



# Using a clustering algorithm to identify patterns of valve-gaping behaviour in mussels reared under different environmental conditions

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## ABSTRACT

Physiological adaptations for inhabiting transitional environments with strongly variable abiotic conditions can sometimes be displayed as behavioural shifts. A striking example might be found in bivalve species that inhabit estuaries characterised by fluctuations in environment. The opening and closing of their valves, so called gaping activity, represents behaviour that is required for two key physiological functions: food intake and respiration. Linking valve-gaping behaviour to environmental drivers can greatly improve our understanding and modelling of bivalve bioenergetics. Nowadays large data sets on gaping activity can be collected with automated sensors, but interpretation is difficult due to the large amount of environmental drivers and the intra-individual variability. This study aims to understand whether an unsupervised machine learning method (k-means clustering) can be used to identify patterns in gaping activity.

Two commercially important congener mussels, *Mytilus galloprovincialis* and *Mytilus edulis* inhabiting two transitional coastal areas, the Venice Lagoon and the Wadden Sea, were fitted with sensors to monitor valve-gaping, while a comprehensive set of environmental parameters was also monitored. Data were analysed by applying three times a k-mean algorithm to the gaping time series. In the 1st analyses, the algorithm was applied to the overall gaping time series, including daily variations. We identified at both sites three clusters that were characterised by different average daily gaping aperture. The algorithm was subsequently reapplied to relate daily means of gaping to environmental conditions, being temperatures, oxygen saturation and chlorophyll levels. This 2nd analyses revealed that mean gaping aperture was mainly linked to food availability. A 3rd follow-up analysis aimed at exploring daily patterns. This third analysis again revealed consistent patterns amongst the two sites, where two clusters emerged that showed different degrees of oscillatory behaviour. There was however no obvious relationship between this fine scale oscillatory behaviours and environmental variables, but in the Venice Lagoon there was a site effect. Overall, we show that clustering algorithms can disentangle behavioural patterns within complex series of big data. The latter offers new opportunities to improve site-specific bioenergetic bivalve models by rephrasing the clearance and respiration terms based on the mean gaping aperture, provided that further laboratory experimentations are conducted to extrapolate parameters linking aperture with energy inputs and outputs.

## 1. Introduction

Lagoons and deltas are highly heterogenous transitional systems, subject to multiple pressures, from nearby on-land activities (e.g. presence of agriculture and industry resulting in nutrient and chemicals discharges), fisheries, naval traffic often leading to dredging of canals,

and faster spread of invasive species (Kennish and Paerl, 2010). The spatially heterogeneous nature of these habitats is evident as shallow and deeper areas form a mosaic which results in complex hydrodynamics. These transport processes can influence the temporal dynamics of important variables such as temperature or salinity likely exacerbating the effects of climate change in these systems (Amos et al., 2017;

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Scanes et al., 2020). Despite being harsh environments, lagoons and deltas are inhabited by many sessile benthic species, that must cope with the variability in stressors. Bivalves are a common example of a species that is both important for the ecosystem functioning (water filtration, food-webs, etc) and local economies (aquaculture). Physiological and behavioural responses to coastal environmental stressors are key in allowing so many sessile bivalve species to inhabit transitional zones.

There are various known physiological responses by which bivalves can cope with coastal environmental stressors. For example, mussels adapt their gill-palp ratio in response to changes in food quality, (Capelle et al., 2021) and can depress their metabolism or even switch from aerobic to anaerobic metabolism under hypoxia (Stevens and Gobler, 2018). Oysters can slow the rate of shell building to cope with acidification (Waldbusser et al., 2016)). All these kinds of responses can be energetically costly, with consequences that will be damaging albeit not being lethal. For example, chronic exposure to hypoxia was found to impair reproduction, immune responses and growth (Breitburg et al., 2009). Behavioural responses are another way by which bivalves can cope with coastal environmental stressors, and may in fact underlie some of the observed physiological responses. For example, mussel gaping is linked to key metabolic functions like filtering water necessary for feeding and respiration.

Various models that relate clearance to environmental variables have been proposed and reviewed (Ehrich and Harris, 2015). Given the increasing emphasis on understanding the ecosystem services provided by bivalves and their potential role in restoration aquaculture (e.g. nutrient removal, carbon storage.), it is important for clearance and respiration to be modelled correctly (Ehrich and Harris, 2015). Ideally, gaping behaviour should thus be introduced in bioenergetic process-based individual models to correctly simulate clearance rate and respiration. As filtration is costly in terms of energy expenditure (Riisgård and Larsen, 2000), mussels might close their valves during environmental stressful periods or when food concentrations or food quality is low (Dolmer, 2000). This may lead to large differences in feeding rates as predicted from laboratory experiments and as occurs in situ.

