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RESEARCH ARTICLE

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Applicability of the gape monitor to study flat oyster (*Ostrea edulis*) feeding behaviour

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Abstract – Innovative techniques are needed to assess oyster performance in flat oyster reef restoration projects. A valve gape monitor, a device that continuously measures opening and closing of live bivalves, can potentially be used as an effective method to determine survival and behaviour of the European flat oyster *Ostrea edulis*. The method has been successfully used in combination with a number of bivalve species to investigate valve gape activity in response to environmental factors. In this study, eight *O. edulis* were equipped with valve gape sensors in order to relate gape to environmental conditions such as food availability. Valve gape activity was monitored under controlled laboratory conditions, with and without food, in a concrete basin in the Oosterschelde and in the field (Voordelta, Dutch North Sea). Under controlled laboratory conditions, oysters clearly responded to changes in food availability. Starved oysters closed their valves significantly longer than oysters that received food, and the relative gape width in fed oysters was larger. In the concrete basin (Oosterschelde), a positive correlation between valve opening and Chlorophyll-*a* was found. Additionally, valve gape activity and tidal movement appeared to be linked. When exposed to a full tidal cycle (Voordelta), a negative correlation between valve opening and Chlorophyll-*a* was found. However, there was no correlation between valve gape and current velocity. In autumn, longer periods of inactivity were seen, but when valves opened, the valve gape was larger. These data indicate that valve gape can provide valuable information on behaviour (gape frequency and gape width), but also show that it is not necessarily a good proxy for feeding rate. Nevertheless, these results show that the gape monitor can be used to determine the natural behaviour of flat oysters under field conditions, and that gape opening provides information on behaviour and the stress response of bivalves to environmental conditions.

Keywords: *Ostrea edulis* / oyster reef restoration / North Sea / valve gape monitor / Voordelta

1 Introduction

Flat or common oyster (*Ostrea edulis*) beds were once an important habitat in the North Sea that provided natural hard substrate in an otherwise soft-sediment environment (Olsen, 1883) cited in (Kamermans et al., 2018). These natural reef-like structures have virtually disappeared from the Dutch part of the North Sea as a result of intensive flat oyster fishery at the end of the 19th century, followed by other types of bottom trawling fisheries (Gercken and Schmidt, 2014). Although some *O. edulis* individuals have managed to survive in estuaries around the North Sea, many of the ecosystem services that oyster reef structures originally provided, such as food and habitat, have disappeared accordingly (Lenihan and Peterson, 1998; Bouma et al., 2009; Gercken and Schmidt, 2014). Since 2016, several flat oyster restoration projects have commenced in the North Sea (Smaal et al., 2017; Kamermans et al., 2018; Pogoda et al., 2019). Moreover, flat oysters have sporadically been found on artificial hard substrates such as shipwrecks, buoys and wind mono-piles in wind farms in Europe, indicating their ability to survive, reproduce and distribute in the open ocean (Bouma and Lengkeek, 2013; Kerckhof, 2018). The quest for renewable energy sources in recent years has led to investments in offshore wind farms, particularly in the North Sea. Multi-use of offshore wind

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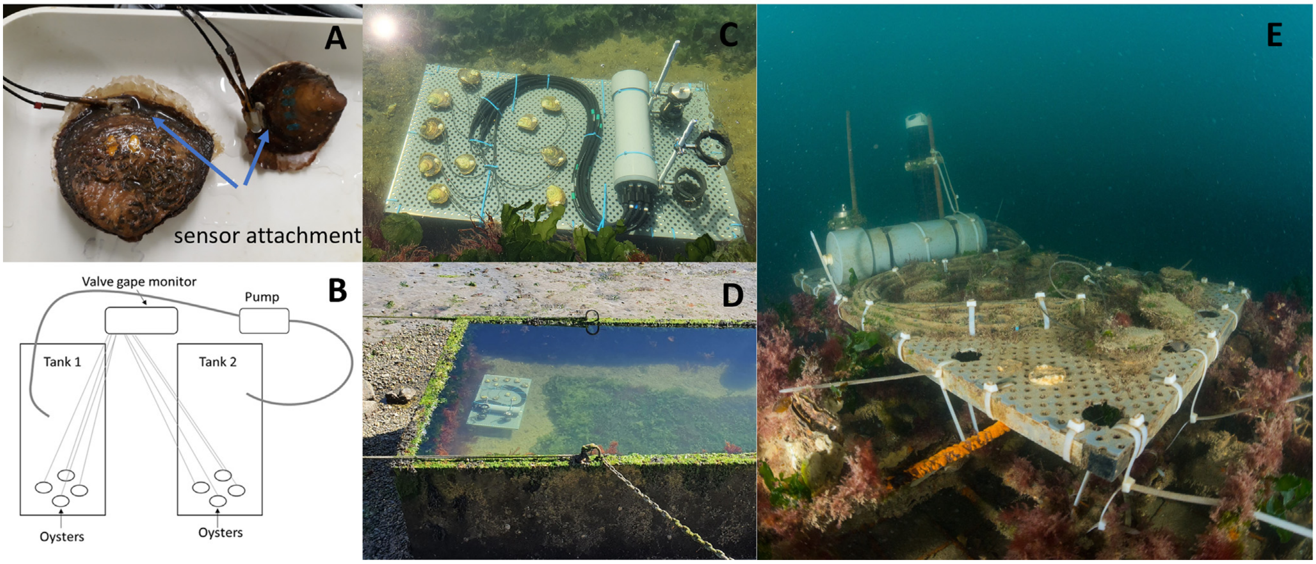

Fig. 1. (A) Oysters (*Ostrea edulis*) from Norway (large) and Grevelingen (small) with sensors attached. (B) Schematic presentation of the tank 1 and tank 2 containing the oysters connected to the valve gape monitor. (C) Oysters attached to the valve gape monitor in situ, (D) the valve gape monitor and attached oysters in the concrete basin in the Oosterschelde and (E) in the Voordelta. Photos by Pim van Dalen (A, C, D) and Joost Bergsma (E).

farms, such as nature-inclusive development, in which the design of wind farms aims to enhance biodiversity and natural resources, provide an opportunity for flat oyster reef restoration. Several farms have already been indicated as potential restoration locations in the Dutch North Sea.

Survival, growth, reproduction and recruitment of the flat oyster are four important performance parameters in restoration projects. Monitoring these parameters is often hard to achieve, given the remote, offshore locations and requires novel techniques that reduce the frequency in which such locations can be visited. One way to monitor the performance of transplanted oysters is by using valve gape monitors. This is a device that “continuously” measures the opening and closing (gape or gaping activity) of the shells of living bivalves and can be seen as an effective method to monitor feeding behaviour and survival. Especially in combination with the simultaneous measurement of environmental parameters (temperature, salinity, current speed, suspended particulate matter, or SPM, Chlorophyll-*a*), it can give valuable insights on the reasons for the failure or success of restoration projects. In other bivalve species, valve gaping behaviour has been related to key physiological processes such as respiration, feeding, metabolic activity and reproduction, and it has also been used as an indicator of environmental stress (Bernard et al., 2016; Ballesta-Artero et al., 2017; Clements et al., 2018; Hubert et al., 2022). Moreover, this methodology has been successfully used in combination with the razor clam (*Ensis leei*) (Witbaard and Kamermans, 2009), the giant clam (*Hippopus hippopus*) (Schwartzmann et al., 2011), the Icelandic scallop (*Chlamys islandica*) (Berge et al., 2015), the ocean quahog (*Arctica islandica*) (Ballesta-Artero et al., 2017), the blue mussel (*Mytilus edulis*) (Berge et al., 2005), the Mediterranean mussel (*Mytilus galloprovincialis*) (Comeau et al., 2018; Bertolini et al., 2021), the Pacific oyster (*Crassostrea gigas*) (Sow et al., 2011) and eastern oysters (*Crassostrea virginica*) (Clements et al., 2018). *O. edulis* is a filter-feeder and must open its valves to feed and respire by means of water filtration. Results from studies measuring valve gape using bivalve species have shown that food (Chlorophyll-*a*), temperature and light conditions are key drivers of valve activity (in Ballesta-Artero et al., 2017). While wide, open valves suggest feeding and respiration, partly closed or closed valves suggest a reduction or halt in filtration (Riisgård and Larsen, 2015; Hubert et al., in press). Thus, by using a valve gape monitor, insight into the differences in activity and stress response of individual bivalves can be obtained. Gape monitor data could also be used to retrospectively determine mortality and the response of bivalves to extreme environmental conditions. When the oyster dies, the muscle that is used to control valve opening and closure no longer functions, leaving the oyster wide open (beyond the maximum valve gape of living oysters). Potentially, the periods of spawning can also be determined. The application of this method can therefore provide valuable information on the performance of *O. edulis* in restoration projects.

