characterized by a higher residence time (Alvarez-Borrego and Alvarez-Borrego, 1982; Pivato et al., 2020). These spatial gradients can vary in time, due to both seasons and tidal cycles (Alvarez-Borrego and Alvarez-Borrego, 1982; Falcao, 2003). Such spatio-temporal variability can have consequences for the biology and ecology of these coastal ecosystems, due to individual physiology and interspecific interactions (predation, grazing, competition). Behavioural adaptations may enable, especially sessile and highly sedentary, organisms to deal with such spatiotemporal variable conditions, but may, however, come at a possible cost for individuals fitness (Helmuth et al., 2010; Monaco and McQuaid, 2018). As it is expected that these kinds of fluctuations will be exacerbated in the future due to climate change (Pesce et al., 2019), this raises the question to which extent sessile organisms can cope with future conditions.

Bivalves offer an ideal model system to study environmental drivers of behavioural responses, due to their highly sedentary nature and because valve-gaping behaviour can be investigated in non-invasive way using low cost sensors (Andrade et al., 2016; Andrewartha et al., 2015; Ballesta-Artero et al., 2017). Valve-gaping, the opening and closing movement of the two valves, is linked to both feeding and respiration, and hence thought to be a physiological process, which could be regulated to respond to changes in the environmental conditions (Ballesta-Artero et al., 2017; Nicastro et al., 2012). Riisgård and Larsen (2014) state that valve-closing should be interpreted as 'an energy-saving adaptation or as an exaptation' and that a 'starvation opening-closing behaviour' could be a mechanism to maintain low oxygen uptake avoiding less efficient anaerobic metabolism (Nicastro et al., 2010). Feeding and associated activities can account for as much as 38% of standard metabolic demands (Hawkins and Bayne, 1985). A regulation of activity based on food availability may be an advantage, as seen with a decrease in feeding activity over winter for *Mytilus edulis,* but this may be an endogenously regulated mechanism indicative of time-average rather than immediate optimization (Hawkins and Bayne, 1985). While gaping cannot be interpreted as a synonymous of clearance rate, it is deemed as a useful method to provide a continuous measurement that can reveal general trends in feeding behaviour (Cranford et al., 2011). Bivalve gaping can be disrupted by environmental stressors such as metal contamination (Curtis et al., 2000), low salinity (Addis et al., 2020; Davenport, 1981) and low oxygen (Porter and Breitburg, 2016) and it can be related to thermal experience and history of an individual (Gleason et al., 2017).

Both circadian and lunar rhythms can affect behaviour of many organisms (e.g. plants, Ruts et al. (2012); insects, Tomioka et al. (2012)), to which a circatidal rhythm is added for those organisms living in the intertidal zone (Morgan, 1991; Neumann, 1981). External inputs are found to synchronize these biological rhythms, and relationships with various parameters in experimental situations seem straightforward. However, results from in situ studies have so far defied simple interpretation of these rhythms (Glass, 2001), and some mechanisms behind the functioning of these clocks were found to be elusive, missing simple explanations (Hirschie Johnson and Hastings, 1986). Bivalve gaping shows some periodicity for most species (*Crassostrea gigas* (Tran et al., 2011), *Pinna nobilis* (Garcia-March et al., 2016), mytilid mussels (Ameyaw-Akumfi and Naylor, 1987)) suggesting they have an internal clock. For mytilid mussels, a circadian rhythm is often found where mussels tend to spend more time closed in daylight conditions (Ameyaw-Akumfi and Naylor, 1987; Comeau et al., 2018; Gnyubkin, 2010). However, whether this is of endogenic or exogenic origin is still debated. Mussels located at greater depths, for example, were found to lose this pattern (Comeau et al., 2018). Laboratory studies seem to show that such circadian pattern is linked to food, either its timing (Robson et al., 2010a, 2010b) or previous feeding conditions (Riisgård et al., 2006). Saurel et al. (2007) suggested a linkage between tide and food availability driving this pattern. The link to tidal patterns for both *M. edulis* and *M. californianus* was first found by Rao (1954), who demonstrated not only that it was an endogenous rhythm, but also showed that the rhythm was more marked when environmental fluctuations were bigger. Miller and Dowd (2017) found a similar tidal 'learnt' behaviour that depended in intensity upon their original position on the shore (becoming less from upper, medium, low) but remained as it was if they were immersed on a rockpool and remained submerged.

Most work on periodic responses in mussels comes from laboratory studies or short-term in-situ studies. Therefore, the effect of spatiotemporal variability in external drivers on behaviour are not accounted, which makes it difficult to use these results for predicting behaviour under climate change. For the purpose of understanding stress responses in face of environmental changes that organisms may experience, it is essential to establish a baseline describing the behaviour of the species studied under non-stressful conditions (Hartmann et al., 2016). Gaining insight into which are the behavioural patterns and how they relate to environmental conditions, requires long-term in situ measurements (Cheng et al., 2015). Moreover, such long-term measurements may also reveal if there are biological rhythms driving behaviour. To address this knowledge gap, we carried out an in-situ, long-term (6 months) and high frequency (minutes) monitoring, to gain insight into how the gaping behaviour of *M. galloprovincialis* on suspended farms in a transitional coastal zone is affected by spatiotemporal variability of the environment. This study specifically aimed to (i) identify the presence of periodicity in gaping behaviour, and (ii) understand the relationships between valve-gaping and environmental variables, in order to disentangle potential drivers of valve-gaping behaviour.

2. Material and methods

2.1. Study site

The study was conducted in the Venice lagoon, which is the largest Mediterranean lagoon and present a microtidal regime with mean tidal excursion from 50 cm on neap tides to 100 cm on spring tides (see Cucco and Umgiesser, 2006) The mean water volume of the lagoon is around $632 \cdot 10^6$ m^3 and the exchange of water through its three inlets in each tidal cycle is about a third of the total volume of the lagoon. Three mussel farms (*Mytilus galloprovincialis*) in the southern part of the lagoon, where the near totality of aquaculture practices take place, and located on a gradient from the sea inlet to a more internal area were chosen for this study (**S1:** 45° 14′ 00.77″ N, 12° 16′ 54.82″ E; **S2:** 45° 13′49.08″ N, 12° 16′ 03.09″ E; **S3:** 45° 13′ 48.83″ N, 12° 15′ 13.03″ E; Fig. 1). The farms are small scale (occupying 0.5 ± 0.3 ha), suspended cultures located at the edges of channels in areas of approximately 3–5 m depth. Mussels are farmed starting from naturally recruited seed collected directly on site on the farm poles, ropes and on older cultivated mussels.

Average water residence time (Cucco and Umgiesser, 2006) was extracted from Atlante della laguna (http://www.atlantedellalaguna.it/?q=maps#tema-4-titolo), and were for S1: 5.9 days, S2: 8.74 days, S3: 11.38 days. Areas closer to the lagoon inlet tend to have lower turbidity values, compared to inner areas: a comparison between two multiparametric buoys located one at the Malamocco inlet (ve-3 in Fig. 1) and one in the inner area near Valle Averto (ve-9 in

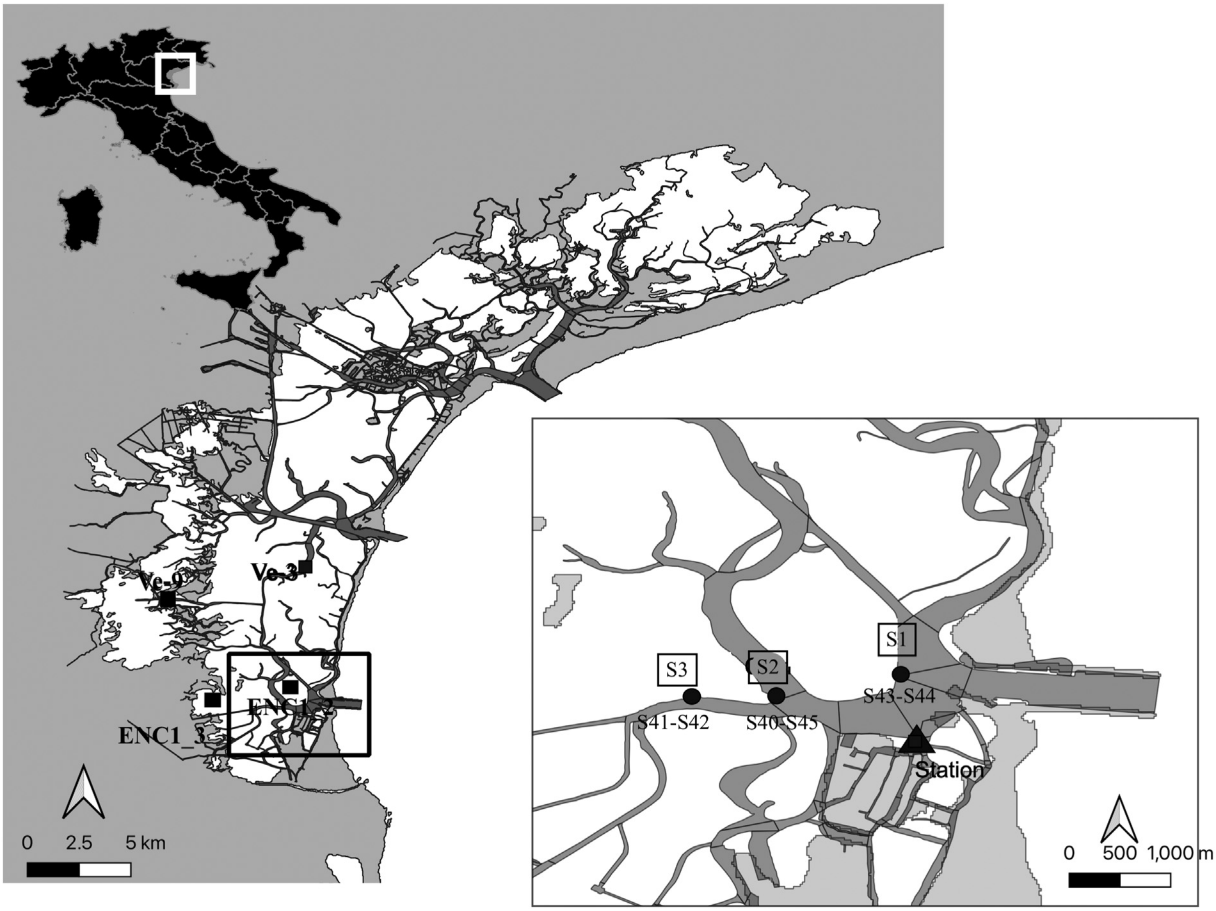

Fig. 1. Study site location.

Fig. 1) for the same time period as the experiment shows turbidity values in the inner area reaching up to 6 fold the values in the inlet (see Table 1). This heterogeneity of the lagoon sites can also be seen when comparing total suspended solids, particulate carbon and particulate nitrogen taken from open access data collected by ARPAV (https://www.arpa.veneto.it/dati-ambientali/open-data/file-e-allegati/soaml/laguna-di-venezia/tss-nutr-acqua), at two siteswhich are also higher moving from the inlet (station ENC1_2 in Fig. 1) to the inner area (station ENC1_3 in Fig. 1) of the southern lagoon (see Table 1).

2.2. Biophys monitoring sensors

Biophys sensors (Fig. 2E) have been developed at the Royal Netherlands Institute for Sea Research (NIOZ) as long-term standalone data logging instrument that can be used for measuring valve-gaping *or* hydrodynamics (using magnetism /hall sensor - DRV5053VAQLPGM), air/water pressure (pressure sensor - MS580314BA01-00) and temperature (temperature sensor - TSIC506F). The valve-gaping measurement is based on the principle described in Ballesta-Artero et al. (2017), but adjusted to measure a single bivalve per sensor, thus working with a magnet and a hall-sensor (see Fig. 2E). All sensors are located in the head of the Biophys sensors. The minimum sampling frequency is 0.1 s. In our experiment, the sampling frequency was set at 1 s for all parameters to avoid unnecessary battery usage, while still obtaining high frequency samples (Robson et al., 2009). In order for the hall sensor to be used for shell gaping, a small magnet was glued on the mussel shell, placed between inhalant siphon and mantle (Fig. 2D). Preliminary studies revealed this was the location where the best opening signal was obtained. Magnet size (Ø = 3 mm) was chosen to be the smallest possible to limit behavioural interference but large enough to give a signal which could be distinguished from Earth magnetism.