Valve gaping has often been used as a proxy for clearance (e.g. Maire et al., 2007; Riisgård et al., 2006; Saurel et al., 2007). Mediterranean mussels (*Mytilus galloprovincialis*) gaping in the Venice lagoon was found to have a circadian rhythm that followed that of tides, which was more evident in internal areas compared to the sea facing site (Bertolini et al., 2021c). However, the question remains as to whether specific environmental stressful conditions could be linked to valve closures, overruling the circadian rhythm. (MacDonald and Ward, 2009). Given the usefulness of machine learning techniques as 'hypothesis-free' approaches (Valletta et al., 2017), an unsupervised algorithm was used to answer the question 'Are there consistent patterns of gaping behaviour that can be linked to patterns in environmental conditions?'. To provide a more robust answer, this study uses two examples with two congeneric mussel species in two transitional coastal zones: *M. galloprovincialis* in the Venice lagoon and *Mytilus edulis* in the Wadden Sea.

## 2. Methods

### 2.1. Biophys sensors description

Biophys sensors were 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 (using magnetism /hall effect sensor - DRV5053VAQLPGM), water pressure (pressure sensor - MS580314BA01-00) and temperature (temperature sensor - TSIC506F). The valve-gaping measurement is based on hall-sensor measurements. All sensors are located in the head of the Biophys sensors. A full description of sensors and data processing to convert sensor output to valve gape amplitude can be found in Bertolini et al. (2021c).

### 2.2. Sites description and experimental design: Venice lagoon

The Venice lagoon is the largest Mediterranean lagoon and presents 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  $632 \cdot 10^6 \text{ m}^3$  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 (*M. galloprovincialis*) in the southern part of the lagoon, where most shellfish farms are located, were chosen for this study. The farms are located along a gradient, from the sea inlet to a more internal area (S1: 45° 14.013 N, 12° 16.914; S2: 45° 13.818 N, 12° 16.051; S3: 45° 13.814, 12° 15.218, see Fig. 1). The farms are small scale (occupying  $0.5 \pm 0.3 \text{ ha}$ ), suspended cultures located at the edges of channels in areas of approximately 3–5 m depth. Mussels are stocked starting from naturally recruited seed collected directly on site on farm poles, ropes and mussel socks.

Six biophys sensors were available for the project, allowing two sensors to be allocated at each site. During 2019 (July to December) sensors were retrieved and redeployed monthly. In 2020 (January to July) they were left to record, and mussels replaced in case of losses, which occurred most frequently at site S1 characterised by stronger currents. A full schedule of deployments is available in Appendix 1. A multiparametric buoy containing sensors for temperature, oxygen, chlorophyll and turbidity (Tecnos S.a.S., Chioggia, Italy), logging data every 12 min was located at the S2 site. While the three sites should be considered as separate sites in analyses due to their location on three different farms on a gradient from the inlet towards a more internal area, with likely differences in tides and currents (see Bertolini et al., 2021c), they are close enough not to experience significant differences in the variables here considered.

### 2.3. Sites description and experimental design: Wadden Sea

The Wadden Sea is a mesotidal basin, with an extensive network of gullies, tidal flats and salt marshes, separated from the North Sea by a range of barrier islands, that stretches along the coasts of The Netherlands, Germany and Denmark. The Wadden Sea can be divided in several adjacent basins, characterised by a system of tidal channels that connect the basin with the North Sea through the deep and narrow tidal inlets along the barrier islands. Sediment and nutrients are mainly transported with the tide from the North Sea. The amount of suspended solids and turbidity sharply increases towards the tidal flats because of sediment resuspension (Condie and Sherwood, 2006).

Benthic mussel (*M. edulis*) culture is carried out on subtidal plots in the most western part of the Wadden Sea in the Netherlands. Mussel productivity is higher on plots that are situated closer to the tidal inlets. Two contrasting culture plots in the Vlietstroom basin (NL) were chosen for this study. One plot (S1: Oos1b) in the northern part bordering the Vlietstroom inlet channel (N53°21.317, E005°15.331). The other plot (S2: S114) is located in the southern part of the basin (N53°12.947, E005°21.164), further away from the inlet channel (19.5 km orthogonal), along a small gully that penetrates a tidal flat (see Fig. 1).

Nine sensors were deployed at S1 and 10 sensors at S2. Sensors were attached to PVC-frames that rested on the sea floor wrapped around wooden poles that were fixed in the sediment. Measurements were run for approximately one month from 28 & 29 Aug 2020 until 30 Sep 2020. Of the initial 19 sensors, two ones were dislodged at S2 and could not be retrieved, also 4 mussels died at S1 and 3 mussels died at S2, yielding successful measurements from 5 individuals over the entire period per location. Simultaneously with the sensors, one calibrated logger-type chlorophyll and turbidity meter (ACLW2-USB, JFE Advantech) was deployed per location and measured temperature (°C), chlorophyll a (ug/l), and turbidity (FTU) with a 5 min sampling period.