In this study, the main objective was to test the applicability of the valve gape monitor for the monitoring of *O. edulis* in reef restoration projects and whether (a) behavioural differences in valve gape exist between oysters in the presence or absence of food and (b) whether differences in valve gape measured in situ could be linked to environmental conditions. Experiments were performed in three different environments, i.e. in the laboratory where all environmental conditions were kept constant except for food availability, in a semi-enclosed basin where oysters were exposed to fluctuations in environmental parameters but partly sheltered from current and tidal movement, and finally in the Voordelta, where the monitor was placed on the sea bottom and oysters were fully exposed to fluctuations in currents. Valve gape activity of eight flat oysters (*O. edulis*) was monitored with and without food in the laboratory in order to investigate the effect of food availability whilst other environmental parameters were kept constant. In addition, the valve gape of eight flat oysters were monitored in the semi-enclosed basin in the Oosterschelde and during a spring and an autumn period in the Voordelta to investigate the effect of environmental parameters such as current speed and food availability in a more natural setting.

2 Material and methods

2.1 Valve gape monitor

The autonomous self-logging gape monitor was originally developed to measure the valve gape width in the American razor clam (*E. leei*) and the blue mussel (*M. edulis*) (Witbaard and Kamermans, 2009), and was later successfully used on the bivalve *A. islandica* in northern Norway (Ballesta-Artero et al., 2017). The principle of the valve gape monitor is based on the measurement of electromagnetic field strength between two sensors. The sensors (electro-coils coated with plastic tubing) were attached to the outside of each of the two valves of the bivalve, directly opposite of one other (Fig. 1A). Light-curing dental resin cement (3M ESPE RelyX Unicem 2 Clicker) was used as the adhesive as it minimizes the handling time of gluing to a few minutes. The sensors were connected to the data logger by means of 0.6 mm coaxial cables. For the energy source, battery packs were placed inside a waterproof PVC housing (Fig. 1C). The measurement principle is based on an electrical pulse which is sent to the excitation sensor, thus creating an electromagnetic field, and the strength of this electromagnetic field is detected by the other sensor. This measured electromagnetic field strength is dependent on the distance between the two valves and this distance can be calculated using the following equation:

$$\mathrm{Dt} \propto \surd\,(1/\mathrm{St}).$$

D_t denotes the distance between valves and S_t denotes the electromagnetic field strength as detected by the sensor (Ballesta-Artero et al., 2017). Although the absolute distance between the sensors can be determined, differences in the positioning of the sensors on the shell and differences in shell morphology make the absolute distance difficult to differentiate between the individuals. Therefore, the absolute distance was scaled (from 0 to 1) to a relative distance and expressed as a percentage of the signal of when the valves are fully opened (indicated by 1). The current valve gape monitor, which was powered by a 9 volt battery pack, can accommodate up to eight bivalves, and the signal strength was simultaneously measured every ten seconds in all eight channels and stored on a 2 GB SD memory card. A resolution of ten seconds was used for technical reasons. While the equipment can measure at approximately 1Hz in an online application, in the standalone application that was used in our experimental set-up, it is not possible to measure at this resolution since it compromises the battery lifespan. Fast valve movements, such as sudden closure and re-opening of the valves, are therefore potentially missed. The signal was stored per channel along with the time, date and battery power.

2.2 Organism

The *O. edulis* individuals for the laboratory experiment were collected from Hafrsfjord, Norway (May 2018) and Lake Grevelingen, The Netherlands (May 2018) and were kept in flow-through tanks in the laboratory under stable conditions ($T = 19.2 \pm 0.32$ °C, Salinity = 29.35 ± 0.2 PSU, pH = 8.36 ± 0.19). Oysters were fitted with valve gape sensors (Fig. 1A, see Ballesta-Artero et al., 2017). The Norwegian oysters ($n = 4$) were denoted: Norw1, Norw2, Norw3, Norw4 and the Dutch oysters ($n = 4$) as: Grev1, Grev2, Grev3, Grev4. The Norwegian oysters were larger (oyster width: 95.59 ± 2.78 mm) than the oysters collected in Lake Grevelingen (oyster width: 79.04 ± 6.59 mm) (Fig. 1A). The *O. edulis* individuals for the experiment in the concrete basin in the Oosterschelde and in the Voordelta (experiments in autumn 2020) were collected from Lake Grevelingen, The Netherlands in March 2020 and March 2021 (Voordelta experiment in spring 2021). These oysters were kept in an outdoor holding tank connected to Oosterschelde water prior to the experiments. Before commencing all experiments, an acclimatisation period of at least 7 days was applied to allow the oysters to acclimatise to the environmental conditions.

2.3 Experimental set-up: laboratory food availability experiment

The oysters were kept in a continuous flow-through set-up (constant in- and outflow of Oosterschelde water filtered with a 5 µm cartridge that was obtained locally, Fig. 1B), in a temperature-controlled room at the research facility of Wageningen Marine Research in Yerseke (The Netherlands). Feeding with the unicellular flagellate *Isochrisis galbana* and the diatom *Chaetoceros calcitrans* ($\pm 5 \times 10^6$ cells mL^{-1}, in the stock concentration) was continuous and controlled by a peristaltic pump (flow rate of 2 $\mathrm{mL\,min}^{-1}$). Fresh algae stock was added every Monday, Wednesday and Friday. Oxygen supply was provided by an aeration stone and pump. Temperature (°C), pH, dissolved oxygen ($\mathrm{mg\,L}^{-1}$) and salinity (PSU) were measured three times per week in both tanks. The experiments were conducted under a constant dark regime to prevent confounding effects from light on oyster behaviour (Wu et al., 2015). The oysters were fitted with valve gape sensors on the 11th of July 2018 and, following a recovery period of 24 hours, the monitoring commenced on the 12th of July. Two sequential starvation runs of at least one week in duration were performed, with a recovery period of 7 days in-between trials, in which all of the oysters were fed (Monday, Wednesday and Friday). The experiments ran from the 20th of August until the 15th of September 2018. The second starvation run was performed under the same conditions as the first starvation run.

In total, eight oysters were divided over two tanks. Oysters in tank 1 functioned as the control group (Norw1, Norw2, Grev1, Grev2), while the oysters in tank 2 were the experimental group (Norw3, Norw4, Grev3, Grev4). During the experiment, the oysters in tank 1 were continuously fed fresh algae three times per week, while the oysters in tank 2 were not fed. However, due to technical issues with one of the sensors (attached to Norw4 in tank 2), the sample size was reduced to seven individuals.

2.4 Experimental set up: concrete basin experiment (semi tidal condition)

Eight oysters from the Lake Grevelingen (collected in March 2020) were attached to the valve gape monitor and placed in the Oosterschelde (a tidal bay in the southwest part of the Netherlands) in a concrete, open-top basin with a dimension of $7.12 \times 2.96 \times 1.5$ m (L × W × H) (N51°29.362′, E004°3.444′) on

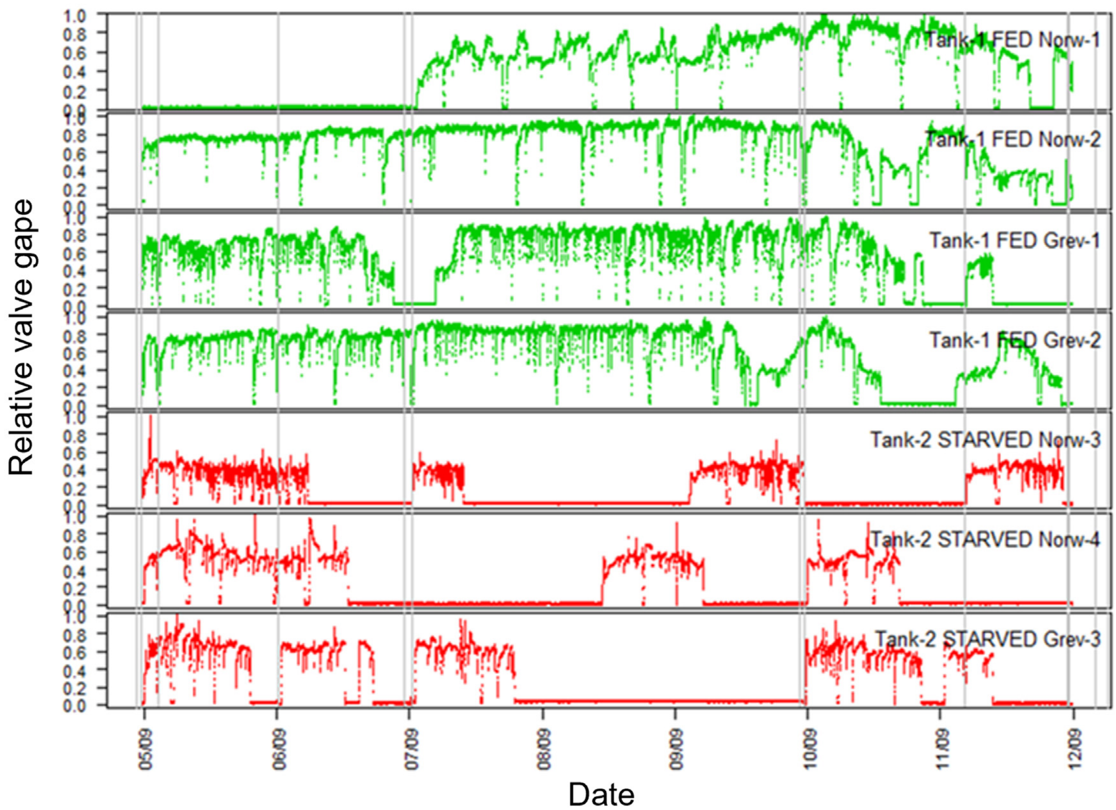

Fig. 2. Gaping activity of seven oysters (*O. edulis*) during the laboratory experiment, 5th to 12th September 2018. Oysters in tank 1 (green) were fed, oysters in tank 2 (red) were starved. The *x*-axis denotes time (days). The y-axis denotes the relative valve gape (0 indicates closed and 1 indicates maximally opened). Time of disturbance is indicated in grey.