Table 1
Medians and standard deviations of turbidity, total suspended matter (TSM), particulate organic carbon (POC), particulate total nitrogen (PTN) comparing outer area of the lagoon to inner areas.

Variable	Outer	Inner
Turbidity (2019)	July: 3.9 ± 3.7 August: 9.2 ± 9.3 December: 6.6 ± 0.13 (ve-3 in map)	July: 22.9 ± 75.4 August: 14.6 ± 46.2 December: 7.04 ± 39.1 (ve-9 in map)
TSM (2016–2018)	2.8 ± 1.8 (ENC1_2)	4.1 ± 3.3 (ENC1_3)
POC (2016–2018)	0.31 ± 0.13 (ENC1_2)	0.43 ± 0.25 (ENC1_3)
PTN (2016–2018)	0.05 ± 0.02 (ENC1_2)	0.07 ± 0.04 (ENC1_3)

2.3. Experimental design

Six sensors were available for use during this project. Two adult mussel specimens (>5 cm) were collected at each farm, glued each into one sensor (Fig. 2) and redeployed. The sensors (see Fig. 2) were attached to the rope just above the farmed mussels, in order for the mussel on the sensor to experience conditions as similar as possible to that of the farmed mussels. Mussels in each farm were oriented in the same direction and all approximately towards the channel.

In order to retrieve the data, the sensors had to be collected and brought into the laboratory. This was done approximately every month, to avoid data loss. The sensors were first deployed on July 16th 2019, where after data were retrieved (sensors collected and

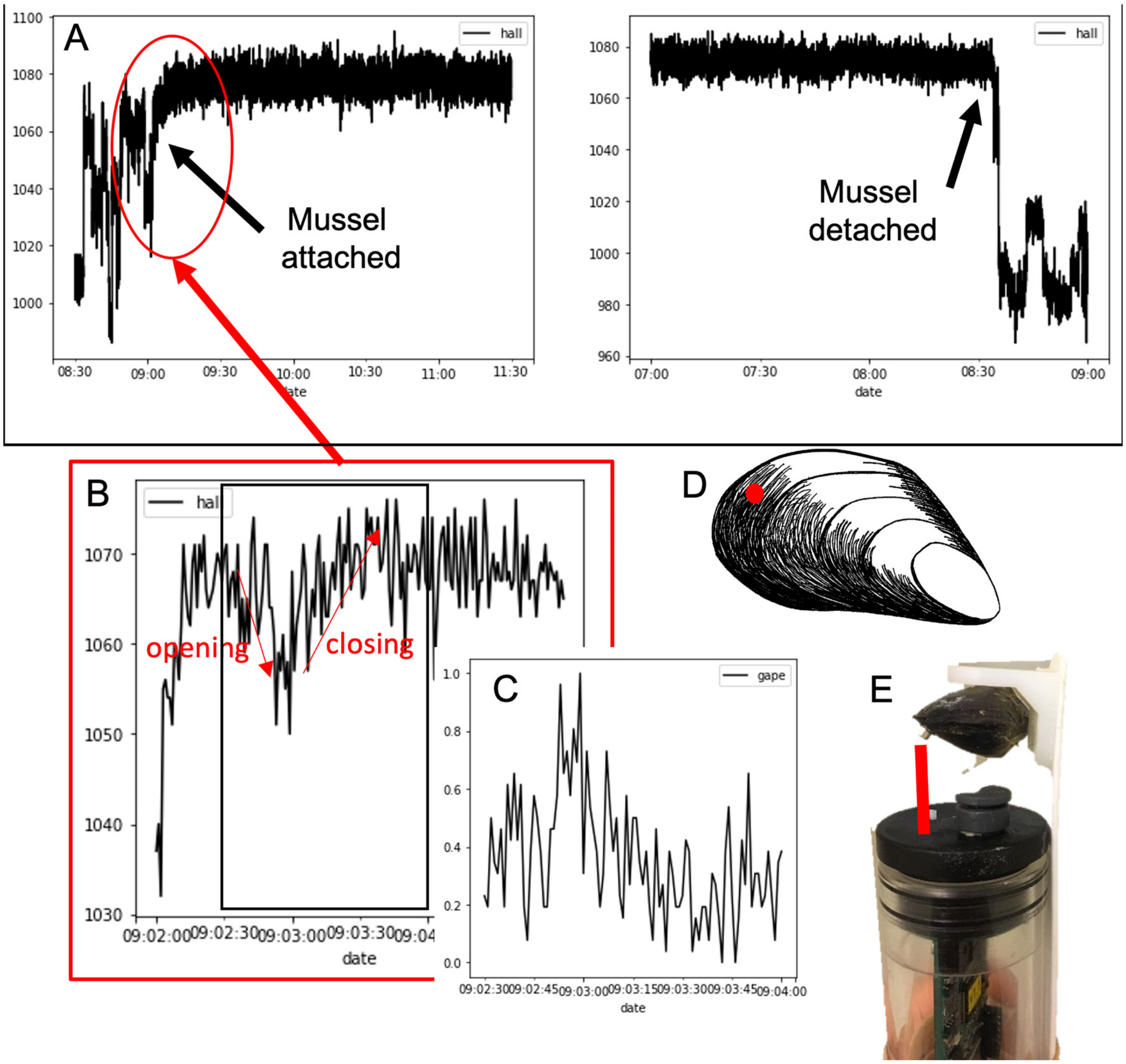

Fig. 2. Processing methods for the gaping data. (A) identification of the moment where mussel was attached to the sensor, jumping occurs because of the presence of the magnet attached to the shell and located directly above the sensor (D, E). Red line in (E) shows the distance being measured to quantify gaping. (B) and (C) represent data series processing descripted in methodology.

then redeployed) on August 20th, September 24th, October 24th and December 18th. For the first deployment, mussels were collected in the morning (between 8 am and 9 am), brought to the laboratory and glued to the sensor using aquarium silicone. They were then submersed in a seawater tank before deployment. The sensors were then deployed around 13:00. For the in-between sampling, sensors and mussels were retrieved between 8:00 and 9:00 and brought to the laboratory where mussels were detached from sensor and left in a seawater tank while sensors were cleaned of fouling, opened, data retrieved, redeployed. Mussels were then reattached to the same sensors and redeployed (around 14:00). From July to October the same specimen was used for each sensor. In October new mussels were used for the October–December deployment, these were collected in the morning and attached following the same procedure used in July.

2.4. Sensor data processing

Raw data were plotted using Phyton 3.6 (Spyder 3.1.4, Anaconda), in order to identify the point in which the mussel with the magnet was positioned (Fig. 2A), and the direction of hall number changes (dependent upon magnet direction, Fig. 2B). Time series were then cut, based on dates of deployment and retrieval, setting the start at 15:00 (circa 1 h after deployment) and ended at 07:00 (circa 1 h before retrieval). Only in two occasions (S44 in July–August period and S40 in August–September period), we found that the mussels detached themselves and moved from their position: therefore, the series were cut shorter (S44 to 27th July and S40 to 10th September). To avoid spikes of noise, which were rare but present, interfering with gape angle calculations, outliers were removed based on lower and upper quantiles (5 and 95%). The gape 'angle' (thus the proportion of valve opened) at each sample was calculated as:

$$\text{Gape} = \text{abs}((\text{hall} - \max\ (\text{hall}))/(\max(\text{hall}) - \min\ (\text{hall}))$$

(if the hall number decreased with opening)

Or

$$\text{Gape} = \text{abs}((\text{hall} - \min\ (\text{hall}))/(\max(\text{hall}) - \min\ (\text{hall}))$$

(if the hall number increased with opening) (Fig. 2C).

The data set was then averaged over a minute to remove potential noise and obtain a manageable time series.

2.5. Environmental data

Temperature at the three locations was measured by the Biophys sensors with the same sampling frequency as the gaping (1 Hz). Data were averaged over a minute to obtain a manageable time series that could be compared to that of gaping.

The model SHYFEM has been used for the characterization of the hydrodynamic variables, i.e. surface elevation (ζ), current speed and direction. This state-of-the art hydrodynamic model was applied to the Venice lagoon and was used in many studies (e.g. Umgiesser et al., 2004). The model was forced using weather data for the same temporal window in which the sensors were deployed (July–December 2019).

Chlorophyll and Turbidity time series, used as proxies for food quantity and quality, were estimated from the data collected hourly by a self-cleaning chlorophyll sensor (Tecnos S.a.S., Chioggia, Italy) within a multiparametric buoy (measuring temperature, oxygen, salinity, pH, chlorophyll and turbidity) deployed as part of the same sampling campaign at the S2: gaps in data from malfunctioning periods were filled in with data retrieved from the multiparametric buoy from the SAMANET network, also recording hourly (Provveditorato, ve-3; 45° 7′ 11.566″ N; 20° 17′ 20.292″ E; square Fig. 1). Other environmental parameters, such as oxygen, salinity, pH that may influence gaping were retrieved from the same multiparametric buoys and averaged over each month (Table 4).

2.6. Descriptive analyses

R was used for descriptive and statistical analyses described below. Descriptive statistics (medians, standard deviations) were estimated on gaping, time spent open or closed, considering as a 'closed threshold' a level of opening of 20% following Comeau et al. (2018) and Miller and Dowd (2017), and environmental parameters (i.e. irradiance, oxygen, pH, temperature, chlorophyll and turbidity) for all deployment periods. The median valve opening by hour was also calculated and used as a response variable in a Generalised Additive Model (GAM in package *mgcv*) to identify if gaping changed through the day in a predictable manner by individual and deployment period. GAM fit was checked by looking at residual plots for homoscedasticity and normality of residuals. The consecutive number of hours in which each individual was closed was also calculated. The longest closure for each day was used in analyses. This value was used to draw empirical cumulative distribution function curves, and following (Dowd and Somero, 2013) their shapes were compared using Anderson-Darling test with pairwise adjustments (in the package *PMCMRplus*) to see effects of site and deployment time. Cross correlation analysis was performed on sea surface elevation (ζ) simulated from the three sites in order to quantify the tidal delays in the three locations.

2.7. Spectral analysis methods

Advanced spectral analysis methods have been applied in order to detect the main periodic components characterizing the gape time series and to study their relationship to environmental conditions. These techniques rely on wavelet methods: since they require a constant time sampling Δt, the gaping series was resampled to a $\Delta t = 5$ min, equal to that of the output of the SHYFEM model. When multivariate techniques were applied between gape and chlorophyll and between gape and turbidity, hourly-mean gape values have been considered. The spectral content of each series was studied using the univariate Continuous Wavelet Transform (CWT; Torrence and Compo, 1998). The significance level has been estimated comparing the GWS with the power spectrum of an lag-1 autoregressive model (AR1). Significant spectral components detected with this method were reconstructed through the Inverse CWT (Alessio, 2016; Torrence and Compo, 1998).

Moreover, the Cross-Wavelet Transform (XWT) and the Wavelet Coherence (WTC; Grinsted et al. (2004)) were used in order to detect spectral features common between gape and ζ and chlorophyll/turbidity. XWT was used to detect regions in time-frequency domain in which the two records have common power and relative phase. WTC allows one to identify significant coherence also at low levels of common power, as this methodology estimates also the confidence levels against red noise backgrounds. Full description of the wavelet methods is reported in Supplementary Material.

Low-frequency modes were removed using a smoothing procedure based on local linear regression over different time intervals(loess method; Cleveland (1979)) before applying the spectral analysis. Since significant long-term trends could affect the results, concerning in particular the estimate of high-frequency oscillations (Alessio, 2016). In more detail, short time span (1 day) was used to remove sharp changes in the gape record (as in the case shown in Fig. 1A), while longer time spans (about 5 days) were occasionally applied for removing long-term components with periods of weeks or longer if their associated variance could affect the detection of high-frequency peaks in the power spectrum.