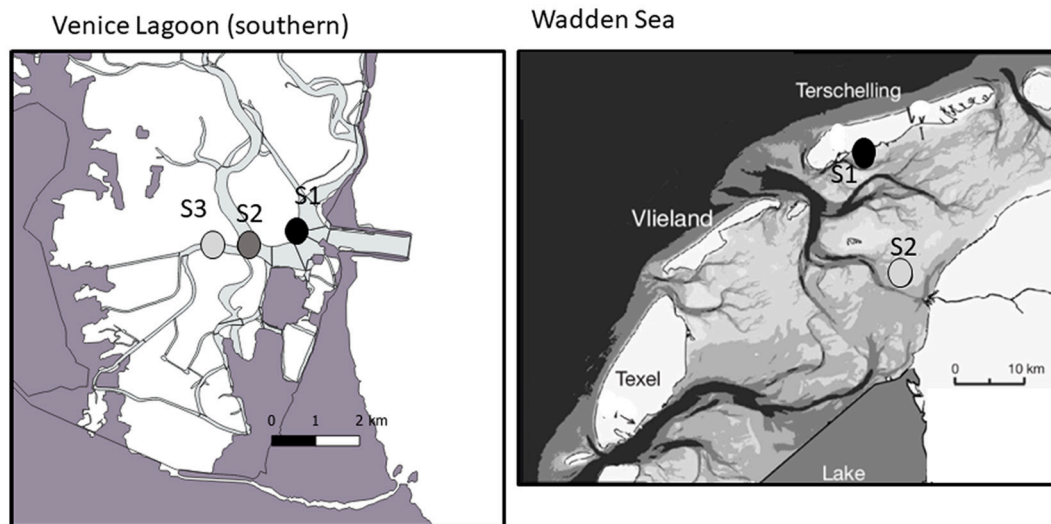


Fig. 1. Maps of the two study sites: the Venice Lagoon (left) and the Wadden Sea (right), with the position of the mussel farms where the studies took place.

#### 2.4. Patterns identification

All data analysis was conducted in R 4.0.5 (R Development Core Team, 2021). Gaping data, expressed in proportion of aperture where 0 is fully closed and 1 is fully opened, and recorded at 1 Hz (see Bertolini et al., 2021c), were averaged (mean) over 5 min in the Venice Lagoon and over 1 min in the Wadden Sea. This difference in averaging time was due to different lengths of deployment periods. Using 1 min averages would make the Venice files too large to easily process, while in the Wadden Sea this higher frequency was technical feasible. Daily curves of gaping for each sensor were then used as input in the analysis yielding 1286 daily curves for the Venice Lagoon and 203 daily curves for the Wadden Sea.

In order to identify the presence of patterns, k-means clustering (*kmeans* from the base package *stats*) was chosen as an appropriate method of unsupervised learning (Hartigan and Wong, 1979; Lantz, 2019). The optimal number of clusters was identified using both the elbow method, choosing number of clusters that optimise both 'within' and 'between' sums of squares, and visually looking at the resulting principal component plot (obtained with *fviz\_clust* in package *factoextra* version 1.0.7) to avoid excessive overlapping between groups. As the initial clustering revealed differences that were primarily linked to the average degree of daily aperture, a second clustering was applied to the residual values after the daily mean gaping value was removed, in order to identify intra-daily behavioural patterns.

#### 2.5. Linking patterns of behaviour to environment

To investigate whether the mean degree of opening was influenced by environmental conditions, the k-means algorithm was applied to mean daily values of gaping and available environmental parameters (water temperature, oxygen concentration, expressed as percentage saturation, turbidity and chlorophyll for the Venice Lagoon; temperature, turbidity and chlorophyll for the Wadden Sea). All environmental parameters were normalized between 0 (min) and 1 (max) over the entire dataset. Each day was therefore characterised by gaping and environment, thus five points (VE) and four points (WS) which were used in the clustering.

To link the finer scale behaviour to the environmental variables, we investigated the predominance of one behaviour over another (wider vs narrower gaping), using the clusters identified in the 1st analyses and looking at the frequency of their occurrence in each site, and in days belonging to the clusters relating mean aperture to environmental conditions (described above). Furthermore, descriptive statistics (means

and standard deviations) of environmental conditions in days belonging to the each of the fine scales cluster were calculated.

