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Gaping behaviour of Blue mussels (*Mytilus edulis*) in relation to freshwater runoff risks

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ABSTRACT

Shellfish grown for food are vulnerable to environmental contamination, potentially rendering them unsafe for human consumption. Non-invasive gape (valve openness) sensing allows in-situ monitoring of bivalve shellfish behaviours, such as feeding, that can result in exposure to contaminated waters. Sensors were attached to Blue mussels and deployed for 10 days on natural mussel beds in Dundrum Bay, Northern Ireland. Data showed a tidally synchronous behaviour pattern of high openness at high water and vice versa. It is likely that this is, at least in part, due to extreme salinity variation (1.8–33.6) resulting from near total water exchange with each tide in the bay. This behaviour is likely to infer a degree of protection from contaminants during periods of low water, a time at which runoff-derived pollutants are most concentrated.

1. Introduction

Traditional monitoring of shellfish to quantify, for example, their growth or health is invasive and often requires lethal sampling (Lucas and Beninger, 1985). Alternatively, non-invasive sensors that allow bivalve shellfish to be monitored remotely can be used (Kramer and Foekema, 2001). These typically monitor the gape (openness) of an organism. Sensors can record gape at a relatively high frequency (e.g. 10 Hz) without interfering with the bivalve's natural behaviour.

Gape monitors have been produced based on various technical principles, discussed in detail by Kramer and Foekema (2001). Sensor units can be designed for extended autonomous or semi-autonomous field deployments or for laboratory use. Gape sensing has been used to monitor several bivalve species for different purposes, as summarised in Table 1.

The monitor utilised in this study was developed at the University of Essex as part of the Non-Invasive Oyster Sensor (NOSy) project (Cameron et al., 2019). The system uses Hall-Effect magnetic sensors in which the voltage across the sensor is proportional to the strength of a magnetic field, and therefore the proximity of a magnet. NOSy was

initially developed to study Pacific oysters (*Magallana (Crassostrea) gigas*), but is also suitable for research on or monitoring of various bivalve species in both laboratory and long-term autonomous field settings.

To date the system has been used in the field and laboratory with both *M. gigas* and the European Flat oyster (*Ostrea edulis*), notably under simulated heatwave and harmful algal bloom conditions (Funesto et al., 2023). In this study we deployed the sensor system on Blue mussels (*Mytilus edulis*) in beds currently deemed unsuitable for harvest due to local contamination. The aims of this study were to demonstrate the utility of NOSy to quantify gaping behaviours in a non-oyster species, and to examine patterns in behaviour across tidal cycles. We further aimed to determine whether the behavioural patterns of *M. edulis* in Dundrum Bay affect their vulnerability to contaminants from freshwater runoff.

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Table 1
Examples from the published literature of studied species and research topics that utilise gape-sensing technology.

Species	Research Topics	Reference
Giant clam (<i>Hippopus hippopus</i>)	Long-term growth monitoring in relation to temperature regime.	Schwartzmann et al., 2011
Ocean quahog (<i>Arctica islandica</i>)	Environmental regulation of gaping activity.	Ballesta-Artero et al., 2017
Eastern oyster (<i>Crassostrea virginica</i>)	Salinity and seasonal impacts on clearance rates. Siltation impacts on gaping behaviours. Feeding responses to food availability.	Casas et al., 2018 Poirier et al., 2021 Higgins, 1980a, 1980b
Razor clam (<i>Ensis leei</i>)	Siltation impacts on growth dynamics.	Witbaard et al., 2012
Icelandic scallop (<i>Chlamys islandica</i>)	Diurnal behaviour patterns. Annual growth patterns.	Berge et al., 2015 Tran et al., 2020
Mediterranean mussel (<i>Mytilus galloprovincialis</i>)	Circadian gaping behaviours in an aquaculture setting.	Comeau et al., 2018
Blue mussel (<i>Mytilus edulis</i>)	Copper exposure impacts on gaping behaviour. Annual growth patterns. Feeding behaviour and predation risk. Oil dispersant impact quantification. Detection of harmful algal blooms.	Curtis et al., 2000 Tran et al., 2020 Robson et al., 2010 Durier et al., 2021 Durier et al., 2022
Akoya pearl oyster (<i>Pinctada fucata</i>)	Detection of harmful algal species.	Nagai et al., 2006
Pacific oyster (<i>Magallana</i> (<i>Crassostrea</i>) <i>gigas</i>)	Bio-monitoring in an aquaculture setting. Automated spawning and stress detection. Water quality assessment.	Andrewartha et al., 2015 Ahmed et al., 2017, 2016 Sow et al., 2011
Asiatic clam (<i>Corbicula fluminea</i>)	Use of bivalves as a biosensor for cadmium detection.	Tran et al., 2003
Sea scallop (<i>Placopecten magellanicus</i>)	Feeding responses to flocculation and sedimentation.	Cranford et al., 2005