CWT, XWT and WTC methods were performed using the MatLab (Mathworks, 2020) software package (Grinsted et al., 2004), available at https://github.com/grinsted/wavelet-coherence. Signal reconstructions through Inverse CWT were obtained using the MatLab code reported in Alessio (2016).

3. Results

3.1. Descriptive analyses

3.1.1. Gaping behaviour

The amount of time mussel spent with their valve closed (< 20%) varied amongst specimen and deployment periods and was as little as 1% of the time in the S2 and as much as 42% in the S3 in September–October (Table 2). Most closures events (75%) lasted for less than 5 h / day, with some exception in September–October at both S2 and S3 and October–December at S3 (Fig. 3, Table 3).

When looking at the timing of gaping with respect to the hour of the day the GAM model described only 5.5% of the variability. Overall, there were constant patterns at the S3, with the two individuals following the same pattern with the greatest periodicity in both summer deployments (July–August and August–September), where mussels tended to be more open in the late morning (circa 10:00), and only one individual keeping some rhythm in autumn and winter (with the maximum opening occurring circa at 5:00 and 18:00). At the S2, patterns were not consistent between the two individuals in July–August, with no rhythm found in August–September and again only one individual keeping some rhythm in autumn and winter (more open around 13:00). In the S1, there were weak oscillations in autumn-winter (10% of mean hourly gaping angle).

3.1.2. Environmental variables

The semidiurnal tidal cycle showed a delay of circa 15 min between site 1 and 2 and 25 min between site 1 and 3. Water velocities diminish progressively moving from the inlet where velocities of more than 20 cm/s up to 40 cm/s are common compared to 10 cm/s in the more

Table 2
Time spent closed in the deployment periods.

Deployment	Time (%) closed S3	Time (%) closed S2	Time (%) closed S1
J-A	11	2	14
	16	10	23
A-S	12	2	15
	24	7	2
S-O	42	1	36
	11	5	8
O-D	33	5	13
	5	5	

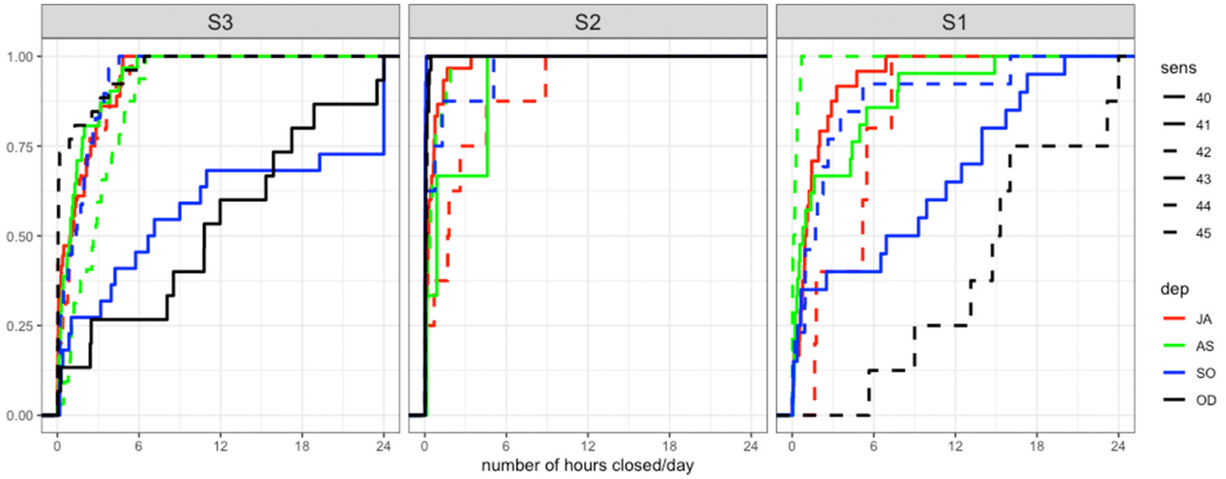

Fig. 3. Cumulative empirical distribution curve of the number of consecutive hours spent closed every day during the four deployment periods at the three locations. Each line represents one mussel.

internal site. Water velocities are higher when tides are going on a westerly direction, thus going out (Fig. 4), and these are more defined by tidal dynamics, with velocities being greater during spring tides compared to neap tides (Fig. 5). Temperature fluctuations associated with the tide were greater in S2 and S3, compared to S1, and resulted in higher temperatures recorded during low tides in summer and lower temperatures recorded during low tides in winter in S2 and S3 (Fig. 6). A summary of all environmental variables is presented in Table 4.

3.2. Time series spectral analyses

The main period common to most mussels and time intervals (all aside from S1 in July–August) and all deployments was 12.3 ± 0.3. The 24.2 ± 0.6 h periodic cycle was also present for 5 out of 6 mussels (i.e., with 1 mussel at the 'S1' site non showing this periodicity) from July until September and up to 2 individuals also displayed a cycle of about 6 h. Chlorophyll always showed 12 and 24 h periodicities but in July–August their associated power was not significant at the 95% c.l. The results of all univariate wavelet analysis for gaping and zeta (tide) are shown in Fig. 7, in which the global wavelet spectra (GWSs) are represented. Results of the analysis of chlorophyll and turbidity time series are also included for the only one site available. In each panel the spectra of a variable referred to the same site are shown for the four periods available. Periods significant at least at 95% confidence level (c.l.) are reported in Table 5.

The results of the cross-wavelet transforms (XWT) presented in the fourth and fifth column of Table 5 showed two main frequency bands, corresponding to periods of about 12 and 24 h (full figures in Supplementary 2). The phase relationship between gaping and the other parameters did not show clear patterns but appears to be site specific, with a tendency for mussels in the 'S3' to be in phase with the tide (opening when tide comes in and closing when it goes out) in summer (until September), with some delays on the tide. Time lags between gaping and zeta, chlorophyll and turbidity are presented in Table 5. Phase examples between gaping and zeta in summer and winter for in-phase (S3) and anti-phase (S2) are shown in the reconstruction of the signals presented in Figs. 8 and 9. For Chlorophyll at S2, XWT does not provide evidence of a clear phase relationship, since the spectra show only patchy areas of signal sharing (Supplementary figures). When XWT is significant, antiphase dominates; nevertheless the average phase lag value over the entire period covered by the series has values of few hours maximum (see Table 5).

WTC analysis between gape and zeta showed coherence located in both the 12 and 24 h spectral bands more well-defined at S3 and S2. The significance of these coherence bands decreased from summer to winter as expected from previous analyses (Supplementary figures). No clear coherence bands were obtained between gape and chlorophyll.

Table 3
Statistical results from the Anderson-Darling test with pairwise adjustments for (a) comparisons between sites and (b) comparisons between deployment periods.

(a)

Sites comparisons	*p*-Value
S1–S2	<0.0001
S1–S3	<0.0001
S2–S3	<0.0001

(b)

Deployment comparisons	*p*-Value
JA-AS	<0.05
JA-SO	<0.001
JA-OD	<0.0001
AS-SO	N.S
AS-OD	<0.0001
SO-OD	<0.0001

4. Discussion

With an in-situ, long-term (6 months) and high frequency monitoring, this study found evidence for the presence of an endogenous circadian rhythm associated with tides. The rhythm was present at all locations and predominant in the summer months, but appeared to be more marked in the site subject to larger environmental fluctuations linked to tidal oscillations. During winter, the relation with environmental drivers was reduced.

4.1. The periodicity of the rhythm and potential drivers

Semidiurnal (~12.4 h) and diurnal (~24.7 h) cycles were identified as dominant periodicities in the gape records for *M. galloprovincialis.* This result describes the same patterns seen by Miller and Dowd (2017) for *M. californianus* in the intertidal shores (12.4 h and 24.8 h). According to the definition by Tran et al. (2020), the combination of these two periods follow what is the 'circadian rhythm', instead of the 'circalunidian' which is when two antiphase 24.8 h cycles meet (Palmer, 1995, 2000) or the circatidal, which is simply a unimodal 12.4 h clock. This same bimodal rhythm was found for the oyster *Crassostrea gigas* (Tran et al., 2020). It was suggested that tidal cues are integrated with daily cues giving rise to a single clock giving either bimodal or unimodal oscillation according to the balance between the two cues in each specific location

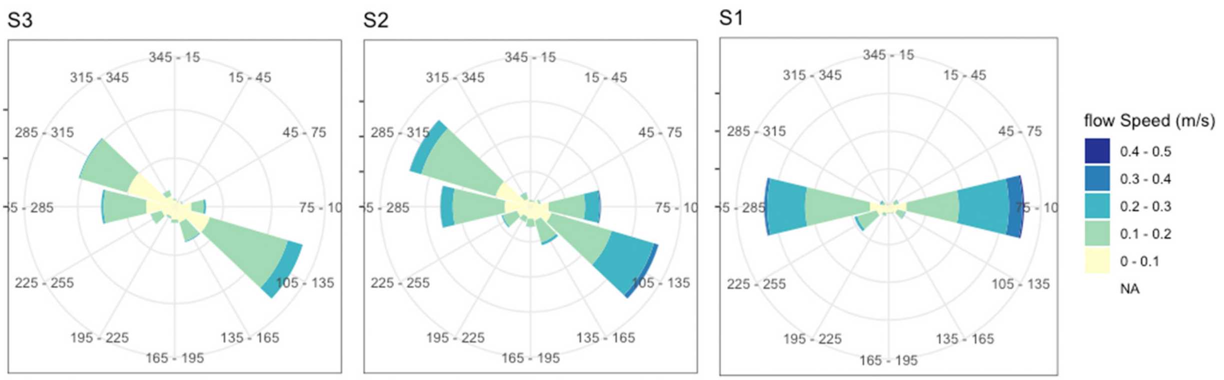

Fig. 4. Rose plot of direction and speed of flow at the three sites.

(Tran et al., 2020). This was also the case in our study, where bimodal oscillations were present in summer months, shifting towards a circatidal, unimodal clock of 12.4 h towards the winter months. These findings indicate that the tidal relationship was modulating the gaping throughout the observation period and appeared to be the driving one, similar as to what reported for *C. gigas* (Tran et al., 2011, 2020).

The circadian cycle (24 h) was the only one found in a study of *M. galloprovincialis* in aquaculture farm off the coast of Spain during a ten days deployment in December, where there was a consistent effect of closures during the daytime (Comeau et al., 2018). In the present study, however, there was no such clear relationship with hour of the day per se. The 24.7 h that occurred more prominently in the summer months could be supportive of a light dependency in these months were solar irradiance is stronger (Table 4), however only three out of six mussels showed predominant closures in the early hours of the morning (3–4 am, Fig. 4). The 'labile' nature of the circadian rhythm vs the circatidal rhythm was already highlighted for *C. gigas* (Tran et al., 2020) and the pattern is here confirmed, where the circatidal 12.4 h is maintained throughout and the amplitude of gaping follows the tidal amplitudes, suggestive of a ubiquitous relationship with tidal patterns in bivalves. This tidal relationship was found even if mussels were continuously submersed, suggesting that this is not only a reaction or adaptation to being exposed to air during low tides.