the 5th of August 2020 (Figs. 1C and 1D). The top edge of the basin was 0.87 m NAP. Due to the tidal movement in the Oosterschelde, the water level dropped beneath the top edge of the basin twice daily. In the concrete basin, which serves as an artificial tidal pool, the oysters were protected from strong tidal currents and isolated from the Oosterschelde water for approximately 9 hours during each tidal cycle. During this time, there is no influx of seawater containing suspended material (or Chlorophyll-*a*). The water was retained in the concrete basin such that the oysters were never exposed to air (Fig. 1D). In addition, Chlorophyll-*a*, turbidity and temperature were measured in the basin at 1-minute intervals (INFINITY-CLW, JFE Advantech Co., Ltd, Tokyo) during the entire period of deployment with the valve gape monitor. A measurement period of 17 days was used for the analyses (from the 12th of August until the 29th of August). Given the conditions in the concrete basin, we were not able to (accurately) measure the current velocity. To test for the influence of current movement (when the basin is submerged and the water inside the basin is subject to the tide), via the proxy from the tidal height, the tidal data from this period was downloaded from the Yerseke buoy (Rijkswaterstaat, 2022).

2.5 Experimental set up: in situ measurement Voordelta

Eight oysters from Lake Grevelingen (collected in March 2020 and March 2021) were attached to the valve gape monitor and placed on the bottom of the Voordelta (N51°46.667′, E003°51.603′), where a natural oyster bed exists at approximately 5 m depth (Fig. 1E). Valve gape activity was measured for 28 days during autumn (from the 20th of October until the 17th of November 2020) and spring (from the 10th of May until the 13th of June 2021), but only 17 days of data were used in the analyses to maintain consistency in the measurement period both within and between the experiments. In both campaigns, data was successfully collected from 3 out of the 8 oysters (spring: channel 1, 2 and 5 and autumn: channel 6, 7 and 8). Only the results from these replicates are presented in this study. The data from the other 5 oysters were either missing or the quality was too low to use in the analyses. The same environmental parameters from the in situ concrete tidal basin were also measured here, with the addition of current velocity (Nortek Aquadopp).

2.6 Analysis and statistics

All analyses were performed in Rstudio version 3.4.3 (R. Core Team, 2022). The relative valve gape (percentage open) was partitioned in ten ranges from 0 to 1 (gape classes: 0-0.1, 0.1-0.2, etc.), with 0 being completely closed and 1 being completely opened (for details, see “Valve gape monitor”). The relative frequency of observations (gape classes) was calculated for each individual oyster as the number of observations in each gape class divided by the total

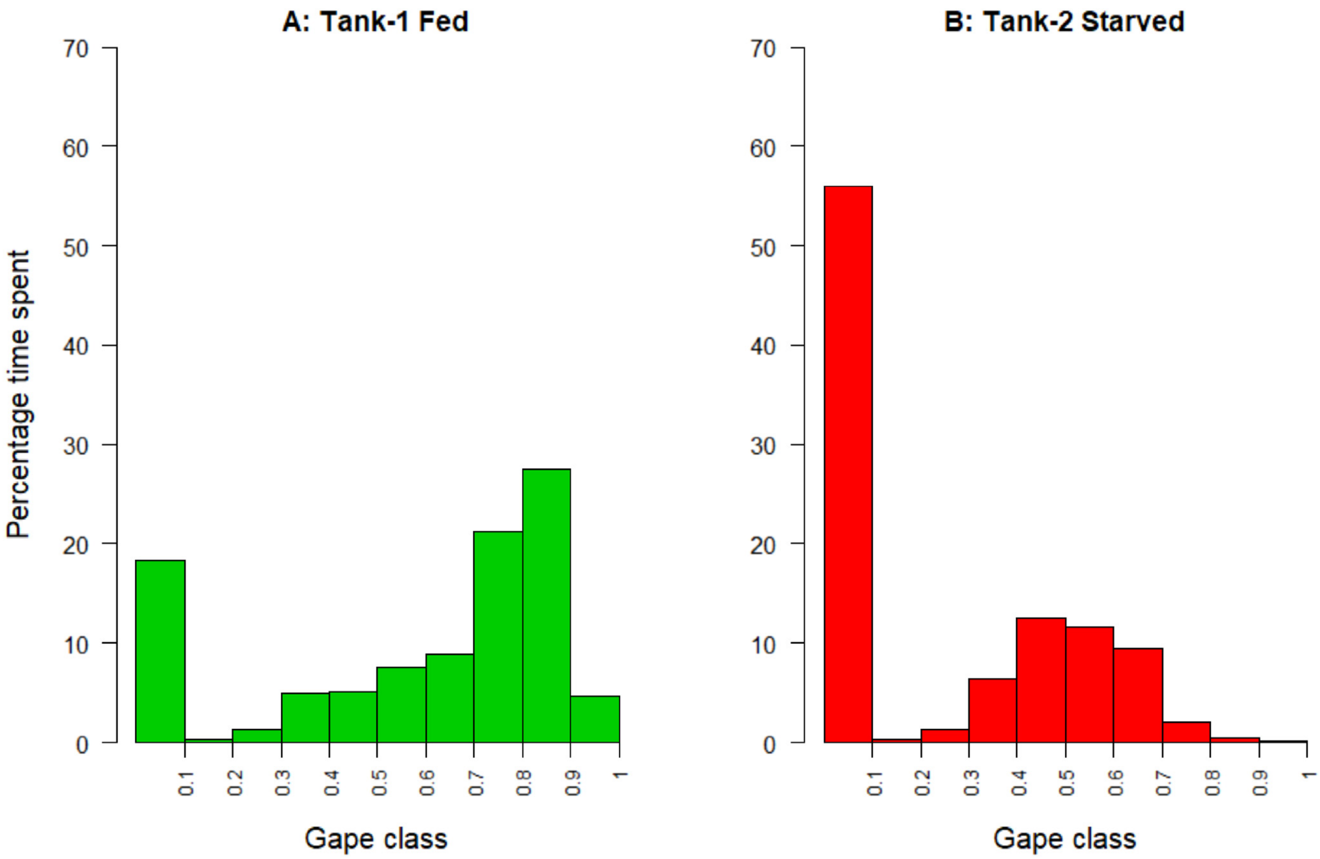

Fig. 3. Gape class frequency distribution of (A) fed (green) and (B) starved (red) oysters (*O. edulis*) during the laboratory experiment. Y-axis indicates the percentage of time spent. The *x*-axis indicates valve opening per gape class (0–0.1, 0.1–0.2, ..., 0.9–1.0).

number of observations. In this way, we can clearly see the differences between the spring and autumn seasons, as well as differences between closing/opening of the valves. The mean frequency of the gape class observations was then calculated for each oyster in each experimental run. Chi-squared test were used to test the similarity in distribution of gape class frequency between oysters with and without food and between ecotypes (Norwegian versus Grevelingen oysters). In order to compare valve gape between treatments excluding the times the oyster was completely closed, a threshold of 0.2 was chosen. This threshold was arbitrarily set, based on the observation that at a gape width of 0.2 the valves are not visibly separated. In addition, to compare the valve gape and environmental parameters between the Voordelta and concrete tidal basins, all datasets (cropped to a period of 17 days) were averaged to 30-minute time intervals to smoothen the data and minimize potential effects from outliers. The valve gape datasets (per each oyster from each experiment) could then be individually compared with the environmental datasets. A linear regression was calculated for each valve gape-environmental parameter comparison to see if there were any statistical correlations. Additional comparisons between the valve gape and environmental parameters were tested by averaging the datasets per day to check for correlations (except for current velocity, in which a 30 minute average was used).

3 Results

3.1 Valve gape monitor – laboratory food availability experiment

3.1.1 Environmental parameters

The average temperature was 20.7 °C in both tanks (SD = 1.8 and SD = 1.9 in tank 1 and 2, respectively). The average salinity (PSU) was 30.5 and 30.6 in tank 1 and 2, respectively, and slightly increased throughout the experiment (SD = 0.4 and SD = 0.3), and the average pH in both tanks was comparable, fluctuating between approximately 7.9 and 8.5 (SD = 0.2 in both tanks). Moreover, the average concentration of dissolved oxygen was 7.7 (SD = 0.3) and 8.0 mg L^{-1} (SD = 0.4) in tank 1 and 2, respectively.

3.1.2 Valve gape

To explore the response of valve gape to the presence or absence of food (and the effect of starvation on gaping behaviour), the oysters were divided into two groups. One group was fed, the other group was starved. In both groups, valve gape was measured for 1 week (from the 5th to the 12th of September 2018) (Fig. 2). The results show that in both groups (starved and fed), periods of valve closure occurred but these periods lasted longer in the group without food.