2. Materials and methods

2.1. Study area

Dundrum Bay, Northern Ireland (Fig. 1) covers an area of approximately 450 ha and has a tidal range of ~ 4 m. The inner bay, connected to the Irish Sea by a narrow, 1 km long channel is almost completely intertidal, experiencing near total water exchange with each tidal cycle (Snodden and Roberts, 1997). At low tide, the water that remains in the bay's drainage channels is virtually fresh, whilst it reaches nearly full-marine salinity at high tide. The restriction of water to drainage channels at low tide suggests that contamination entering the bay would be relatively concentrated at low tides.

2.2. Sensor instrument

As part of the NOSy project, we developed a sensor unit capable of simultaneously monitoring gape in up to 16 animals per unit. The unit records the gape of each individual at a frequency of 3.7 Hz (Cameron et al., 2019). In contrast to active electromagnets used in some previous studies (e.g. Tran et al., 2003), we utilised passive permanent neodymium magnets to reduce power consumption. Previous trials indicated that sensor and magnet attachment site selection was key to successful deployment. Hence individuals with a thin shape at the posterior margin were required for maximum signal strengths to be obtained.

NOSy requires a single 12 V battery (RS Pro 12 V 13 Ah, RS Pro, Corby, UK), supplemented by a solar panel on the unit case. Sensor

management, power and data storage were carried out by a BeagleBone Black (BeagleBoard.org, Michigan, USA) and a custom-printed circuit board (PCB). Hall-effect sensors (SS495A Hall Effect Sensor, Honeywell, Charlotte, NC, USA) were connected to the PCB with cabling sheathed in a silicon tube, sealed at the sensor end with flexible silicon sealant (Elastosil E43, Wacker, Munich, Germany). The instrument is capable of remote data transfer via mobile networks but in this instance a USB connection was used for setup and data download to a laptop in the field. The overall design is similar to that detailed in Nagai et al. (2006).

2.3. Ancillary monitoring

Conductivity, temperature and depth (CTD) data were recorded with two types of CTD instrument (Sea-Bird 19 CTDi on 16–18 September 2020; Sea-Bird Electronics, Bellevue, WA, USA; YSI 6080 on 18–25 September 2020; Xylem Analytics UK, Letchworth, UK). These were deployed on the seafloor in close proximity to the sensor instrument, logging depth and salinity every 15 min (Seabird 19 CTDi) or 20 min (YSI 6080) throughout the deployment (Fig. 2). Depth data from the second CTD instrument were adjusted by – 27 cm to calibrate with the readings of the first instrument. All CTD data were linearly interpolated between available data points (either 15 or 20 min apart) to calculate an estimated value for each minute of the deployment.

2.4. Animals and experimental conditions

Mussels for the study were retrieved from the study site, a mussel bed in the south west of the bay (Fig. 1C), on 15 September 2020. They were stored overnight in the high intertidal zone adjacent to the bed for easy retrieval on the following day. The use of mussels that have grown in situ reduced the need for an acclimation period to the conditions in the area. The next day, 15 mussels (54 mm average length, range 50–60 mm,) were selected for optimal shape and prepared for sensor attachment by cleaning and roughing the shell.

Sensors and magnets were attached to opposite valves with a UV-curing fibre-reinforced polyester resin (Solarez Ding Repair, Vista, CA, USA). Live data from the NOSy unit were used to optimise attachment site selection for maximal signal strength. Sensor attachment disturbance was kept small by keeping the emersion time to under 3 h. One sensor was left mussel-free for monitoring of background noise for quality control. Sensors/mussels were designated as M1 – M15.