Summer gaping is mostly dual, whereas in autumn and winter the 24.7 disappeared. Chlorophyll also peaks at 12 h and 24 h becoming more evident from the end of summer, although the oscillations are less marked than for the other variable considered. Since the critical algal concentration below which the mussel closes its valves is between 0.5 (Riisgård et al., 2006) and 0.9 μg Chl-a l^{-1} (Pascoe et al., 2009), and these values were seen in July, it may seem that mussels are more likely to display periodic gaping to be open only when food is available. This would be in line with the starvation regulation of gaping proposed by Riisgård and Larsen (2014) and by Tang and Riisgård (2016). However, in July–August, when chlorophyll levels were the lowest, and with no periodicity, mussels showed the strongest signal, suggesting that the driving mechanism may be food pdelivery rather than concentration, and thus an interaction between chlorophyll and local hydrodynamic could be considered as a possible driver. In the other deployment periods, where chlorophyll showed periodicity, gaping and chlorophyll measured at site S2 were found to be mostly in anti-phase, meaning mussels tended to close as chlorophyll increased and vice versa, in the site where chlorophyll was measured. In this period, chlorophyll levels were never below the threshold, but it may be that mussels become food saturated with increasing levels, closing their valves, as seen for example with *Mytilus edulis* filtration rate diminishing in periods of high concentration (Riisgård et al., 2013) or with abrupt changes in *M. galloprovincialis* clearance when exposed to high chlorophyll diets (Filgueira et al., 2009), although the values of chlorophyll required for shutting of valves reported in the latter (26 μg/l) were not recorded here.

It should be considered that chlorophyll, while often used as a proxy for food, is not the only 'trophic' indicator, and plankton cells and species may be highly variable. For example, while for most of the year the phytoplankton charge is higher at flood tide sometimes the opposite occurs when more plankton is found during ebb tides, due to high abundances of a few species, enhanced by the favourable environmental conditions of inner areas (Bernardi Aubry and Acri, 2004), and due to resuspension of microphytobenthos (Facca et al., 2002). Secondly, the

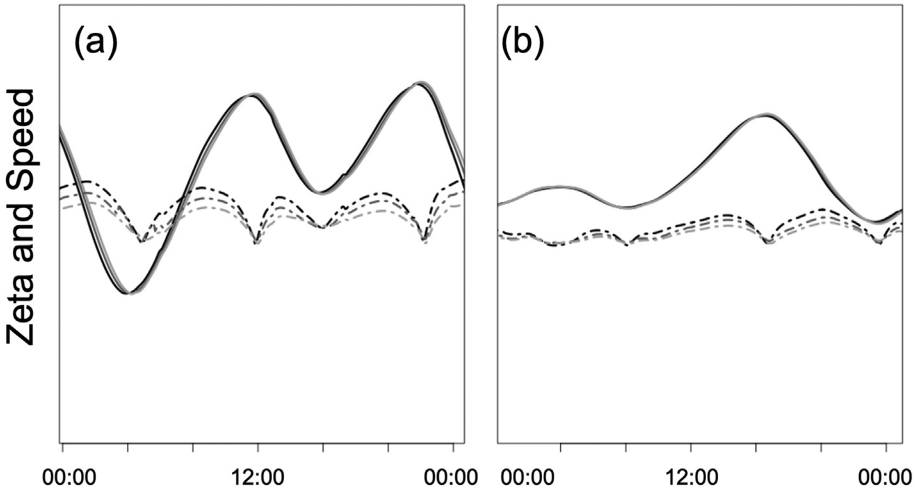

Fig. 5. Example of relationship between zeta and velocity during (a) spring and (b) neap tides, in July at the three sites (S1:black, S2:dark grey, S3: grey).

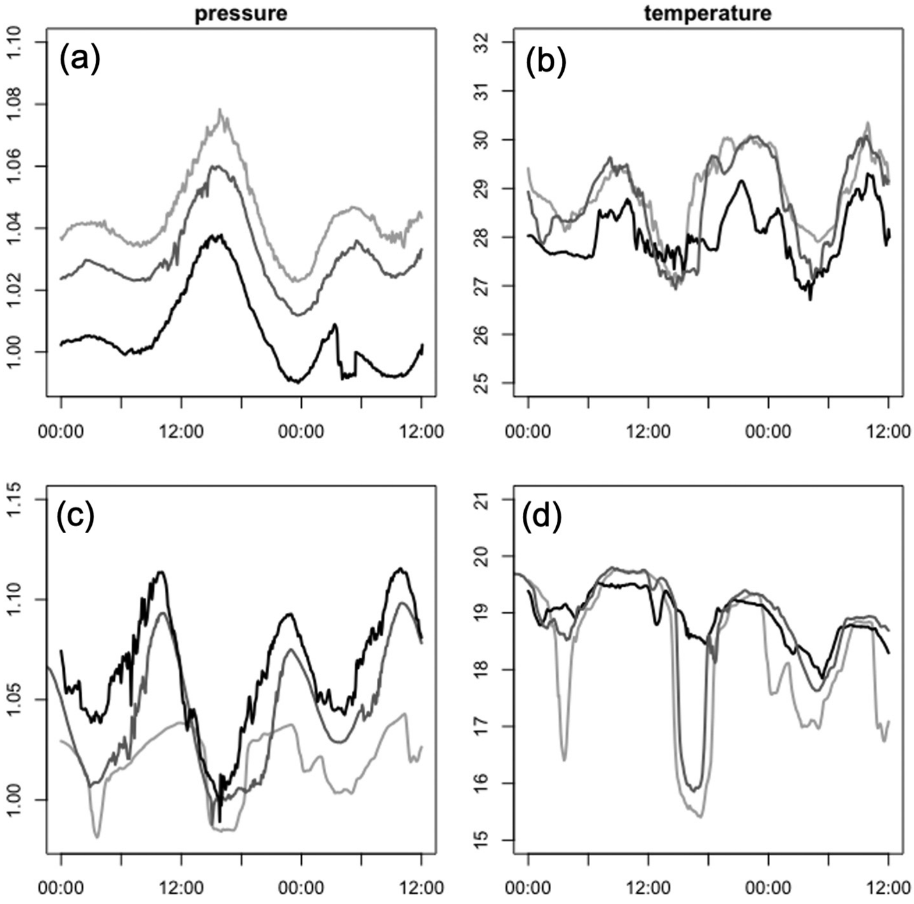

Fig. 6. Pressure and temperature recorded by the biophys sensors during 25–26 July 2019 (a, b) and during 29–30 October 2019 (c, d) at the three sites (S1:black, S2:dark grey, S3: grey).

composition of suspended particulates influence food quality, as the higher the fraction of inorganic particles, which may be related to sediment resuspension, the higher the sorting time, which leads to a lower efficiency in converting bioavailable organic matter into energy. As a result, the SPM:Chl-a ratio can influence clearance rate and frequency of gaping (Filgueira et al., 2010), due to the need of excreting more particles as seen by an increased production of pseudofaeces during high turbidity seasons (Zúñiga et al., 2014). Food quality, here intended as the amount of bioavailable organic material in the suspended particles and its seasonal variability, influences the amount of energy ingested by individuals (Fernández-reiriz et al., 2017) and thus site specific data on other food proxies, such as biopolymeric organic carbon (C-BPC) and their interactions with tidal fluxes could be relevant for improving the understanding of the dynamic of gaping in relation to food quality.

Further, while valve gaping was found to be mostly correlated with other measurement of clearance (e.g. siphon area), it also showed some inconsistencies in published literature (e.g. Maire et al., 2007). This is likely due to the fact that gaping is necessary for other activities besides feeding, such as gas exchange. For example, as organisms adapted to intertidal living, mussels close their valve to retain water, but some species, such as *Perna perna* are also gaping while air exposed resulting in cooler body temperatures (Nicastro et al., 2012). This versatile behaviour may therefore display a rhythm linked to rhythm of other environmental variables (e.g. oxygen, temperature), as seen with oysters closing during diel cycle hypoxia (Porter and Breitburg, 2016) that while are showing mostly a diurnal pattern may also be influenced on a local scale by more oxygenated seawater influx, particularly in the more restricted 'inner' areas.

4.2. Spatiotemporal effects on rhythms

If the circadian rhythm was fixed, then all sites would be strongly periodic. However, in this study periodicity was variable not only over different seasons, as already observed for oysters (Tran et al., 2011, 2020), but also across different locations. Miller and Dowd (2017), with a manipulative experiment where mussels were moved across the intertidal area, found similar patterns where gaping 'periodicity' was related to the origin of the mussels, with a stronger signal arising from individuals coming from the upper shore. In this study, which was subtidal, location also was found to be important, suggestive of the idea that a gradient in environmental signal strength could drive the strength of behavioural

Table 4
Medians and standard deviations of environmental parameters for the 6 months of deployment.

Month	Solar irradiance (MJ/m^2/day)	Chlorophyll (μg/L)	Salinity	Oxygen (mg/L)	Turbidity (NTU)	pH	Temp
July	22 ± 5.8	0.79 ± 0.26	33.5 ± 0.94	8.05 ± 1.71	3.30 ± 1.55	8.53 ± 0.08	26.7 ± 1.63
August	19.3 ± 3.82	1.47 ± 0.64	34.24 ± 0.75	7.77 ± 2.12	3.77 ± 2.35	8.64 ± 0.07	26.9 ± 1.53
September	15.3 ± 5.21	1.77 ± 0.56	35.81 ± 0.65	6.96 ± 1.87	5.7 ± 3.83	8.67 ± 0.09	22.7 ± 2.44
October	10.7 ± 3.82	1.27 ± 0.38	35.8 ± 0.86	7.49 ± 1.91	4.09 ± 3.12	8.29 ± 0.03	19.4 ± 1.43
November	3.48 ± 2.94	2.18 ± 0.8	33.81 ± 2.63	7.46 ± 0.99	8.3 ± 7.09	8.27 ± 0.04	14.1 ± 1.34
December	3.04 ± 2.6	1.87 ± 0.95	32.29 ± 2.5	9.02 ± 0.55	4.9 ± 4.22	8.3 ± 0.06	9.98 ± 1.89

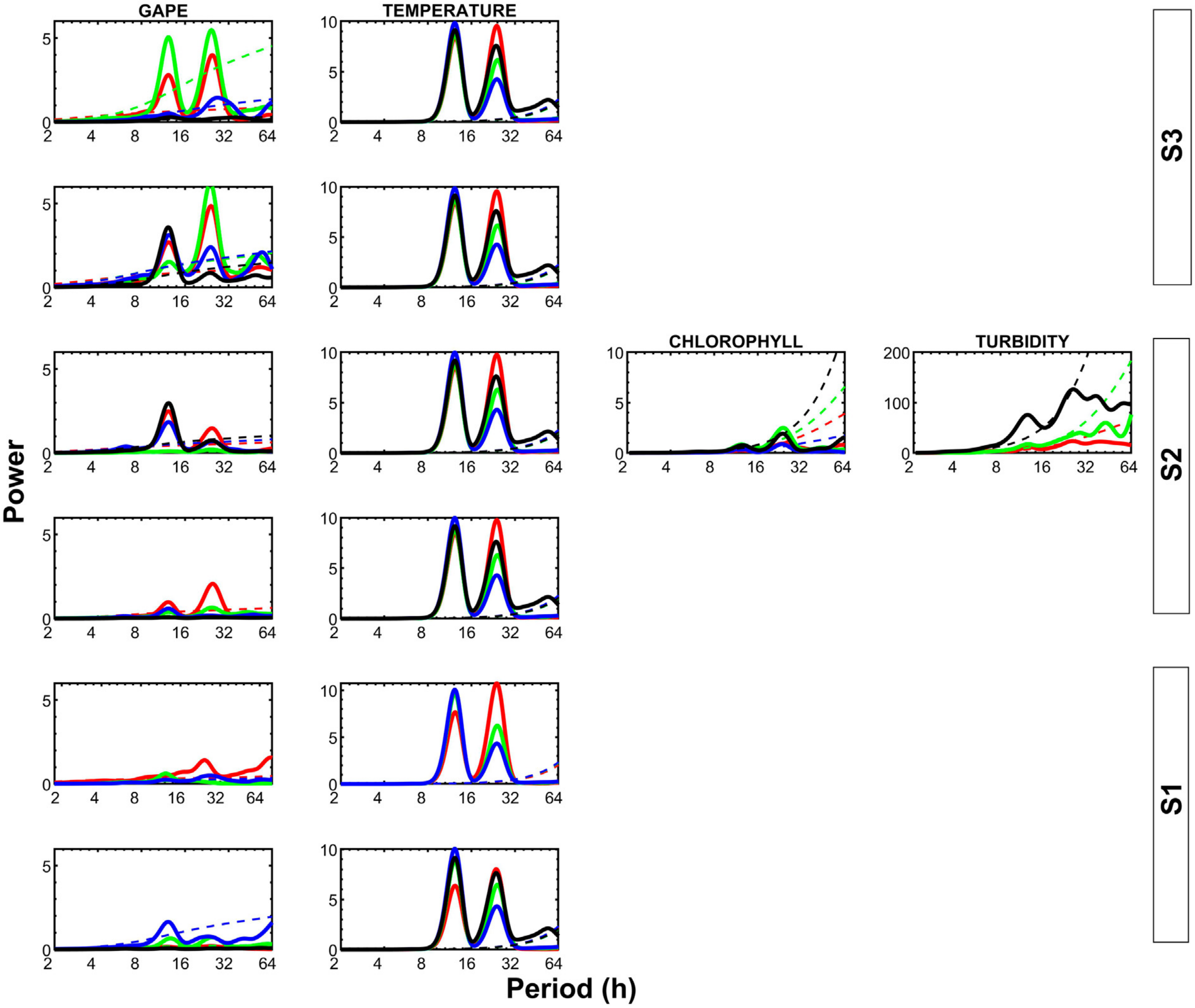

Fig. 7. : Global wavelet spectra of gape (first column), ζ (second column), chlorophyll (third column) and turbidity (fourth column) for each considered site and time interval available (J-A: red line, A-S; green line; S-O: blue line and O-D: black line). Dotted lines represent the 95% c.l. for each spectrum obtained considering a red noise background as null-hypothesis.