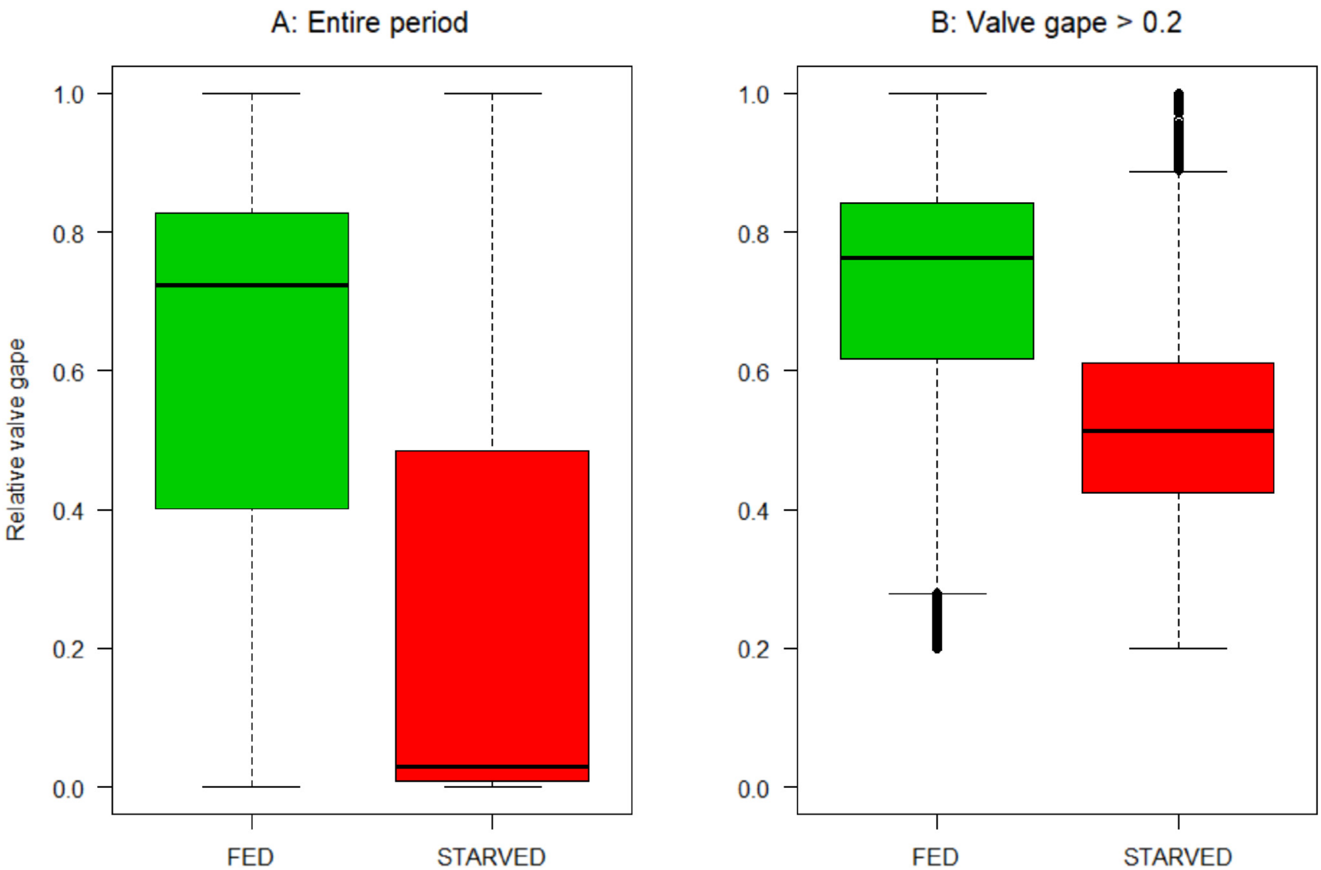

Fig. 4. Boxplot of the relative valve gape in *O. edulis* in the laboratory experiment during the entire period of starvation (A) compared to the period of valve gape > 0.2 (B) during fed (green) and starved (red) conditions. The *y*-axis (valve gape) indicates how wide the oysters open.

Table 1. Mean valve gape and standard deviation under conditions with (fed) and without (starved) food. Mean valve gape was measured during the entire run and during periods with open valves only, to determine whether gape width is larger when valves are opened under conditions with and without food). Norwegian oysters are indicated N1 to N4 and oysters from the Grevelingen G1 to G3.

Date (day/month)	Experiment	Treatment		Entire Run		Valve gape > 0.2	
		Tank1	Tank2	MeanTank1 (SD)	MeanTank2 (SD)	MeanTank1 (SD)	MeanTank2 (SD)
20/08–27/08	Starvation	Fed (N1,N2,G1,G2)	Starved (N3,N4,G3)	0.50 (0.34)*	0.17 (0.25)*	0.67 (0.22)	0.53 (0.09)
27/08–03/09	Recovery fed	Fed (N1,N2,G1,G2)	Fed (N3,N4,G3)	0.34 (0.29)	0.46 (0.32)		
03/09–12/09	Starvation	Fed (N1,N2,G1,G2)	Starved (N3,N4,G3)	0.58 (0.31)*	0.23 (0.26)*	0.71 (0.17)	0.52 (0.12)
01/10–10/10	Recovery fed	Fed (N1,N2,G1,G2)	Fed (N3,N4,G3)	0.57 (0.30)	0.50 (0.30)		
		Tank1	Tank2	Mean Nor (SD)	Mean Grev (SD)		
27/08–03/09	Recovery fed	Fed (N1,N2,G1,G2)	Fed (N3,N4,G3)	0.38 (0.33)	0.39 (0.27)		
01/10–10/10	Recovery fed	Fed (N1,N2,G1,G2)	Fed (N3,N4,G3)	0.58 (0.30)	0.49 (0.30)		

[*] Indicates that a significant difference was found.

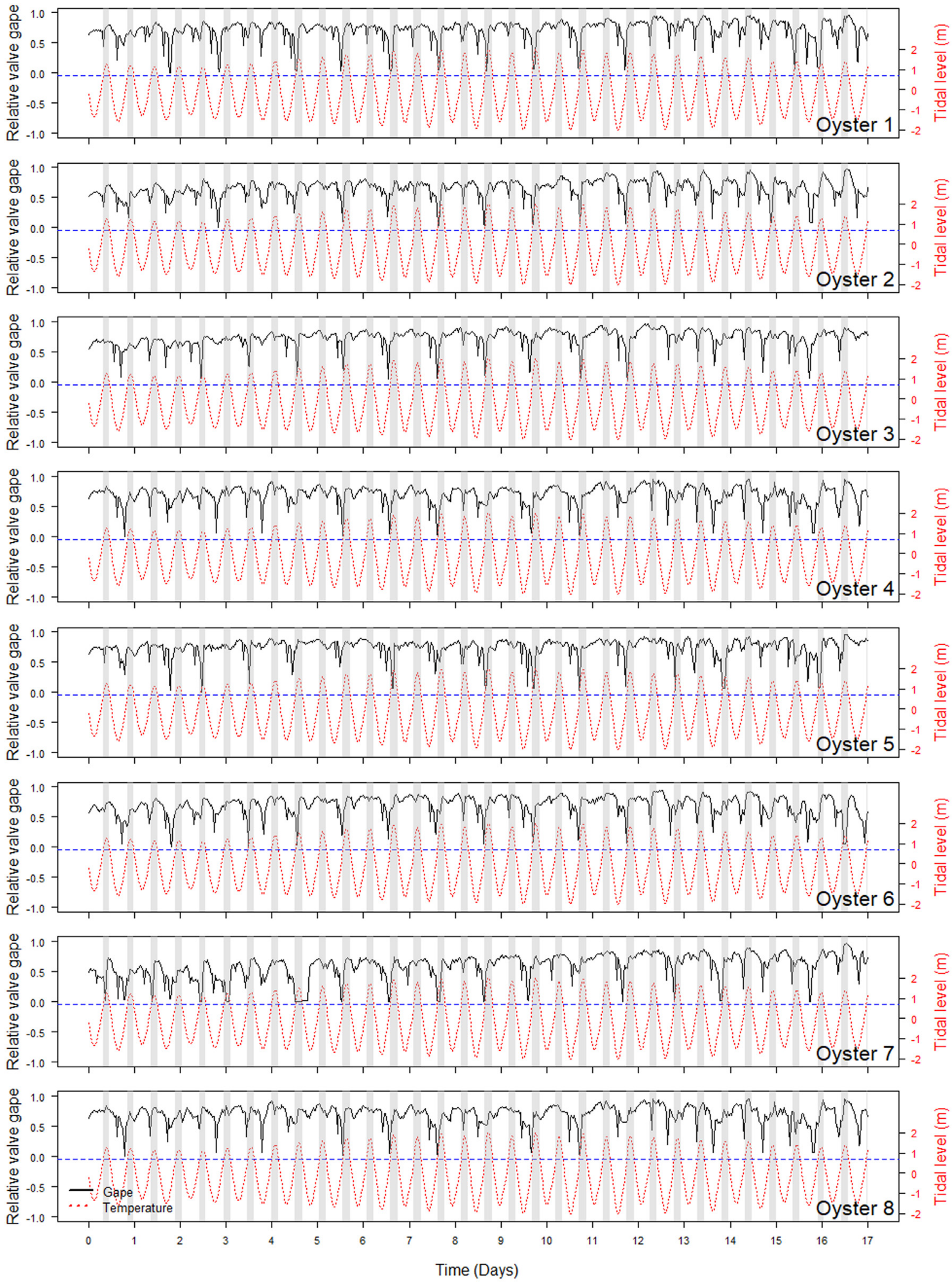

Fig. 5. Gaping activity of eight *O. edulis* (oyster 1 to 8) deployed in a concrete basin in the Oosterschelde. The *x*-axis denotes time (days). The left *y*-axis and black solid line denotes the relative valve gape (0 indicates closed and 1 indicates maximally opened) and the right *y*-axis and red dotted line denotes the height of the tidal level in meters. The dotted blue line indicates the point at which the tidal level drops below the edge of the concrete basin (no connection to surrounding water). The grey polygons indicate the time at which the tidal level is above the edge of the concrete basin.