### 3. Results

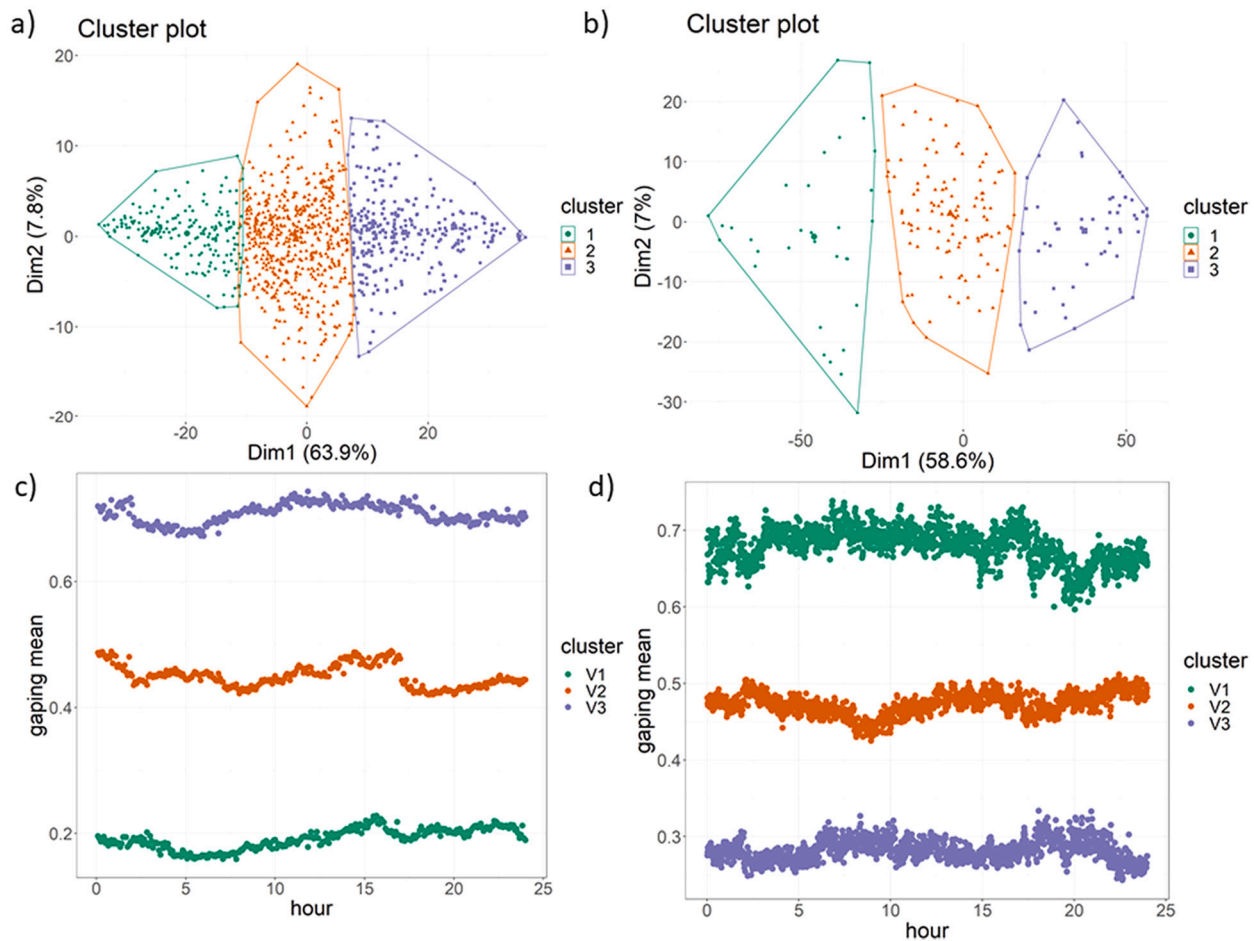
The 1st analyses in which the k-mean algorithm was applied to the overall gaping time series, including daily variations, revealed that the optimal number of clusters for both the Venice Lagoon and the Wadden Sea was 3. The clusters were separated on the axis that explained 64% variability (VL, Fig. 2a) and 59% (WS, Fig. 2b) and their differentiation was related mostly to daily mean apertures (Fig. 2c,d) with a wider (averaging 70%), a medium (45%) and a narrower (20%) gaping angle.

The 2nd analyses, in which daily means of gaping and environmental parameters were clustered, 3 new clusters were identified both in Venice Lagoon and Wadden Sea (Fig. 3 a, b). In Venice the days with wider mean daily opening were clustered with higher temperatures, higher oxygen concentration and medium mean chlorophyll levels, whereas occasions in which mussels had a narrower gaping were colder days with less chlorophyll and a high oxygen percentage saturation (Fig. 3c). Low levels of dissolved oxygen were associated with a medium gaping angle, even when associated to high chlorophyll (Fig. 3c). In the Wadden Sea, mussels were more open on days which had higher chlorophyll and turbidity levels (Fig. 3d).