Once sensor attachment was completed the NOSy unit was secured on a floating pontoon in one of the drainage channels in the south of Dundrum Bay (Fig. 1C). The mussels were then attached to pontoon mooring lines, sensors M1 to M8 on one line, sensors M9 to M15 and the control sensor on another. All mussels were suspended throughout the tidal cycle, remaining submerged at all times (Fig. 2).

Deployment of sensor unit and mussels started on 16 September and ended 9 days later on 25 September 2020. Gape was logged at 3.7 Hz throughout the deployment. The NOSy unit was checked daily and battery replacements were carried out regularly to ensure sufficient power supply.

2.5. Data treatment and statistical analyses

Data are available as [supplementary material online](#) [dataset] (Shakspeare, 2023).

Gape data affected by disturbance during sensor attachment and associated movement prior to 17:00 on 16 September 2020 were excluded. Low power levels after 00:00 on 25 September 2020 resulted in reduced quality data which were also excluded. Quality checks indicated short (<1 s) spikes in sensor output throughout the deployment, believed to be as a result of electronic noise. These were manually removed by deleting data points outside of the expected range. Data for mussels 5, 12 and 13 in the latter parts were low quality or missing as a result of either mussel mortality, mussel detachment or sensor failure

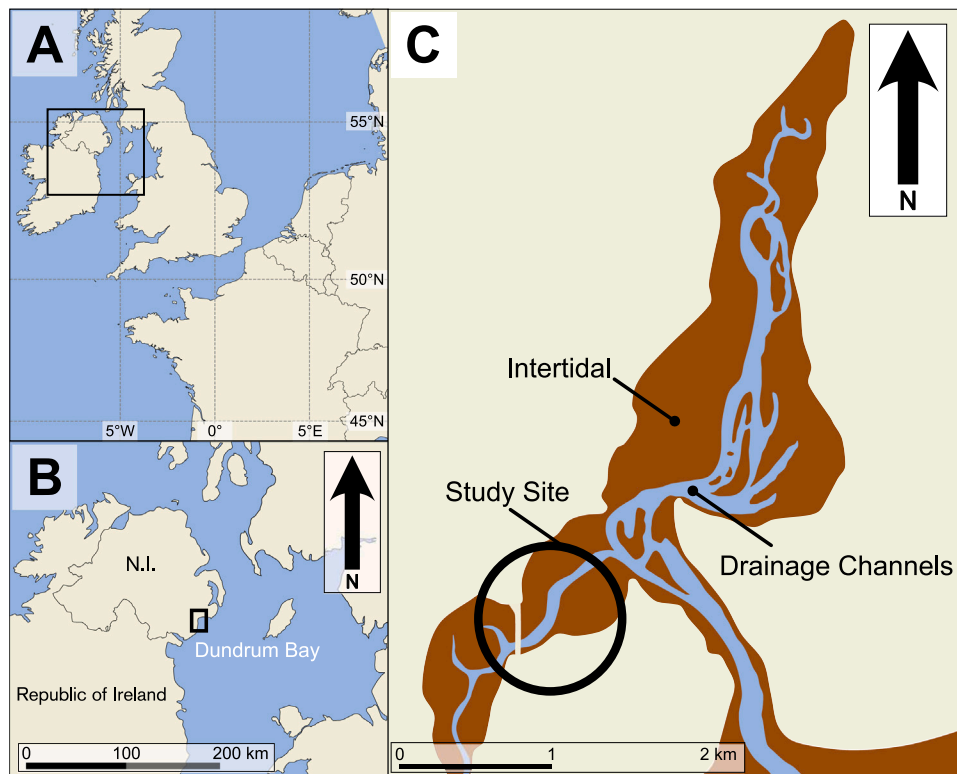


Fig. 1. Study site location. **A** – Large-scale location of the site. **B** – Location of Dundrum Bay within Northern Ireland (54.250°N, 5.850°W). **C** – Detail of Dundrum Bay with the study site highlighted. The intertidal zone (brown) and drainage channels (blue) are also shown as they would appear at mean low water.