signal (Rao, 1954). Tran et al. (2020) also suggested that that '*integrating the tidal cues with the daily cues might occur in a single clock that would give bimodal or unimodal oscillation outputs according to the balance between tidal and daily cues in each specific location inhabited by oysters'*. The site which displayed the greater power in the periodicity of gaping, was in fact also the site where environmental conditions, such as temperature, were also the most variable and most tidally-dependent. Since clearance rate, which clearly depends on gaping, is incorporated in bioenergetic individual models and used to estimate shellfish growth and ecosystem functioning such as carbon and nitrogen outputs (e.g. Brigolin et al., 2009) and it is usually kept constant, including a tidal component to the clearance rate may give more accurate 'site specific' predictions, which could be used for improving shellfish farm management.

Organisms inhabiting a tidal environment can be exposed to high levels of environmental fluctuations. Gudimov (2006) found that blue mussels can respond to natural changes in water temperature and salinity as small as 0.1 °C and 0.1–0.3‰, respectively, and changes in seston and phytoplankton concentrations of 0.1 mg/l and 100–300 cells/l, respectively. These fluctuations in transitional coastal zones can be significant and can be linked to tidal and other periodic fluctuations (e.g. day/night). Chlorophyll availability, for instance, may also depend on flow velocity (found in a study by Dolmer, 2000). Therefore understanding responses to 'fluctuations' driven by periodic cycles present in nature is the first step to understand responses to environmental changes. Gaping periods were mostly in phase with the tide in the most internal site, and as seen by a greater amplitude during spring tides which are associated with greater velocity (see example in Fig. 5 and time series reconstruction in Figs. 8 and 9).

Speed was always lower in the most internal site and it may be that flow brought in by the movement of the tide was the instigating mechanism for initial opening, since a unimodal response of siphon area and valve gape was observed with an optimum at medium velocities (Sénéchal et al., 2008) and at low flow velocities algal depletion is maximised (Widdows et al., 2002). It can be that at maximum water height mussels were fully open until algal depletion occurred and they started to close again as tide was going out, suggestive of an interaction with flow and productivity. This can be highly context dependent and influenced by the tide-plankton relationship. Plankton abundance and species composition vary considerably between flood and ebb (Bernardi Aubry and Acri, 2004), with a seasonal shift with spring and summer having richer floods and autumn and winter richer ebbing. The location of the sites along different channels may be therefore enough for different species and dynamics to be present, but this can only be speculated here and investigated further with a specific design.

Suspended matter can have impacts on gaping activity, as particles rejection rate increases as its concentration increases (Hawkins et al., 1998). Suspended matter dynamics are complex in the Venice lagoon and their distribution is highly heterogeneous: their concentration tend to be higher in the inner areas of the lagoon, with highly variable spatial and temporal dynamics (Rolinski and Umgiesser, 2005). Mussels

Table 5
Results of the wavelet analysis performed on the gape, water height, chlorophyll and turbidity. The first three column refers to periods (in hours) of spectral components significant at 95% c.l. In brackets the full width at half maximum (FWHM) for each peak of the power spectrum is reported. The fourth and fifth columns refer to the phase lags and relative standard errors, expressed in hours, for the dominant shared periods (reported in bold) between Gape, Zeta, Chlorophyll and Turbidity. If 'n.s.' then no significant periods or no significant shared periods were found. If 'n.a.' then data were not available.

JULY-AUGUST

Site	Gape	ζ	Chlorophyll	Turbidity	Gape-ζ	Gape-Chlorophyll	Gape-Turb
S3	12.2(3.7), 24.7(7.6)	12.4(3.4), 24.(6.9)	n.a.	n.a	**12:** 0.2±.6 **24:** 0.3±2.7	n.a	n.a.
	12.4(4.1), 24.(7.8)	12.4(3.4), 24.(6.9)			**12:**0.9±1.4 **24:** 2.5±2.2		
S2	12.2(3.6), 24.7(8.2)	12.4(3.4), 24.(6.9)	n.s	7(4) 12 (5)	**12:** -4.3±.9 **24:** -11.8±4.7	n.s.	12: -2±1
	12.2(3.6), 24.7(7.9)	12.4(3.4), 24.(6.9)			**12:** -4.7±1.1 **24:** -10.7±2.2	n.s.	12: 4 ± 1
S1	17.2(9.3), 24.4(11.8)	12.4(3.5), 24.(7.)	n.a.	n.a.	**24:** 6.2±3.1	n.a	n.a.
	5.4(2.2), 7.9(3.2)	12.4(3.5), 23.7(7.1)			n.s.		

AUGUST-SEPTEMBER

	Gape	ζ	Chlorophyll		Gape-ζ	Gape-Chlorophyll	Gape-Turb
S3	12.4 (3.6), 24.4(8.)	6.3(1.6), 12.4(3.4), 24.4(7.2)	n.a.	n.a.	**12:** 0.1±1.3 **24:** 0.2±1.8	n.a.	n.a.
	12.4(5.4), 24.(7.4), 50.2(34.5)	6.3(1.6), 12.4(3.4), 24.4(7.2)			**12:** 0.2±2.1 **24:** 1.4±1.6		
S2	6.4(1.9), 8.5(2.8), 12.5(6.9), 24.4(9.6)	6.3(1.5), 12.4(3.4), 24.4(7.1)	12 (5), 24 (8.4)	3(3) 12 (5)	**12:** 2.1±2.5 **24:** .6±5.3	12: 5 ± 1 24: 5 ± 6	12: -4 ± 2
	12.4(4.5), 24.4(9.8), 42.8(28.8)	6.3(1.5), 12.4(3.4), 24.4(7.1)			**12:** 4.9±2.6 **24:** -9.9±4.4	12: -5 ± 3 24: 1 ± 4	12: -4 ± 3
S1	12.4(4.2)	6.3(1.5), 12.4(3.4), 24.(7.)	n.a.	n.a.	**12:** -2.7±2.	n.a.	n.a.
	12.7(4.3), 24.7(10.1), 59.7(45.1)	6.3(1.4), 12.4(3.4), 24.4(7.1)			**12:** 6.1±2.7 **24:** 10.9±4.3		

SEPTEMBER-OCTOBER

	Gape	ζ	Chlorophyll	Turbidity	Gape-ζ	Gape-Chlorophyll	Gape-Turb
S3	27.(15.4)	12.2(3.4), 24.(7.3)	n.a.	n.a	n.s.	n.a	n.a.
	12.4(4.), 24.(8.6), 54.7(20.7)	12.2(3.4), 24.(7.3)			**12:** -2.9±1.3 **24:** -1.1±3.4		
S2	6.3(2.4), 12.4(3.8), 24.7(9.7)	12.2(3.4), 24.(7.3)	12 (4), 24 (8)	n.a.	**12:** -3.1±1. **24:** -4.3±4.8	n.s.	n.a.
	6.2(2.7), 12.2(3.7), 23.7(14.6), 47.4(25.5)	12.2(3.4), 24.(7.3)			**12:** -3.8±.6 **24:** -7.5±2.8	n.s.	
S1	12.4(5.9), 27.(16.1)	6.5(1.4), 12.2(3.4), 24.(7.4)	n.a	n.a	**12:** -1.3±2.1	n.a.	n.a.
	12.2(4.2)	6.5(1.4), 12.2(3.4), 24.(7.4)			**12:** 8.2±6.		

OCTOBER-DECEMBER

	Gape	ζ	Chlorophyll	Turbidity	Gape-ζ	Gape-Chlorophyll	Gape-Turb
S3	12.7(6.9), 35.5(28.9)	12.4(3.5), 23.7(7.6)	n.a.	n.a	**12:** -6.1±2.6	n.a.	n.a.
	12.2(3.5)	12.4(3.5), 23.7(7.6), 54.(35.9)			**12:** 3.1±.6		
S2	12.4(3.5)	12.4(3.5), 23.7(7.5), 54.(33.9)	13 (4), 24 (7)	12 (6)	**12:** -3.8±.5	12: 5 ± 2	12: 6 ± 3
	6.3(2.4), 8.1(1.6), 12.4(4.6), 25.1(21.7)	12.4(3.5), 23.7(7.5), 54.(33.9)			**12: -**5.1±3.6 **24**: -11.4 ± 4.2	12: 5 ± 3 24: 2 ± 1	12: -2 ± 4
S1	n.a.	n.a.	n.a.	n.a	n.a.	n.a.	n.a.
	6.2(3.3), 12.2(4.2), 25.1(10.9), 37.1(11.5)	12.4(3.5), 23.7(7.5), 54.(33.4)			**12:** 2.3±2.3 **24:** 5.6±4.2		

may respond by closing at ebb if sediment is resuspended, when gills clog and pseudofaeces are produced. For *Crassostrea virginica,* for example, higher turbidity resulted in a greater amount of micromovements of shell but the mean gape opening tended to be lower (Suedel et al., 2014) and for *Pinna nobilis* individuals did not open their valves in some events of high turbidity (Garcia-March et al., 2016). In this study it was also observed that turbidity, although not as strongly periodic as the other parameters, tends to have a 12 h period and to be out of phase with mussels gape opening, peaking when they are closing. Resuspension events show a stronger periodicity in the winter periods. A specifically designed experiment, given the intrinsic link between speed and other tidal aspects (i.e. spring, neap, flood, ebb…) should be designed to investigate the interaction between flow speed and particles, and may be an important aspect to understand especially in areas where coastal engineering work may lead to changes in tidal amplitude and speed.