The 3rd analyses, in which residual gaping behaviour was analysed, revealed two clusters for both Venice Lagoon and the Wadden Sea, that explained 21% (VE) and 16% (WS), respectively (Fig. 4 a, b). The difference between the two clusters was related to the amplitude of oscillations of the residual gaping (Fig. 4 c, d) being either more (cluster 1) or less (cluster 2) pronounced. In the Venice Lagoon, there was a higher frequency of days belonging to cluster 2, with a lower amplitude of oscillation, which was particularly evident in the S1 site (Fig. 5a), while in the Wadden Sea there was not a clear site effect or clear predominance of one behaviour (Fig. 5b) In terms of links between the environmental condition and the predominance of these fine scale behaviours, however, there were no significant differences in either daily means or daily standard deviation of any of the environmental parameters measured in the days belonging to different fine scale behaviour clusters.

### 4. Discussion

The application of an unsupervised clustering algorithm led to identify consistent behavioural patterns amongst two mussel congeners (*Mytilus galloprovincialis* and *Mytilus edulis*) inhabiting two coastal



**Fig. 2.** Principal component representation of k-means clustering of daily gaping curves (a & b) and pattern of gaping for each cluster centroid (c & d). With respect to locations, a & c refer to Venice Lagoon while b & d refer to Wadden Sea. Colours of each cluster in the principal component representation are matching the colours of their pattern representation.

transitional areas (Venice lagoon and Wadden Sea) emerged. The data-driven clustering approach allowed us to draw some inferences on the mussel gaping behaviour. *Firstly*, it could clearly detect days when mussels had a wider, narrower or medium gaping aperture, and this was the first level of classification between days. This main finding was the same for both species and regions. *Secondly*, reapplying the algorithm in this new light allowed the detection of some links between the mussels gaping aperture and the main measured environmental variables, showing a relationship with food availability, where mussels tend to be more open in days with more food supply. *Thirdly*, clustering of the residuals after removal of the daily mean gaping aperture revealed the presence of an oscillatory behaviour. This was likely the circadian rhythm that followed the tidal rhythm that was already shown in Bertolini et al. (2021c) for the Venice Lagoon, and Miller and Dowd (2017) for intertidal californian mussels. The different amplitudes found in this study showed that this pattern also applies to *M. edulis* in the Wadden Sea.

In terms of links between gaping and environment: mussels in the Venice Lagoon were found to have a wider opening gape in warmer temperature days and a narrower gaping in colder days. This is consistent with observations for other bivalves, such as the oyster *Crassostrea virginica*, both under natural conditions at its cold distribution limits and during heat shock experiments (Clements et al., 2018; Comeau et al., 2012), and the deep water clam *Arctica islandica* (Ballesta-Artero et al., 2017). More generally, this observation is consistent with the temperature – pumping rate relationship found for mussels by (Jørgensen et al., 2007). Mytilid mussels that experience heatwaves during emersion, also