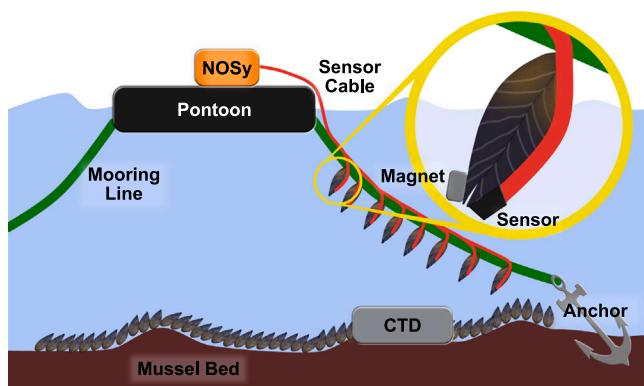


Fig. 2. Schematic of the NOSy installation for this deployment. Shown are the principle set-up of the NOSy sensor with mussels suspended from the pontoon mooring line, and the location of autonomous CTD (conductivity, temperature and depth). The inset illustrates the relative positioning of the magnet and Hall-Effect sensor on a mussel.

(not directly verified during NOSy retrieval). These data were also excluded (Appendix A).

The raw gape data for each mussel were averaged every minute (typically 222 data points per minute). Data were normalised using the Python package ‘sklearn’ (Pedregosa et al., 2011) such that ‘0’ represents the least open (assumed to be ‘closed’) position for each mussel and ‘1’ the most open position over the collected dataset. Population averages of the normalised gape data for each minute were then calculated from all available mussel specimens ($n = 12\text{--}15$).

Inflection point analysis (Muggeo, 2003) was carried out using the Python package ‘piecewise-regression’ (Pilgrim, 2021) to explore the relationship between depth (as a proxy for all tidally influenced factors) and gaping behaviour. The package used an iterative algorithm to

identify inflections in the linear relationship between variables, testing for the significance of inflections with Davies test. Spearman’s rank testing was carried out using the Python package ‘SciPy’ (Virtanen et al., 2020) and used to examine the correlation between depth and salinity. Data were plotted using the Python packages matplotlib (Caswell et al., 2022) and seaborn (Waskom, 2021).

3. Results and discussion

This study demonstrated the successful field deployment of the NOSy system with *M. edulis*. Complete data coverage was achieved for all 15 mussels for 83% of the deployment period. A figure showing processed gape data for all individuals is provided in Appendix A. There was a difference in raw-data quality between mussels, as inter-individual morphological variation affected the distance between sensor and magnet (Appendix B). However the averaging and normalisation procedures ensured that data were smoothed and directly comparable between mussels.

During the sensor deployment, water depths at the study site ranged from 0 to 3.5 m, and salinities from 1.8 to 33.6 (mean = 24.1 ± 10.1). Mean temperature was 14.3 ± 1.3 °C, with a range of 9.7–16.9 °C (Fig. 3). There was a rapid increase and decrease in salinity with the tidal height, with the highest temperatures recorded during high water. A clear pattern of behaviour synchronous with tidal height was found across the study population, with high gape readings occurring at high tides and vice versa (Fig. 3). There was a clear trend of increasing average gape with depth, most notably between the < 0.5 m and 0.5–1 m bands, where the average gape increased from 0.09 to 0.43 (Fig. 4).

The relationship between gaping behaviour and depth was markedly different between high and low depths. Population-averaged gape had a significant inflection point in this relationship at 0.92 m depth (Davies Test, $df = 11937$, $p = 0.021$) (Fig. 5). Below this depth, gape was strongly positively related to depth, above it there was a positive but