Behavioural variability appeared to be present within individuals in each time period but also more strongly between time periods and location. Despite a high variability within time periods, however, there appeared to be some consistency with regards to average time lags between series within locations, suggestive of some location dependent effects of water movements at a fine scale which was not captured here. It should be noted that sensors were oriented so that mussels at the same site would point in the same direction, approximated to be into incoming tide but this was not verified during the experiment, and orientation could have had some influence on gaping activity (Newell et al., 2001). Moreover, there may be local fine scale processes, such as the structural presence of the farm, which may have an influence (Newell

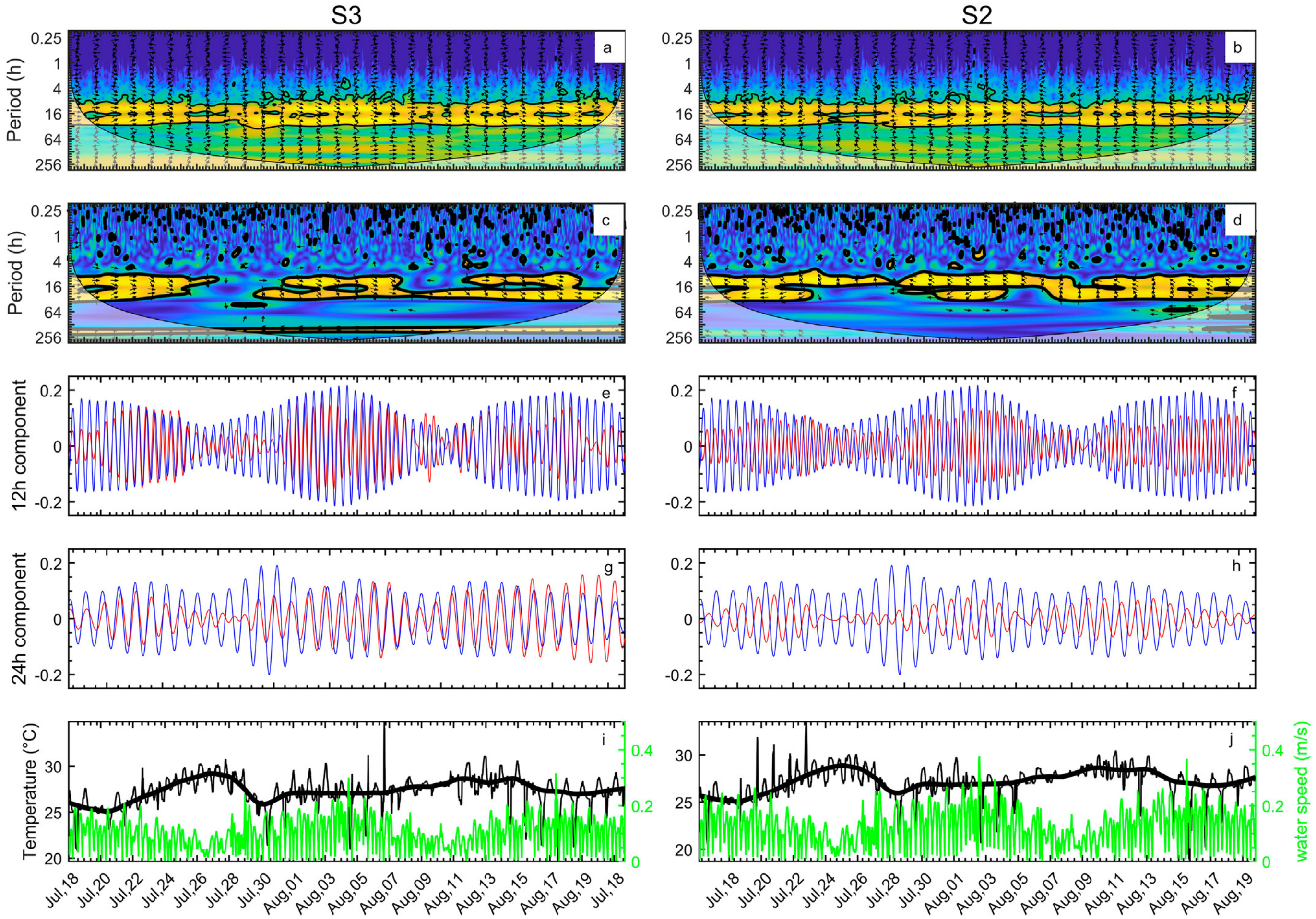

Fig. 8. : Phase analysis between gaping and ζ at S3 (in phase) and S2 (anti- phase) sites for the July–August period. (a-b) cross-wavelet spectra. (c-d) wavelet coherence spectra. (e-h) reconstruction of 12- and 24-h components from gape (red curve) and zeta (blue curve) times series through Inverse-CWT from S3 and S2. Reconstructions have been normalized with respect the standard deviation of the original series in order to compare amplitude modulation. (j-k) Measured temperature (black curve) and simulated water speed (green curve). The temperature long-term trend, obtained with a smoothing procedure (loess method, 4-day time span), is also shown superposed on the original record (heavy black curve).

and Richardson, 2014). With respect to the variability observed within time series, it may be related to the changes in the degrees of fluctuation to which organisms may respond differently. Wong and Cheung (2001), for example, found that green lipped mussels' clearance rate is anti-phase from tide, but it varies from neap to spring tides, with a greater clearance rate observed at low neap tides.

The site differences in the phasing with tidal phase may be due to the heterogeneity of lagoon. 'S3' is located in a channel that connects the lagoon to a FW channel (see Fig. 1), and it is part of a 'restricted euhaline' water body as opposed to the other two sites which are considered 'open euhaline' (Ghezzo et al., 2011). This area is characterized by a higher residence time: therefore, mussels may be more dependent upon 'fresh' oxygenated seawater, particularly in summer months, when oxygen fluctuations are larger and tend to reach lower 'minimum' values in the lagoon (Ciavatta et al., 2008). Some of the between-sites effects may also be explained by the fact that the mussels used came from the same site and may have been acclimated to the conditions, showing some 'endogenous' behaviour (Rao, 1954; Miller and Dowd, 2017). Genetic analyses should be performed, as quantitative differences in feeding behaviour are strongly influenced by genotype (Bayne, 2004), thus investment in this line of research can answer questions that can be harnessed to optimise mussel culture in heterogeneous transitional areas. At each location, individuals tended to exhibit similar patterns of behaviour, however, investigation of only two organisms per site is a limitation of this study, since clearance behaviour has been previously observed to have some great inter-individual variability (e.g. Filgueira et al., 2010). This limitation should be taken into consideration and prevents strong conclusions to be made. Methods that would allow the monitoring of gaping on more individuals simultaneously could be beneficial to continue this line of research.

Overall the periodic closures tended to be longer in the autumn/winter period, despite food concentration being sufficiently high in this period (Table 4). Long periods of closures were also observed by Dowd and Somero (2013) for three *Mytilus* species when exposed to control 13 °C waters, which were disrupted and shortened if mussels had been previously exposed to thermal stress. Moreover, *M. galloprovincialis* is less cold tolerant than other congeners, and its heart rate drops at lower temperatures, which may also explain the longer closures in this period. *M. galloprovincialis* has also low metabolic capacity at high salinity (around 35 ppm), low pH levels (7.3) (Freitas et al., 2017) while *M. edulis* is sensitive to low oxygen levels, spending more time resting when exposed to low saturation (Jakubowska and Normant, 2015). In September and October, the periods with longest continuous valve closures, salinity was the highest around 35 PSU, pH the lowest although still above 8, and average oxygen was the lowest, thus a lowered metabolism may explain the longer closure periods (Anestis et al., 2007), but this deserves to be further investigated with specific analyses.

The relationship between gaping and environmental variables such as temperature, salinity, oxygen and pH is especially important in the context of climate change which is expected to alter some of these

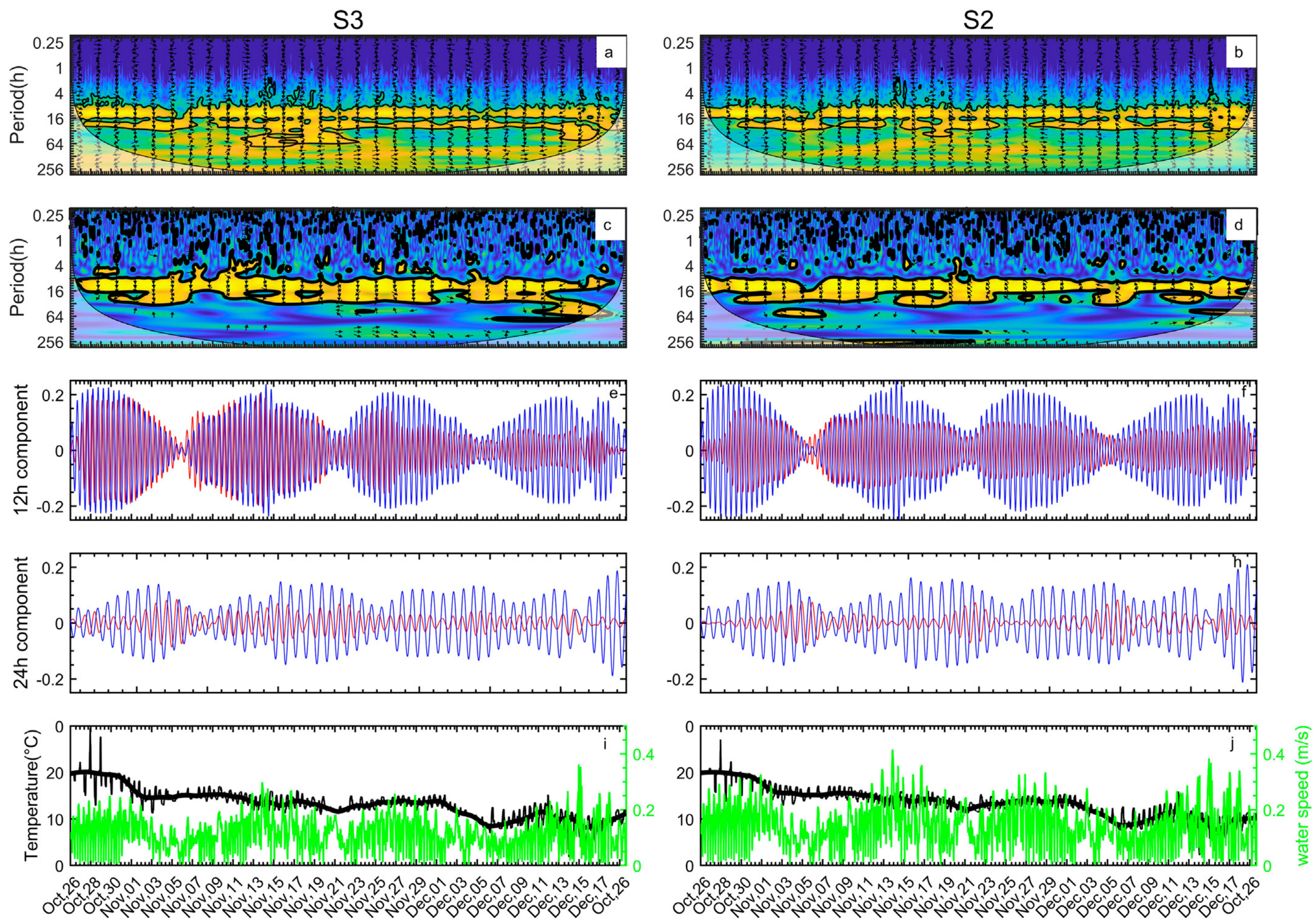

Fig. 9. : Phase analysis between gaping and zeta at S3 and S2 for the October–December period. In this case the clear phase agreement pattern which characterized gape and ζ at S3 in summer is not clear in this period. (a-b) XWT spectra. (c-d) WTC spectra. (e-h) reconstruction of 12- and 24-h components from gape (red curve) and zeta (blue curve) times series through Inverse-CWT from S3 and S2. Reconstructions have been normalized with respect the standard deviation of the original series in order to compare amplitude modulation. (j-k) Measured temperature (black curve) and simulated water speed (green curve). The temperature long-term trend, obtained with a smoothing procedure, is also shown superposed on the original record (heavy black curve).

environmental parameters. This is because gaping is a necessary behaviour, likely to influence the physiological status, growth and condition of individuals The investigation of such relationships would require a comprehensive monitoring network across transitional areas to understand how they will change and whether changes will be more marked in particular areas such as the shallow internal sites. Stress responses would then have to further be addressed after removing the basal behaviours that, as shown here, can be site specific. Stress response may be investigated by subsetting the longer time series on shorter time scales (e.g. when a likely stressing time, such as heatwave, flash flood, storm occurs) and investigating deviations from basal cycles, taking further into consideration micro-closures (e.g. Robson et al., 2009) to further address the suitability of using this parameter in early warning monitoring systems beyond pollutant stressors once it has been established that location will have a non-negligible effect.