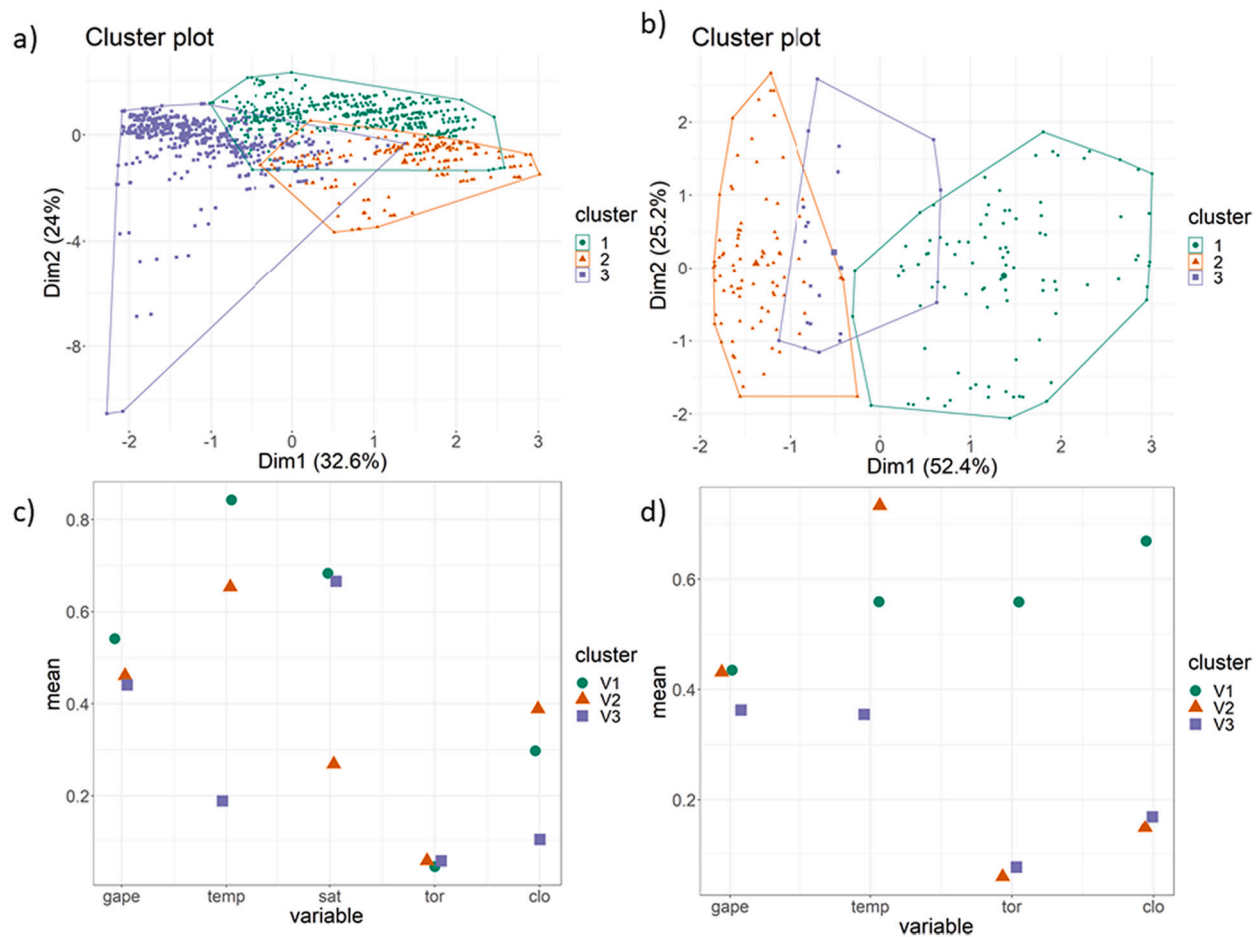
show wider apertures during immersion, with a concomitant increase in respiration rates, indicative of stress (Olabarria et al., 2016).

Temperature and chlorophyll availability are both low in winter time in the lagoon of Venice (Ciavatta et al., 2008): thus the narrower gaping aperture can be also due to a lower food availability. The gaping-clearance-food relationship is well established in the bivalve literature (Riisgård and Larsen, 2014; Robson et al., 2010; Saurel et al., 2007). In the Wadden Sea the temporal span of the data does not allow us to identify the effects of temperature, as they were collected over only one month, where daily mean temperature ranged only between 13.6 °C and 18.7 °C. More data over a longer time period is needed to confirm or reject this hypothesis for the Wadden Sea. In the Wadden Sea there was a clear difference in the site characteristics with S2 having three-fold higher levels of chlorophyll and turbidity values than S1. Here, S2, mussels had wider opening in this ‘high chlorophyll, high turbidity’ condition, pointing again to the potential role of food availability and quality in triggering valve opening when food is available, as already seen for other species (e.g. *A. islandica*, Ballesta-Artero et al., 2017) and narrowing to avoid high metabolic costs when food is low (Riisgård et al., 2011; Tang and Riisgård, 2016). Parallel experimentation showed higher levels of biodeposit production and lower grow rate at S1 (Capelle, pers. comm.).

#### 4.1. Conclusions and outlook

Given the causal relationship between valve gape and pumping rate, which exists both for actively and passively controlled gaping angle





**Fig. 3.** a & b) Principal component representation of k-means clustering of average daily gaping and rescaled environmental variables; and c & d) values of average gaping and rescaled environmental variables (temp = temperature, sat = saturation, tor = turbidity, clo = chlorophyll-a) for each cluster centroid. a & c refer to Venice Lagoon while b & d refer to Wadden Sea. Colours and shapes of each cluster in the principal component representation are matching the colours of their pattern representation.

(Jørgensen et al., 1988), changes to gaping can have long term consequences on organisms energetics. Obtaining values of energetic expenditure associated with valve movements would be highly valuable to gain a deeper understanding and to be able to model the consequences of gaping changes, not only in terms of its modulation of energy input, but also to account for associated energetic loss. Bioenergetic models can be used to inform both aquaculture practices and management measures: e. g. allocation of space for aquaculture (Bertolini et al., 2021b; Brigolin et al., 2017); quantification of ecosystem functioning and services associated both with aquaculture (Bertolini et al., 2021a; Brigolin et al., 2009) and with naturally occurring populations. It would thus be useful to apply this clustering methodology, which yielded some consistent results amongst two species and two areas, to analyse gaping data from also other bivalve species and other locations. This may go so far as even including bivalves in offshore environments. Overall, this would improve bioenergetic models, where clearance and respiration terms are inherently linked with gaping. The current formulations of models however do not account for linkages with food, aside from considering a half saturation constant, nor the different degrees of daily oscillations. Further experimentation, under constrained lab conditions, linking gaping activity with actual clearance rate and respiration rates under different settings will also help devising indices that could be then extrapolated to use gaping directly in the models. An indication of energy consumption during valve movement would also be useful to infer the consequences of having a more or less pronounced amplitudes of gaping, to better formulate this term in energetic based growth models.