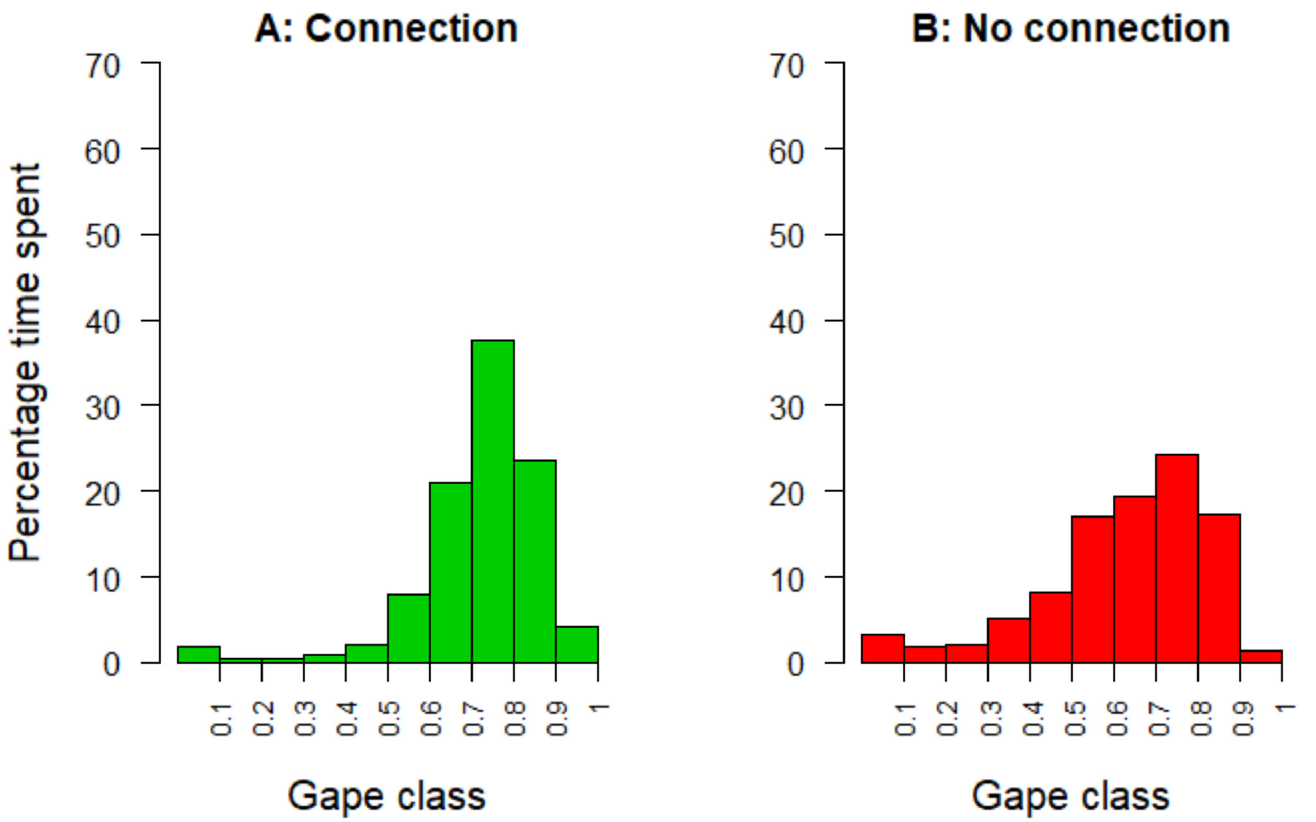

Fig. 6. Gape class frequency distribution of oysters in the concrete basin in the Oosterschelde during the period that water inside the basin is (A) connected (green) and (B) not connected (red) to the water outside the basin. *Y*-axis indicates the percentage of time spent. The *x*-axis indicates valve opening per gape class (0–0.1, 0.1–0.2,...,0.9–1.0).

A comparison of the valve gape frequency distributions clearly shows this difference (Fig. 3) ($\chi^2 = 256720$, df = 9, $p < 0.01$). The frequency distribution of the valve gape of fed specimens showed valves were closed during approximately 20% of the time and longer periods with wider open valves in the higher gape size classes (0.7–0.8, 0.8–0.9 and 0.9–1.0) (Fig. 3). The relative valve gape during the entire period (both open and closed valves) was compared between the period with and without food in Figure 4A. The data showed that oysters opened up wider in the presence of food, and were also found with open valves more frequently (Fig. 4A). In addition, the non-fed oysters opened their valves less wide above a gape size of 0.2 (Fig. 4B and Tab. 1). During the recovery period (when both batches were fed again) (Suppl. Fig. S1 and S2), no significant differences were detected in the valve gape (time spent, and gape class) between tanks ($\chi^2 = 2.989$, df = 9, $p = 0.965$).

3.2 Valve gape monitor – concrete basin experiment (semi tidal condition)

3.2.1 Environmental parameters

The daily average temperature was 25 °C at the start of the experiment and decreased to 20 °C during the 17 days of monitoring (Suppl. Fig. S4). Daily average Chlorophyll-*a* concentration increased from 3 $\mu g\,L^{-1}$ to 12 $\mu g\,L^{-1}$ over the course of the 2.5 weeks, with peaks at day 14 and day 17, while the daily average turbidity fluctuated between 2 and 8 formazine turbidity unit (FTU) (Suppl. Fig. S4).

3.2.2 Valve gape

The valve gape of eight oysters deployed in a concrete basin in the Oosterschelde showed a regular pattern of opening and closing (Fig. 5) during 17 days of monitoring (from 12th until 29th of August 2020). Valves appeared to open when the edge of the concrete basin was submerged and flushing of the seawater within the basin took place. Valve gape often decreased when the basin emerged and lost connection with the surrounding water during low tide, which resulted in stagnant water. This is visible in the higher frequency of valve gape classes between 0.7 and 1 when the concrete basin was submerged (Fig. 6). The mean valve gape during the period in which the basin was completely under water was slightly, but significantly, greater than during the period when the basin lost connection with the surrounding water (Fig. 7A, Connection: mean valve gape = 0.73 ± 0.1 and No connection: mean valve gape = 0.66 ± 0.2, *p*-value < 0.05).

A positive correlation was found between valve gape and Chlorophyll-*a* for four out of eight oysters (oyster 1, 2, 7 and 8; Tab. 2), and a negative correlation was found between valve gape and temperature for five out of eight oysters (oyster 1, 2, 3, 7 and 8; Tab. 2). Only one out of the eight oysters showed a negative correlation with turbidity (oyster 6; Tab. 2).