5. Conclusion

Gaping behaviour of *Mytilus galloprovincialis* in a lagoon ecosystem has a strong periodic component, strictly linked with tides. The present study shows the importance of long term studies in multiple sites to address behaviours that may be influenced by environmental conditions. Circadian rhythm was found to be ‘weak’ (sensu Mat et al., 2012) and of an endogenous nature. Location within a transient zone was found to be a key aspect to consider when investigating behaviour linked to physiology and leads to critical implications for management and further research as detailed:

1) The link between gaping and tidal aspects should be taken into consideration when considering optimal managing strategies for a lagoon ecosystem, evaluating carefully the effects of coastal planning on tidal alterations, especially if water velocity will be altered in areas where organisms are reliant on it, to prevent limiting the suitable habitat area.
2) Culturing mussels in areas with different tidal conditions may result in different growth rates due to different amount of time spent on feeding, if organisms only feed when tide comes in, with the likely consequence of a reduced metabolism. Thus the relationships found in this study should be harnessed to develop improved growth models for transitional coastal zones, which may be employed for spatially explicit models that consider clearance rate and respiration as a function of gaping-tide relationships.
3) The complex relationship between gaping and environment mediated by tides should be taken into account when using changes to gaping as an index of stress, considering site specificities.

CRediT authorship contribution statement

C. Bertolini: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review &

editing, Visualization. **S. Rubinetti:** Methodology, Investigation, Formal analysis, Software, Writing – original draft, Writing – review & editing, Visualization. **G. Umgiesser:** Formal analysis, Software, Writing – review & editing. **R. Witbaard:** Methodology, Writing – review & editing. **T.J. Bouma:** Methodology, Resources, Writing – review & editing. **A. Rubino:** Writing – review & editing. **R. Pastres:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are thankful to the technical staff at the hydrobiological station of Chioggia: Cristina Breggion and Andrea Sambo, and to Ilaria Bernardini and Luciano Boffo for providing help with fieldwork and sensors deployment. And to the NIOZ engineering department for providing technical help with the sensors. The scientific activity was performed in the Research Programme Venezia2021, with the contribution of the Provveditorato for the Public Works of Veneto, Trentino Alto Adige and Friuli Venezia Giulia, provided through the concessionary of State Consorzio Venezia Nuova and coordinated by CORILA. We are also thankful to the four anonymous reviewers which helped improving the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.145085.

References

Addis, P., Angioni, A., Pasquini, V., Giglioli, A., Andreotti, V., Carboni, S., Secci, M., 2020. Flash flood simulation and valve behavior of Mytilus galloprovincialis measured with hall sensors. Integr. Zool., 1–11 https://doi.org/10.1111/1749-4877.12471.

Alessio, S.M., 2016. Digital Signal Processing and Spectral Analysis for Scientists, Signals and Communication Technology. Springer International Publishing, Cham https://doi.org/10.1007/978-3-319-25468-5.

Alvarez-Borrego, J., Alvarez-Borrego, S., 1982. Temporal and spatial variability of temperature in two coastal lagoons. Calif. Coop. Ocean. Fish. Investig. Rep 23, 188–197.

Ameyaw-Akumfi, C., Naylor, E., 1987. Temporal patterns of shell-gape in Mytilus edulis. Mar. Biol. 95, 237–242. https://doi.org/10.1007/BF00409011.

Andrade, H., Massabuau, J.-C., Cochrane, S., Ciret, P., Tran, D., Sow, M., Camus, L., 2016. High frequency non-invasive (HFNI) bio-sensors as a potential tool for marine monitoring and assessments. Front. Mar. Sci. 3, 187. https://doi.org/10.3389/fmars.2016.00187.

Andrewartha, S., Elliott, N., McCulloch, J., Frappell, P., 2015. Aquaculture sentinels: smart-farming with biosensor equipped stock. J. Aquac. Res. Dev 6, 393. https://doi.org/10.4172/2155-9546.1000393.

Anestis, A., Lazou, A., Pörtner, H.O., Michaelidis, B., 2007. Behavioral, metabolic, and molecular stress responses of marine bivalve Mytilus galloprovincialis during long-term acclimation at increasing ambient temperature. Am. J. Phys. Regul. Integr. Comp. Phys. 293, R911–R921. https://doi.org/10.1152/ajpregu.00124.2007.

Ballesta-Artero, I., Witbaard, R., Carroll, M., Van Der Meer, J., 2017. Environmental factors regulating gaping activity of the bivalve Arctica islandica in northern Norway. Mar. Biol. 164, 1–15. https://doi.org/10.1007/s00227-017-3144-7.

Bayne, B.L., 2004. Phenotypic flexibility and physioloagical tradeoffs in the feeding and growth of marine bivalve molluscs. Integr. Comp. Biol. 44 (6), 425–432.

Bernardi Aubry, F., Acri, F., 2004. Phytoplankton seasonality and exchange at the inlets of the lagoon of Venice (July 2001-June 2002). J. Mar. Syst. 51, 65–76. https://doi.org/10.1016/j.jmarsys.2004.05.008.

Bianchi, F., Acri, F., Aubry, F.B., Berton, A., Boldrin, A., Camatti, E., Cassin, D., Comaschi, A., 2003. Can plankton communities be considered as bio-indicators of water quality in the lagoon of Venice? Mar. Pollut. Bull. 46, 964–971. https://doi.org/10.1016/S0025-326X(03)00111-5.

Brigolin, D., Dal, G., Rampazzo, F., Giani, M., Pastres, R., 2009. An individual-based population dynamic model for estimating biomass yield and nutrient fluxes through an off-shore mussel (Mytilus galloprovincialis) farm. Estuar. Coast. Shelf Sci., 1–12 https://doi.org/10.1016/j.ecss.2009.01.029.

Cheng, B.S., Bible, J.M., Chang, A.L., Ferner, M.C., Wasson, K., Zabin, C.J., Latta, M., Deck, A., Todgham, A.E., Grosholz, E.D., 2015. Testing local and global stressor impacts on a coastal foundation species using an ecologically realistic framework. Glob. Chang. Biol. 21, 2488–2499. https://doi.org/10.1111/gcb.12895.

Ciavatta, S., Pastres, R., Badetti, C., Ferrari, C., Beck, M.B., 2008. Estimation of phytoplanktonic production and system respiration from data collected by a real-time monitoring network in the lagoon of Venice. Ecol. Model. 212, 28–36. https://doi.org/10.1016/j.ecolmodel.2007.10.025.

Cleveland, W.S., 1979. Robust locally weighted regression and smoothing scatterplots. J. Am. Stat. Assoc. https://doi.org/10.1080/01621459.1979.10481038.

Comeau, L.A., Babarro, J.M.F., Longa, A., Padin, X.A., 2018. Valve-gaping behavior of raft-cultivated mussels in the Ría de Arousa, Spain. Aquac. Reports 9, 68–73. https://doi.org/10.1016/J.AQREP.2017.12.005.

Cranford, P.J., Ward, J.E., Shumway, S.E., 2011. Bivalve filter feeding: variability and limits of the aquaculture biofilter. Shellfish Aquac. Environ., 81–124 https://doi.org/10.1002/9780470960967.ch4.

Cucco, A., Umgiesser, G., 2006. Modeling the Venice lagoon residence time. Ecol. Model. 193, 34–51. https://doi.org/10.1016/j.ecolmodel.2005.07.043.

Curtis, T.M., Williamson, R., Depledge, M.H., 2000. Simultaneous, long-term monitoring of valve and cardiac activity in the blue mussel Mytilus edulis exposed to copper. Mar. Biol. 136, 837–846. https://doi.org/10.1007/s002270000297.

Davenport, J., 1981. The opening response of mussels *(Mytilus edulis)* exposed to rising sea-water concentrations. J. Mar. Biol. Assoc. United Kingdom 61, 667–678. https://doi.org/10.1017/S0025315400048116.

Dolmer, P., 2000. Feeding activity of mussels Mytilus edulis related to near-bed currents and phytoplankton biomass. J. Sea Res. 44, 221–231. https://doi.org/10.1016/S1385-1101(00)00052-6.

Dowd, W.W., Somero, G.N., 2013. Behavior and survival of Mytilus congeners following episodes of elevated body temperature in air and seawater. J. Exp. Biol. 216, 502–514. https://doi.org/10.1242/jeb.076620.

Facca, C., Sfriso, A., Socal, G., 2002. Changes in abundance and composition of phytoplankton and microphytobenthos due to increased sediment fluxes in the Venice lagoon, Italy. Estuar. Coast. Shelf Sci. 54, 773–792. https://doi.org/10.1006/ecss.2001.0848.

Falcao, M., 2003. Nutrient dynamics in a coastal lagoon (Ria Formosa, Portugal): the importance of lagoon?Sea water exchanges on the biological productivity. Ciencias Mar 29, 425–433. https://doi.org/10.7773/cm.v29i4.173.

Fernández-reiriz, M.J., Labarta, U., Zúñiga, D., 2017. Bioavailable organic matter in seston modulating di ff erential absorption rates by mussels. Aquaculture 479, 161–168. https://doi.org/10.1016/j.aquaculture.2017.05.038.

Filgueira, R., Fernández-Reiriz, M.J., Labarta, U., 2009. Tasa de aclaramiento del mejillón Mytilus galloprovincialis. I. Respuesta a intervalos extremos de clorofila. Ciencias Mar 35, 405–417. https://doi.org/10.7773/cm.v35i4.1645.

Filgueira, R., Fernandez-Reiriz, M., Labarta, U., 2010. Clearance rate of the mussel Mytilus galloprovincialis. II. Response to uncorrelated seston variables (quantity, quality, and chlorophyll content). Ciencias Mar 36, 15–28.

Freitas, R., De Marchi, L., Bastos, M., Moreira, A., Velez, C., Chiesa, S., Wrona, F.J., Figueira, E., Soares, A.M.V.M., 2017. Effects of seawater acidification and salinity alterations on metabolic, osmoregulation and oxidative stress markers in Mytilus galloprovincialis. Ecol. Indic. 79, 54–62. https://doi.org/10.1016/J.ECOLIND.2017.04.003.

Garcia-March, J.R., Jiménez, S., Sanchis, M.A., Monleon, S., Lees, J., Surge, D., Tena-Medialdea, J., 2016. In situ biomonitoring shows seasonal patterns and environmentally mediated gaping activity in the bivalve, Pinna nobilis. Mar. Biol. 163, 29. https://doi.org/10.1007/s00227-016-2812-3.

Ghezzo, M., Sarretta, A., Sigovini, M., Guerzoni, S., Tagliapietra, D., Umgiesser, G., 2011. Modeling the inter-annual variability of salinity in the lagoon of Venice in relation to the water framework directive typologies. Ocean Coast. Manag. 54, 706–719. https://doi.org/10.1016/J.OCECOAMAN.2011.06.007.

Glass, L., 2001. Synchronization and rhythmic processes in physiology. Nature 410, 277–284. https://doi.org/10.1038/35065745.

Gleason, L.U., Miller, L.P., Winnikoff, J.R., Somero, G.N., Yancey, P.H., Bratz, D., Dowd, W.W., 2017. Thermal history and gape of individual Mytilus californianus correlate with oxidative damage and thermoprotective osmolytes. J. Exp. Biol. 220, 4292–4304. https://doi.org/10.1242/jeb.168450.

Gnyubkin, V., 2010. The circadian rhythms of valve movements in the mussel Mytilus galloprovinci. Russ. J. Mar. Biol., 36 https://doi.org/10.1134/S1063074010060039.

Grinsted, A., Moore, J.C., Jevrejeva, S., 2004. Application of the cross wavelet transform and wavelet coherence to geophysical time series. Nonlinear Process. Geophys. https://doi.org/10.5194/npg-11-561-2004.

Gudimov, A.V., 2006. GENERAL BIOLOGY behavior of blue mussels (Mytilus edulis L.) under the fluctuating environmental conditions. Orig. Russ. Text © a.V. Gudimov 409, 419–421. https://doi.org/10.1134/S0012496606040132.

Hartmann, J.T., Beggel, S., Auerswald, K., Stoeckle, B.C., Geist, J., 2016. Establishing mussel behavior as a biomarker in ecotoxicology. Aquat. Toxicol. 170, 279–288. https://doi.org/10.1016/J.AQUATOX.2015.06.014.