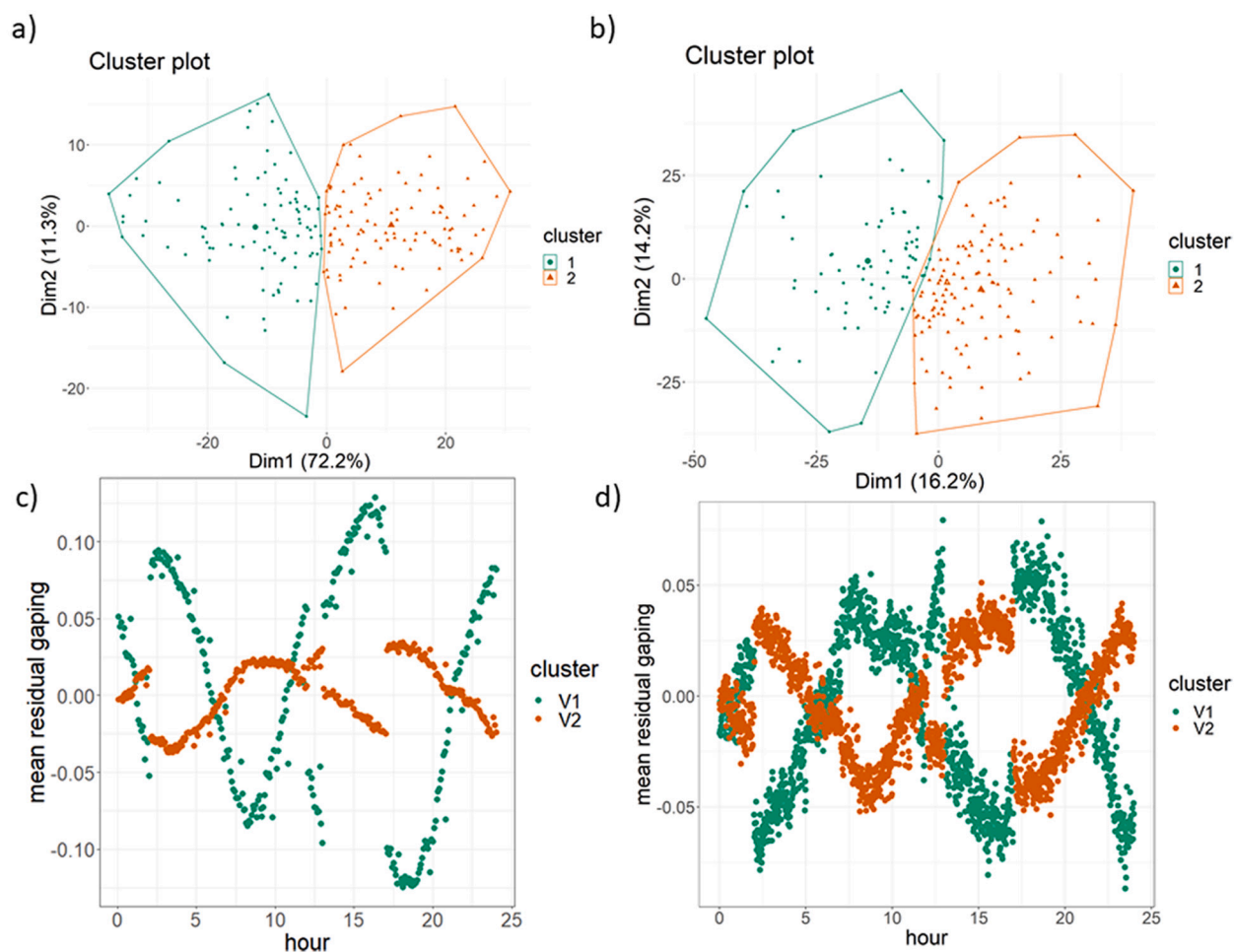
These may be inferred by combining more mechanistic models with the empirical laboratory experiments (e.g. Guarini et al., 2021). Given the availability of sensors and the fast development of technology, having big-data on valve gaping from a few sentinel organisms together with temperature and chlorophyll, becomes more and more feasible these days. Having such big-data should improve predictions on productivity, and be a major aid for aquaculture decision making, for multiple areas and species, especially in the context of 'precision shellfish farming'.