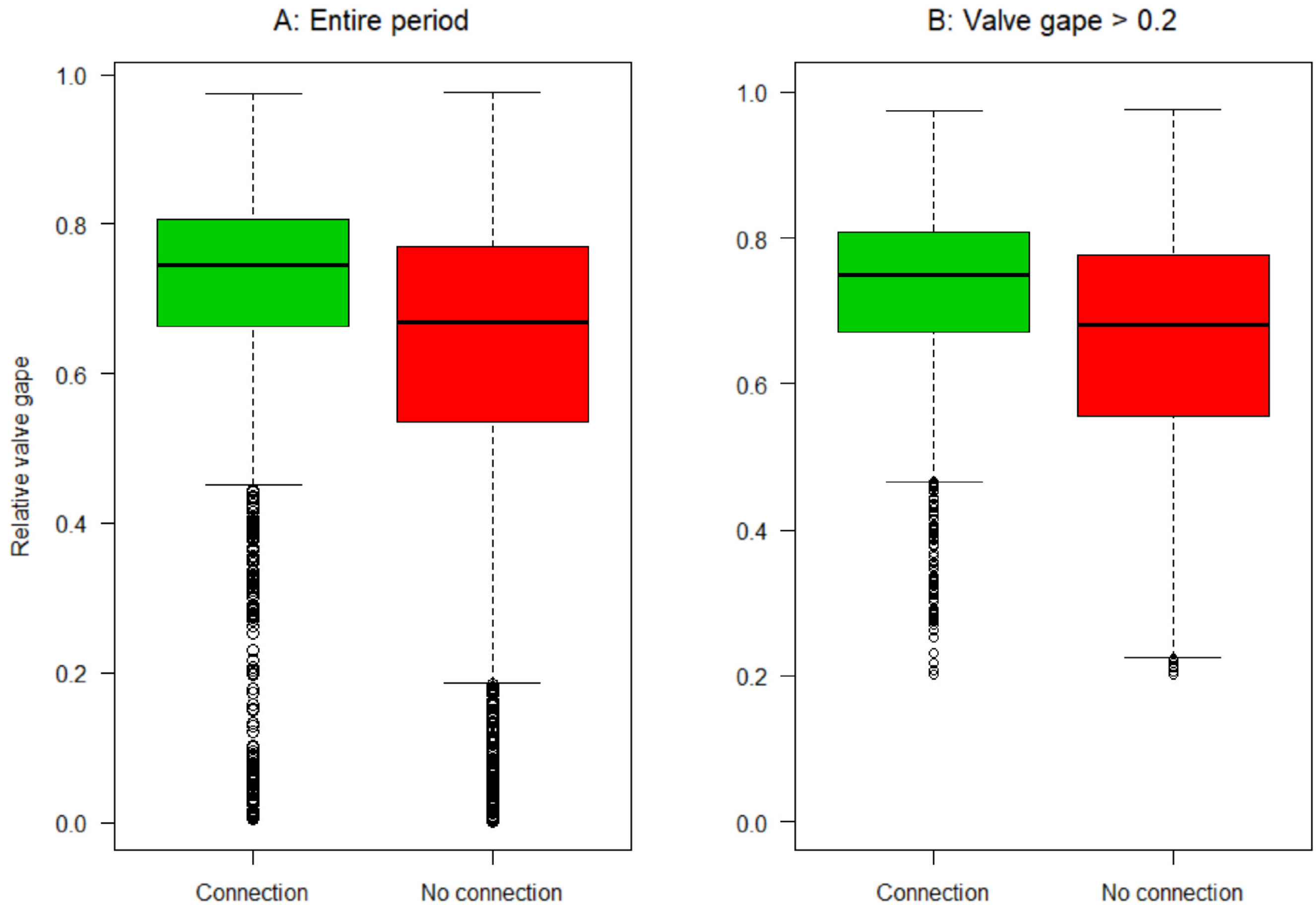

Fig. 7. Boxplot of the relative valve gape in *O. edulis* in the concrete basin in the Oosterschelde during the entire period (A) compared to the period of valve gape > 0.2 (B) during the period that water inside the basin is connected (green) and not connected (red) to the water outside the basin. The *y*-axis (relative valve gape) indicates how wide the oysters open.

Table 2. Overview of the correlation between valve gape and environmental parameters (temperature, Chlorophyll-*a* and turbidity).

Oyster	Chl-*a* ($\mu g\,L^{-1}$)			Temperature (°C)			Turbidity (FTU)		
	Beta	R^2	*p*-value	Beta	R^2	*p*-value	Beta	R^2	*p*-value
1	27.15	0.29	0.03*	–16.38	0.26	0.04*	–1.55	0.00	0.82
2	25.65	0.28	0.03*	–16.75	0.29	0.03*	–4.61	0.04	0.47
3	21.53	0.22	0.06	–16.62	0.30	0.02*	–1.40	0.00	0.82
4	10.89	0.03	0.49	–2.15	0.00	0.83	–8.74	0.08	0.26
5	15.18	0.08	0.27	–6.96	0.04	0.43	–0.99	0.00	0.89
6	–3.39	0.01	0.78	–0.53	0.00	0.95	–14.87	0.42	0.01*
7	16.27	0.46	<0.01*	–12.96	0.72	<0.01*	2.83	0.06	0.37
8	30.56	0.38	0.01*	–18.03	0.33	0.02*	2.03	0.01	0.76
6	–0.70	0.02	0.61	–1.07	0.02	0.57	–1.60	0.00	0.95
7	–0.80	0.21	0.06	–1.90	0.62	<0.01*	–24.16	0.62	<0.01*
8	–1.42	0.43	<0.01*	–1.68	0.31	0.02*	–30.75	0.64	<0.01*
1	–15.84	0.41	0.01*	8.51	0.61	<0.01*	7.76	0.01	0.72
2	–18.32	0.50	<0.01*	9.00	0.62	<0.01*	3.22	0.00	0.89
5	–110.12	0.39	0.01*	49.65	0.41	0.01*	–66.32	0.01	0.67

*Indicates a significant correlation was found with valve gape.

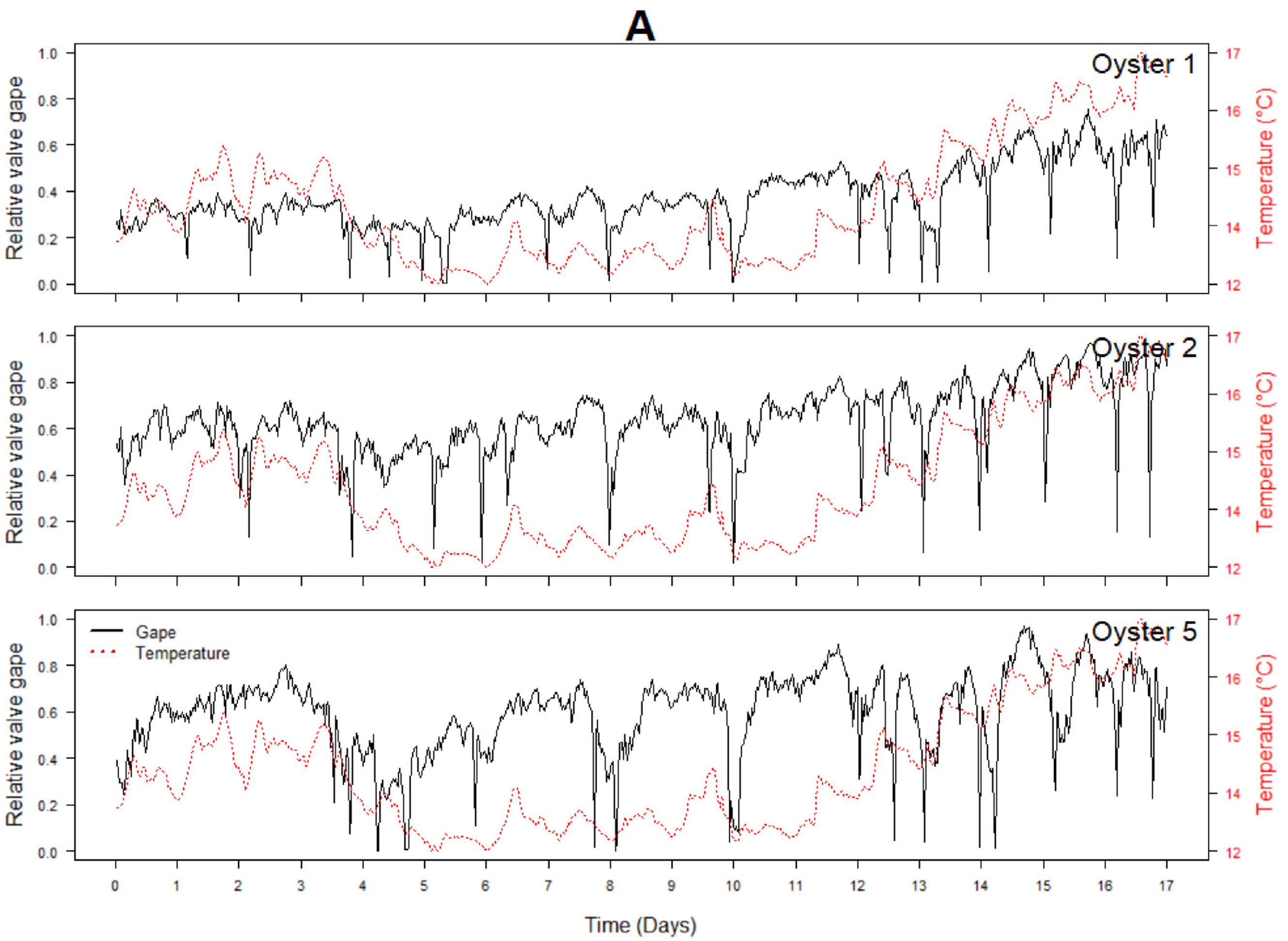

Fig. 8. Gaping activity of oysters (*O. edulis*) deployed in the Voordelta (North Sea) during spring (A) (from 17th of May until the 3rd of June 2021) (oyster 1, 2 and 5) and autumn (B) period (from 27th October to 13th November 2020) (oyster 6, 7 and 8). The *x*-axis denotes time (days). The left *y*-axis and black solid line denote the relative valve gape (0 indicates closed and 1 indicates maximally opened). On the right y-axis temperature (30 min average) is indicated (red dotted line) in °C in Figure 8A. In Figure 8B turbidity is indicated (red dotted line) in FTU on the right *y*-axis

3.3 Valve gape monitor – in situ measurement Voordelta

3.3.1 Environmental parameters

The daily average temperature fluctuated between 14 and 17 °C and between 11 and 13 °C during the 17 days of monitoring in spring and autumn, respectively (Suppl. Fig. S5). The daily average Chlorophyll-*a* concentration fluctuated between 2 and 13 $\mu g\,L^{-1}$ in spring with a peak at day 5, and between 1 and 3 $\mu g\,L^{-1}$ in autumn. The daily average turbidity fluctuated between 1 and 35 FTU in spring and between 1 and 26 FTU in autumn (Suppl. Fig. S5). Current velocity fluctuated between 0 and 0.4 $m\,s^{-1}$ in spring and between 0 and 0.5 $m\,s^{-1}$ in autumn (Suppl. Fig. S5).