Hawkins, A., Bayne, B., 1985. Seasonal variation in the relative utilization of carbon and nitrogen by the mussel Mytilus edulis: budgets, conversion efficiencies and maintenance requirements. Mar. Ecol. Prog. Ser. 25, 181–188. https://doi.org/10.3354/meps025181.

Hawkins, A.J.S., Bayne, B.L., Bougrier, S., Héral, M., Iglesias, J.I.P., Navarro, E., Smith, R.F.M., Urrutia, M.B., 1998. Some general relationships in comparing the feeding physiology of suspension-feeding bivalve molluscs. J. Exp. Mar. Biol. Ecol. 219, 87–103. https://doi.org/10.1016/S0022-0981(97)00176-7.

Helmuth, B., Broitman, B.R., Yamane, L., Gilman, S.E., Mach, K., Mislan, K.A.S., Denny, M.W., 2010. Organismal climatology: analyzing environmental variability at scales relevant to physiological stress. J. Exp. Biol. 213, 995–1003. https://doi.org/10.1242/jeb.038463.

Hirschie Johnson, C., Hastings, J.W., 1986. The elusive mechanism of the circadian clock: the quest for the chemical basis of the biological clock is beginning to yield tantalizing clues on JSTOR. Am. Sci. 74, 29–37.

Jakubowska, M., Normant, M., 2015. Metabolic rate and activity of blue mussel *Mytilus edulis trossulus* under short-term exposure to carbon dioxide-induced water acidification and oxygen deficiency. Mar. Freshw. Behav. Physiol. 48, 25–39. https://doi.org/10.1080/10236244.2014.986865.

Maire, O., Amouroux, J.M., Duchêne, J.C., Grémare, A., 2007. Relationship between filtration activity and food availability in the Mediterranean mussel Mytilus galloprovincialis. Mar. Biol. 152, 1293–1307. https://doi.org/10.1007/s00227-007-0778-x.

Mat, A.M., Massabuau, J.-C., Ciret, P., Tran, D., 2012. Evidence for a plastic dual circadian rhythm in the oyster Crassostrea gigas. Chronobiol. Int. 29, 857–867. https://doi.org/10.3109/07420528.2012.699126.

Miller, L.P., Dowd, W.W., 2017. Multimodal In Situ Datalogging Quantifies Inter-Individual Variation in Thermal Experience and Persistent Origin Effects on Gaping Behavior among Intertidal Mussels (*Mytilus californianus*). https://doi.org/10.1242/jeb.164020.

Monaco, C.J., McQuaid, C.D., 2018. Applicability of dynamic energy budget (DEB) models across steep environmental gradients. Sci. Rep. 8, 16384. https://doi.org/10.1038/s41598-018-34786-w.

Morgan, E., 1991. An appraisal of tidal activity rhythms. Chronobiol. Int. 8, 283–306. https://doi.org/10.3109/07420529109063933.

Neumann, D., 1981. Tidal and lunar rhythms. Biological Rhythms. Springer US, Boston, MA, pp. 351–380 https://doi.org/10.1007/978-1-4615-6552-9_19.

Newell, C.R., Richardson, J., 2014. The effects of ambient and aquaculture structure hydrodynamics on the food supply and demand of mussel rafts. J. Shellfish Res. 33, 257–272. https://doi.org/10.2983/035.033.0125.

Newell, C.R., Wildish, D.J., MacDonald, B.A., 2001. The effects of velocity and seston concentration on the exhalant siphon area, valve gape and filtration rate of the mussel Mytilus edulis. J. Exp. Mar. Biol. Ecol. 262, 91–111. https://doi.org/10.1016/S0022-0981(01)00285-4.

Nicastro, K.R., Zardi, G.I., McQuaid, C.D., Stephens, L., Radloff, S., Blatch, G.L., 2010. The role of gaping behaviour in habitat partitioning between coexisting intertidal mussels. BMC Ecol. 10, 1–11. https://doi.org/10.1186/1472-6785-10-17.

Nicastro, K.R., Zardi, G.I., McQuaid, C.D., Pearson, G. a, Serrão, E. a, 2012. Love thy neighbour: group properties of gaping behaviour in mussel aggregations. PLoS One 7, e47382. https://doi.org/10.1371/journal.pone.0047382.

Palmer, J.D., 1995. Review of the dual-clock control of tidal rhythms and the hypothesis that the same clock governs both circatidal and circadian rhythms. Chronobiol. Int. https://doi.org/10.3109/07420529509057279.

Palmer, J.D., 2000. The clocks controlling the tide-associated rhythms of intertidal animals. BioEssays 22, 32–37. https://doi.org/10.1002/(SICI)1521-1878(200001)22:1<32::AID-BIES7>3.0.CO;2-U.

Pascoe, P.L., Parry, H.E., Hawkins, A.J.S, 2009. Observations on the measurement and interpretation of clearance rate variations in suspension-feeding bivalve shellfish. Aquat. Biol. 6, 181–190.

Pesce, M., Critto, A., Torresan, S., Giubilato, E., Pizzol, L., Marcomini, A., 2019. Assessing uncertainty of hydrological and ecological parameters originating T from the application of an ensemble of ten global-regional climate model projections in a coastal ecosystem of the lagoon of Venice, Italy. Ecol. Eng. 133, 121–136.

Pivato, M., Carniello, L., Viero, D. Pietro, Soranzo, C., Defina, A., Silvestri, S., 2020. Remote sensing for optimal estimation of water temperature dynamics in shallow tidal environments. Remote Sens., 12 https://doi.org/10.3390/rs12010051.

Porter, E.T., Breitburg, D.L., 2016. Eastern oyster, Crassostrea virginica, valve gape behavior under diel-cycling hypoxia. Mar. Biol. 163, 1–12. https://doi.org/10.1007/s00227-016-2980-1.

Rao, K.P., 1954. Tidal rhytmicity of rate of water propulsion in Mytilus and its modifiability by transplantation. Biol. Bull 106, 353–359. https://doi.org/10.2307/1538769.

Riisgård, H.U., Larsen, P.S., 2014. Physiologically regulated valve-closure makes mussels long-term starvation survivors: test of hypothesis. J. Molluscan Stud. 81, 303–307. https://doi.org/10.1093/mollus/eyu087.

Riisgård, H.U., Lassen, J., Kittner, C., 2006. Valve-gape response times in mussels (Mytilus edulis) - effects of laboratory preceding-feeding conditions and in situ tidally induced variation in phytoplankton biomass. J. Shellfish Res. 25, 901–911. https://doi.org/10.2983/0730-8000(2006)25[901:VRTIMM]2.0.CO;2.

Riisgård, H.U., Pleissner, D., Lundgreen, K., Larsen, P.S., 2013. Growth of mussels Mytilus edulis at algal (Rhodomonas salina) concentrations below and above saturation levels for reduced filtration rate. Mar. Biol. Res. 9, 1005–1017. https://doi.org/10.1080/17451000.2012.742549.

Robson, A.A., Thomas, G.R., de Leaniz, C.G., Wilson, R.P., 2009. Valve gape and exhalant pumping in bivalves: optimization of measurement. Aquat. Biol. 6, 191–200. https://doi.org/10.3354/ab00128.

Robson, A.A., Garcia de Leaniz, C., Wilson, R.P., Halsey, L.G., 2010a. Effect of anthropogenic feeding regimes on activity rhythms of laboratory mussels exposed to natural light. Hydrobiologia 655, 197–204. https://doi.org/10.1007/s10750-010-0449-7.

Robson, A.A., Garcia De Leaniz, C., Wilson, R.P., Halsey, L.G., 2010b. Behavioural adaptations of mussels to varying levels of food availability and predation risk. J. Molluscan Stud. 76, 348–353. https://doi.org/10.1093/mollus/eyq025.

Rolinski, S., Umgiesser, G., 2005. Modelling short-term dynamics of suspended particulate matter in Venice lagoon, Italy. Estuar. Coast. Shelf Sci. 63, 561–576. https://doi.org/10.1016/j.ecss.2005.01.002.

Ruts, T., Matsubara, S., Wiese-Klinkenberg, A., Walter, A., 2012. Diel patterns of leaf and root growth: endogenous rhythmicity or environmental response? J. Exp. Bot. 63, 3339–3351. https://doi.org/10.1093/jxb/err334.

Saurel, C., Gascoigne, J.C., Palmer, M.R., Kaiser, M.J., 2007. In situ mussel feeding behavior in relation to multiple environmental factors: regulation through food concentration and tidal conditions. Limnol. Oceanogr. 52, 1919–1929. https://doi.org/10.4319/lo.2007.52.5.1919.

Sénéchal, J., Grant, J., Archambault, M.-C., 2008. Experimental Manipulation of Suspended Culture Socks: Growth and Behavior of Juvenile Mussels (Mytilus spp.)., pp. 811–826 https://doi.org/10.2983/0730-8000(2008)27[811:EMOSCS]2.0.CO;2 27 doi:10.2983/0730-8000(2008)27[811:EMOSCS]2.0.CO;2.

Suedel, B.C., Clarke, J.U., Wilkens, J., Lutz, C.H., Clarke, D.G., 2014. The effects of a simulated suspended sediment plume on eastern oyster (Crassostrea virginica) survival, growth, and condition. Estuar. Coasts 38, 578–589. https://doi.org/10.1007/s12237-014-9835-0.

Tang, B., Riisgård, H.U., 2016. Physiological regulation of valve-opening degree enables mussels &lt;i&gt;Mytilus edulis&lt;/i&gt; to overcome starvation periods by reducing the oxygen uptake. Open J. Mar. Sci. 06, 341–352. https://doi.org/10.4236/ojms.2016.63029.

Tomioka, K., Uryu, O., Kamae, Y., Umezaki, Y., Yoshii, T., 2012. Peripheral circadian rhythms and their regulatory mechanism in insects and some other arthropods: a review. J. Comp. Physiol. B. 182, 729–740. https://doi.org/10.1007/s00360-012-0651-1.

Torrence, C., Compo, G.P., 1998. A practical guide to wavelet analysis. Bull. Am. Meteorol. Soc. https://doi.org/10.1175/1520-0477.

Tran, D., Nadau, A., Durrieu, G., Ciret, P., Parisot, J.P., Massabuau, J.C., 2011. Field chronobiology of a molluscan bivalve: how the moon and sun cycles interact to drive oyster activity rhythms. Chronobiol. Int. 28, 307–317. https://doi.org/10.3109/07420528.2011.565897.

Tran, D., Perrigault, M., Ciret, P., Payton, L., 2020. Bivalve Mollusc Circadian Clock Genes Can Run at Tidal Frequency. https://doi.org/10.1098/rspb.2019.2440.

Umgiesser, G., Canu, D.M., Cucco, A., Solidoro, C., 2004. A finite element model for the Venice Lagoon. 824 Development, set up, calibration and validation. J. Mar. Syst. 51 (1-4), 123–145.

Widdows, J., Lucas, J.S., Brinsley, M.D., Salkeld, P.N., Staff, F.J., 2002. Investigation of the effects of current velocity on mussel feeding and mussel bed stability using an annular flume. Helgol. Mar. Res. 56, 3–12. https://doi.org/10.1007/s10152-001-0100-0.

Wong, W.H., Cheung, S.G., 2001. Feeding rhythms of the green-lipped mussel, Perna viridis (Linnaeus, 1758) (Bivalvia: Mytilidae) during spring and neap tidal cycles. J. Exp. Mar. Biol. Ecol. 257, 13–36. https://doi.org/10.1016/S0022-0981(00)00327-0.

Zúñiga, D., Castro, C.G., Aguiar, E., Labarta, U., Figueiras, F.G., Fernández-Reiriz, M.J., 2014. Biodeposit contribution to natural sedimentation in a suspended Mytilus galloprovincialis Lmk mussel farm in a Galician Ría (NW Iberian Peninsula). Aquaculture 432, 311–320. https://doi.org/10.1016/j.aquaculture.2014.05.026.