## Funding

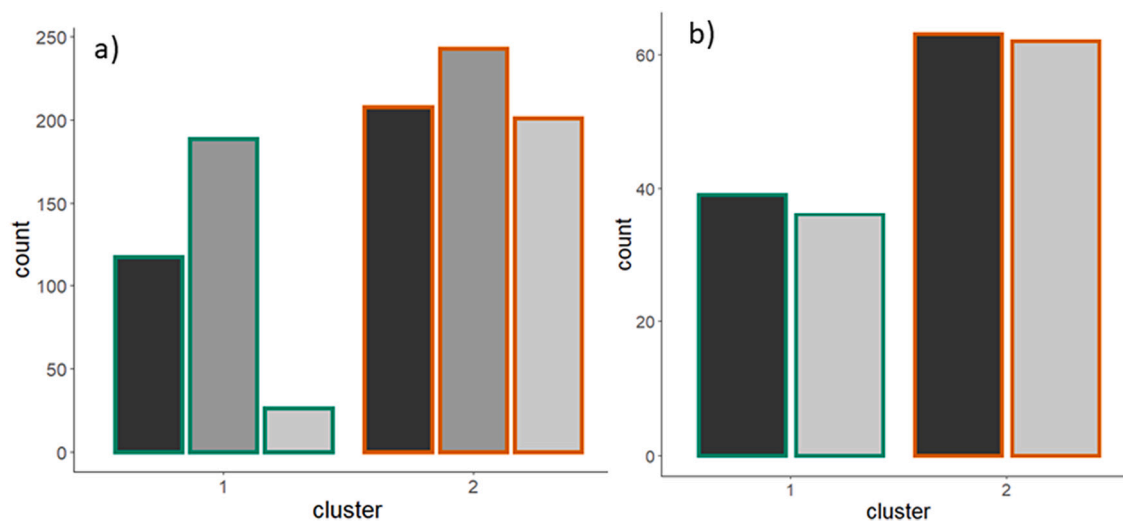
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## Research data for this article

The script and a set of example data are available for use doi: <https://doi.org/10.5281/zenodo.5884284>



**Fig. 4.** a & b) Principal component representation of k-means clustering of daily residual gaping curves; and c & d) daily pattern of residual gaping for each cluster centroid. a & c refer to Venice Lagoon while b & d refer to Wadden Sea. Colours of each cluster in the principal component representation are matching the colours of their pattern representation.



**Fig. 5.** Frequency of occurrence of the two clusters of daily residual gaping in (a) the three Venice sites (black: S1, dark grey: S2, light grey: S3) and (b) the two Wadden Sea sites (black: S1, grey: S2).

#### CRediT authorship contribution statement

C. Bertolini: Conceptualization, Methodology, Software,

Investigation, Formal analysis, Data curation, Visualization, Writing – original draft. J. Capelle: Investigation, Resources, Writing – review & editing. E. Royer: Methodology, Writing – review & editing. M. Milan:

Investigation, Resources, Writing – review & editing. **R. Witbaard:** Writing – review & editing. **T.J. Bouma:** Resources, Writing – review & editing. **R. Pastres:** Conceptualization, Methodology, Writing – review & editing, Supervision, 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.

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**Appendix 1**

Table of deployments with reliable measurements in the Venice lagoon.

site	start	end
S1	2019-07-16	2019-08-20
S1	2019-07-16	2019-08-20
S1	2019-08-20	2019-09-24
S1	2019-08-20	2019-09-24
S1	2019-09-24	2019-10-24
S1	2019-09-24	2019-10-24
S1	2019-10-24	2019-12-18
S1	2020-01-15	2020-01-23
S1	2020-01-17	2020-01-27
S1	2020-02-01	2020-03-14
S2	2019-07-16	2019-08-20
S2	2019-07-16	2019-08-20
S2	2019-08-20	2019-09-24
S2	2019-08-20	2019-09-24
S2	2019-08-20	2019-09-24
S2	2019-09-24	2019-10-24
S2	2019-09-24	2019-10-24
S2	2019-10-27	2019-12-18
S2	2019-10-28	2019-12-18
S2	2020-01-15	2020-04-30
S2	2020-01-17	2020-02-05
S2	2020-02-15	2020-03-10
S2	2020-05-12	2020-07-20
S2	2020-07-28	2020-08-02
S2	2020-07-28	2020-08-14
S3	2019-07-16	2019-08-20
S3	2019-07-16	2019-08-20
S3	2019-08-20	2019-09-24
S3	2019-09-24	2019-10-24
S3	2019-09-24	2019-10-24
S3	2019-10-24	2019-12-18
S3	2019-10-24	2019-12-18
S3	2020-01-16	2020-03-19
S3	2020-03-21	2020-05-19
S3	2020-06-06	2020-06-16

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