3.3.2 Valve gape

The oysters that were deployed during autumn showed 4.1 times longer time fraction in the gape classes 0 to 0.2 than the oysters that were deployed in spring (Figs. 8 and 9, p-value < 0.05). However, a comparison of the valve gape frequency distributions showed higher percentages of time spent in the lower gape size classes in spring (0.2–0.5) (Fig. 9A). In contrast, there were longer periods with wider open valves in the higher gape size classes in autumn (0.5–0.9) (Fig. 9B, p-value $<$ 0.05). The relative valve gape during the entire period (gape class 0–1) was compared between spring and autumn (Fig. 10A). On average the oysters appeared to open slightly less wide in spring (Spring: mean valve gape = 0.47 ± 0.19 and Autumn: mean valve gape = 0.52 ± 0.27, p-value < 0.05). By excluding the periods when valve gape < 0.2 the difference in mean valve gape between spring and autumn is larger and it becomes clear that when the oysters opened they open wider in autumn (Fig. 10B).

In spring, a negative correlation was found between the daily average valve gape with Chlorophyll-*a* (oyster 1, 2 and 5; Tab. 2) and a positive correlation was found with temperature (oyster 1, 2 and 5; Tab. 2). In autumn, a negative correlation was found between the daily average valve gape and Chlorophyll-*a* (oyster 1 and 8; Tab. 2), temperature (oyster 1, 7 and 8; Tab. 2) and turbidity (oyster 1, 7 and 8; Tab. 2). For current velocity, a 30 minute average was used but no correlations were found with the valve gape.

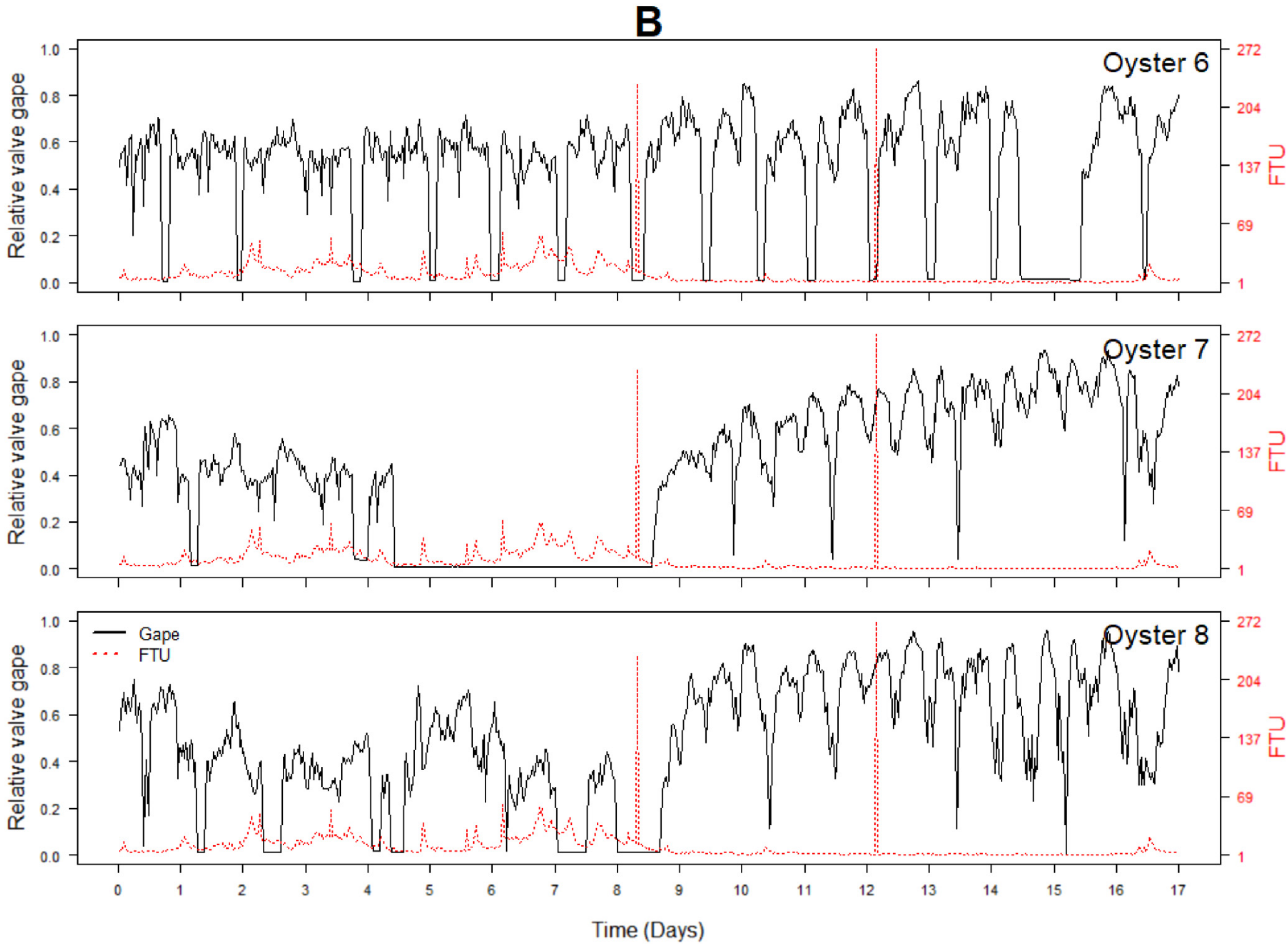

Fig. 8. (Continue).

3.4 Discussion

Frequency distribution of gape classes under controlled laboratory conditions clearly show that oysters that are not fed close their valves for significantly longer periods than oysters that receive food. The relative gape width is also larger in conditions with food than without food. In addition, oysters that were not fed opened less wide when they did open. Our results show that the oysters responded to changes in food availability. In the concrete basin in the Oosterschelde, where the oysters were sheltered from the current but still in connection with the water outside the basin during high tide, a positive correlation between valve gape and Chlorophyll-*a* was found. This confirms the observations on food availability and valve gape from the laboratory experiment. Although fast valve movements, such as sudden closure and re-opening of the valves, are potentially missed due to the resolution of valve gape measurement more general patterns of open and closed valves can be detected. In addition, a pattern of opening of the valves when the basin was under water and gradual closing of the valves when there was no contact with the water outside the basin (no current) was observed. Unlike the starvation experiment in the laboratory where food was absent from the water, it is unlikely that the available food source in the basin was exhausted since the clearance rate of adult flat oysters is about 3 L h^{-1} (Wijsman and Smaal, 2017), and the volume of the concrete basin is about 31612 L. Therefore, eight adult oysters can clear an estimated 0.7% of the volume in the concrete basin during the 9 hours that the basin is isolated from the surrounding water. These results are in line with other studies, which suggest that valve gape activity is related to food availability (Riisgard et al., 2006; Frank et al., 2007; Saurel et al., 2007; Ballesta-Artero et al., 2017: Hubert et al., in press). Ballesta-Artero et al. (2017) showed seasonal changes in valve gape activity and a positive correlation between valve gape and Chlorophyll-*a* concentration and identified the latter as the main driver of valve gape activity in a field situation. However, in the Voordelta, where the oysters experienced full tidal currents, a negative correlation of valve opening and Chlorophyll-*a* was found in both spring and autumn. In spring, valve gape activity was higher while in autumn, the periods of inactivity were longer. Note that a lower, but constant, influx of available food in the Voordelta is different from the situation in the laboratory experiment, where valve gape width was lower during the complete absence of food. Kamermans et al. (2018) determined that phytoplankton availability influences the growth of *O. edulis*. However, food availability can vary substantially both seasonally and spatially, particularly in coastal areas. Moreover, stratification can limit the access of oysters to food supply even in highly productive areas

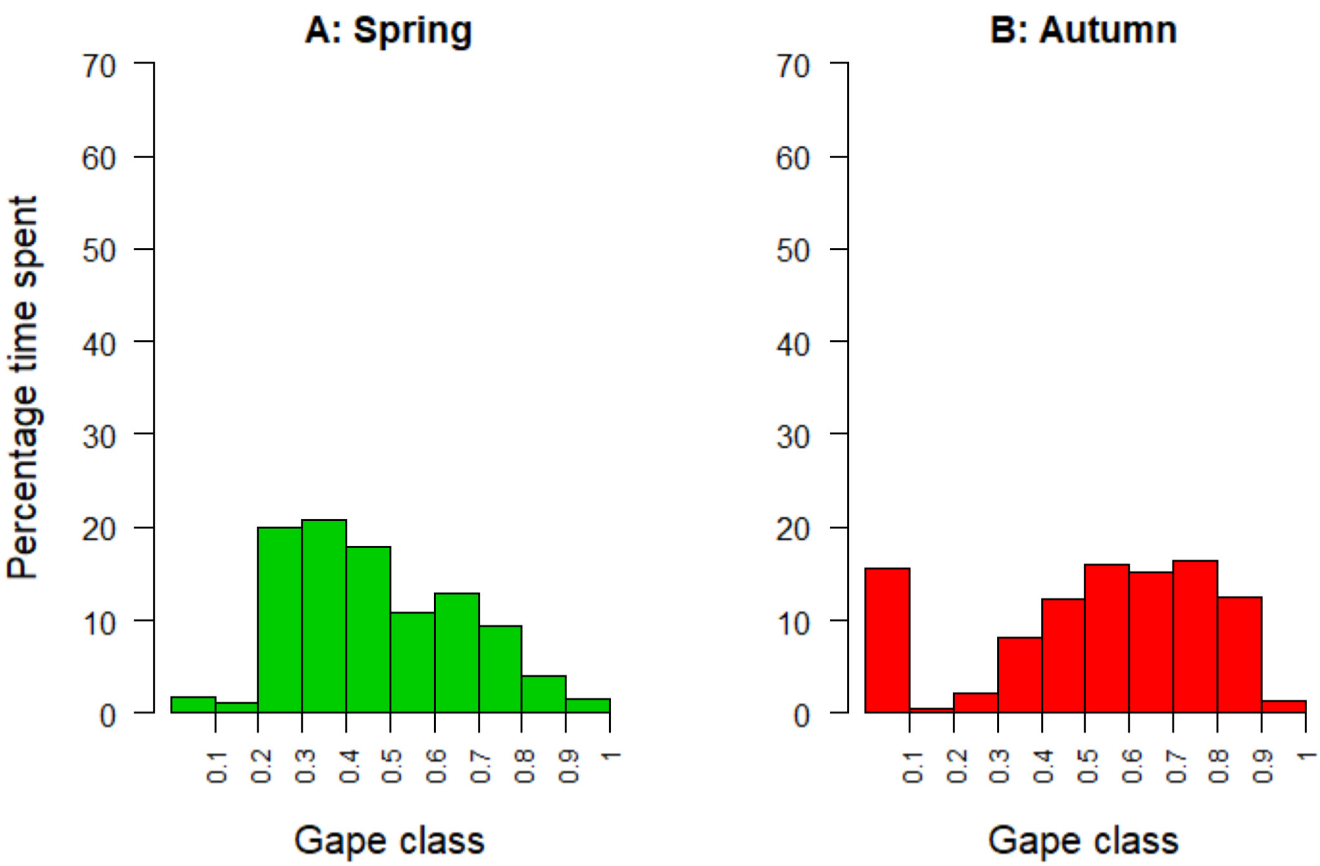

Fig. 9. Gape class frequency distribution of oysters (*O. edulis*) during (A) spring (green) and (B) autumn (red) in the Voordelta. *Y*-axis indicates the percentage time spent. The *x*-axis indicates valve opening per gape class (0–0.1, 0.1–0.2, ..., 0.9–1.0).

(Millican and Helm, 1994), and is considered a potential inhibitor (Kamermans et al., 2018).

A negative correlation was seen between valve gape and temperature and also between valve gape and turbidity (only in autumn). However, no correlation was found between valve gape and current velocity. In our study, both positive and negative correlations with valve gape and Chlorophyll-*a* were found in situations where the oysters were either sheltered or exposed to the current (Oosterschelde versus Voordelta). Perhaps the higher current velocity in the Voordelta influenced the valve gape (although no direct correlation between valve gape and current velocity was found). Other variables or compounding factors that were not included in this study, such as light intensity, could also have influenced the valve gape. Comeau et al. (2018) observed a maximum valve opening during night-time and minimum opening during daytime with *M. galloprovincialis*. Other physiological functions such as respiration have also been shown to determine gape activity in bivalves (Newell et al., 2001), as well as the temperature and light conditions being important driving factors (Schwartzmann et al., 2015). This suggests that gape width is affected by many factors and needs to be treated with caution when used to explain feeding behaviour. Other studies suggest that gape activity is a rather indirect measure of feeding, and additional information such as knowledge on the prior feeding history, particle concentration over time and the exhalant siphon area are needed in order to interpret valve gape activity more accurately (Newell et al., 2001; Frank et al., 2007; Maire et al., 2007).

The application of the gape monitor could, however, greatly assist in oyster reef restoration projects on, for instance, offshore pilot locations, provided that measures for adequately securing the gape monitor and sensors are taken as an extra precaution against the exposed conditions. On the basis of the results presented here, we show that under field conditions, the gape monitor can be used to detect the natural behaviour of flat oysters and that it provides information on behaviour and stress response of the bivalves against a natural set of environmental conditions. Knowledge gathered from such experiments can be beneficial for future flat oyster restoration projects. Moreover, the gape monitor data could also be used to retrospectively determine flat oyster mortality, the response to extreme environmental conditions and perhaps even spawning (Bernard et al., 2016; Gray et al., 2022; Tran et al., 2020). However, when explaining feeding behaviour based on valve gape information and Chlorophyll-*a*, the clearance rates and siphon area should also be known.

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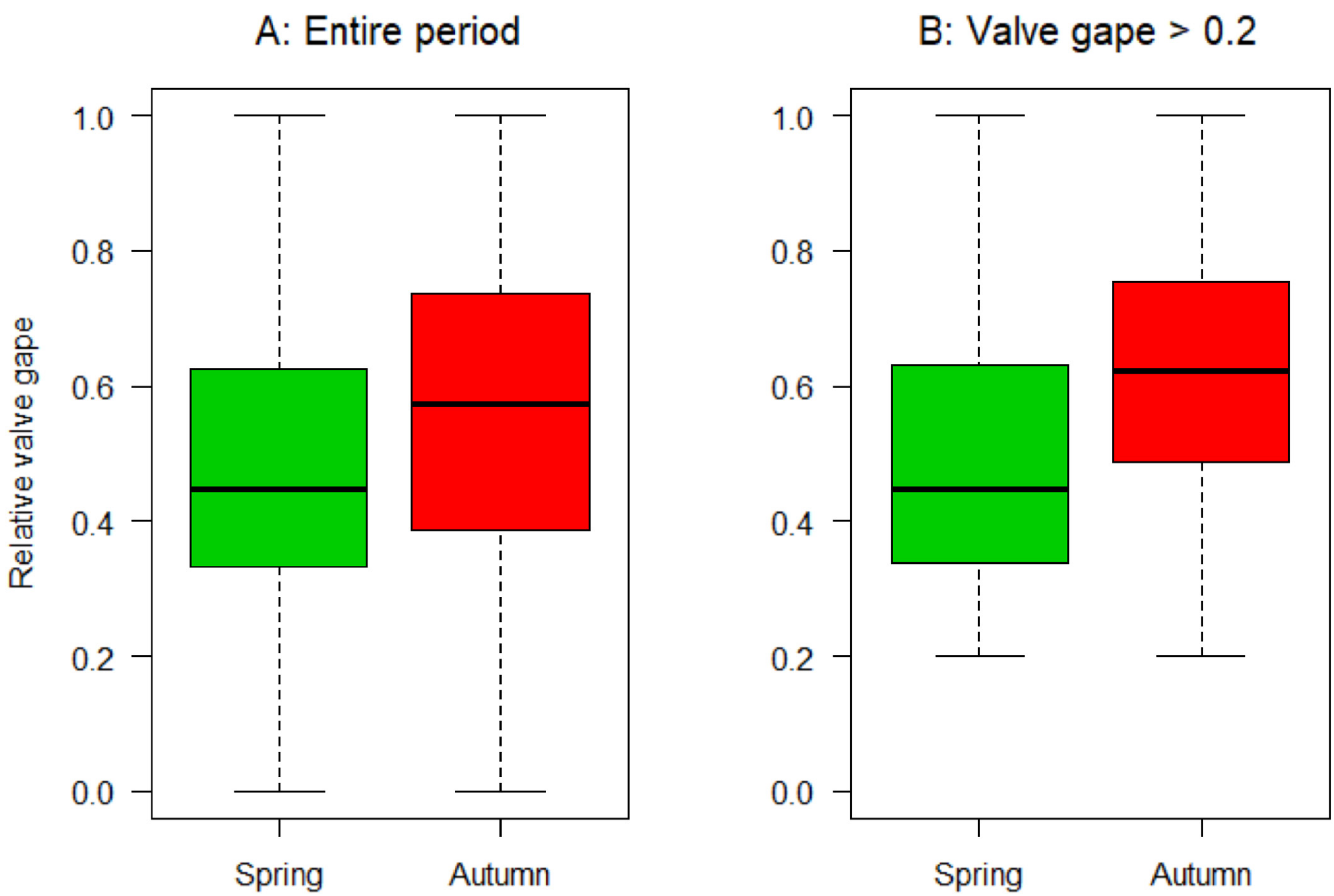

Fig. 10. Boxplot of the relative valve gape in *O. edulis* during the entire period (A) compared to the period of valve gape > 0.2 (B) during spring (green) and autumn (red) conditions. The *y*-axis (relative valve gape) indicates how wide the oysters open.

Supplementary Material

The Supplementary Material is available at https://www.alr-journal.org/10.1051/alr/2022021/olm.

Fig. S1 Gaping activity during the recovery run of the laboratory food availability experiment.
Fig. S2 Gape class frequency distribution during the recovery run of the laboratory food availability experiment.
Fig. S3 Gaping activity during the first laboratory food availability experiment.
Fig. S4 Daily average gaping activity of oyster 1 during the concrete basin experiment (semi tidal condition).
Fig. S5 Daily average gaping activity of oyster 1 during the in situ measurement in the Voordelta.

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