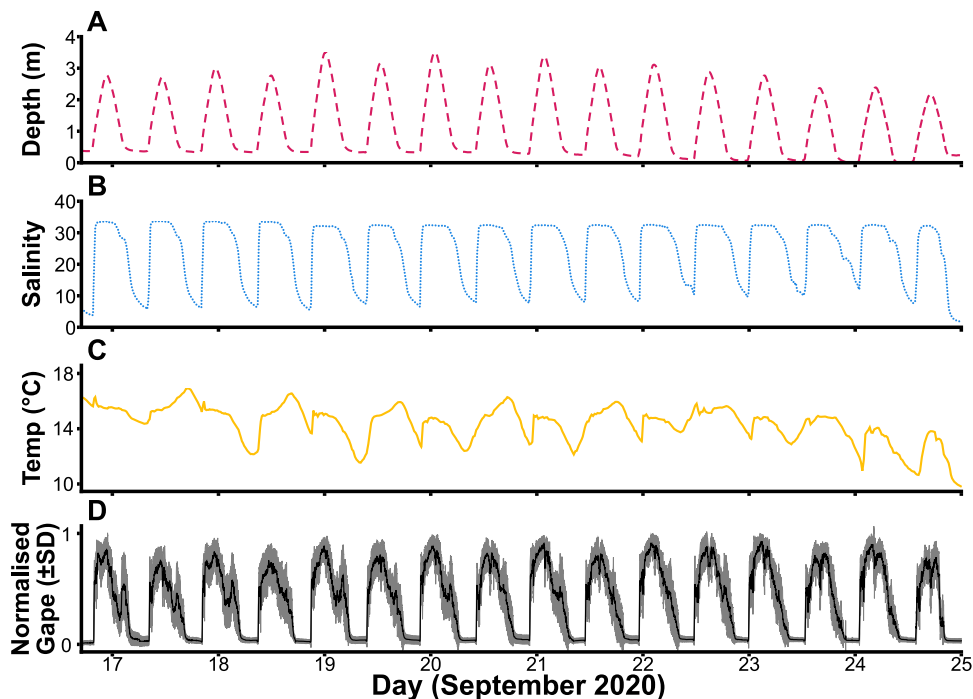


Fig. 3. CTD (conductivity, temperature and depth) and gape data summary for the full September 2020 sensor deployment. Shown are depth (A), salinity (B) and temperature (C) recorded at the seabed directly under the monitored mussels and a summary of the population-averaged mussel gape (\pm one standard deviation) (D).

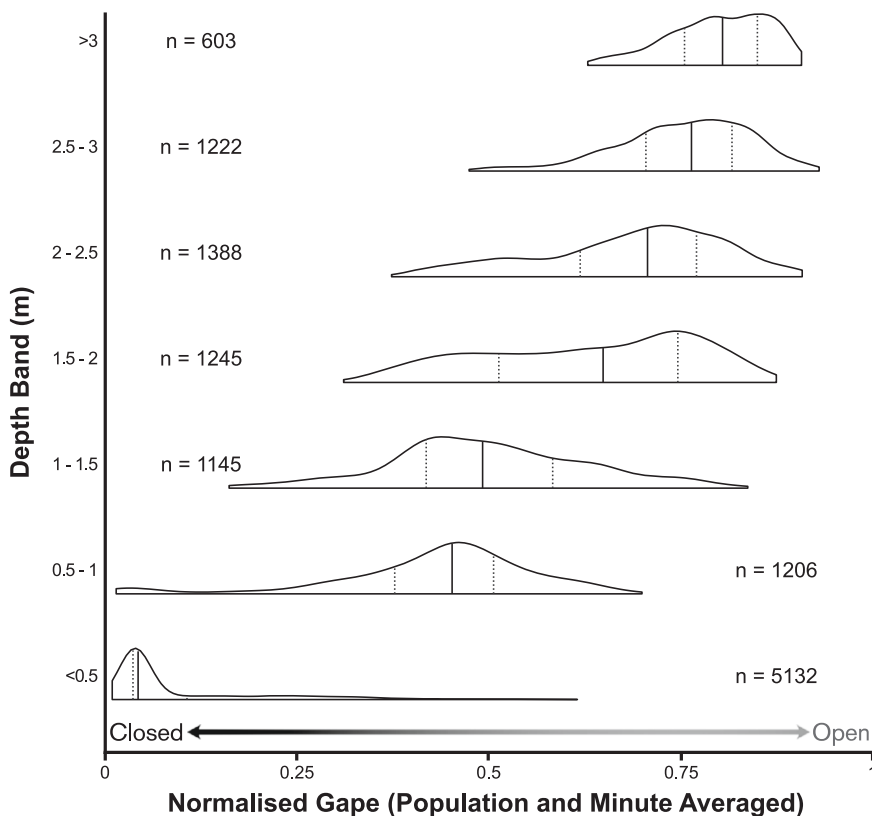


Fig. 4. Population average gape frequency distributions split by 0.5 m depth bins. Vertical lines indicate the mean (solid line) and quartiles (dashed lines) for each depth bin. Values for n indicate the number of minutes spent at depths within each bin over the full deployment.

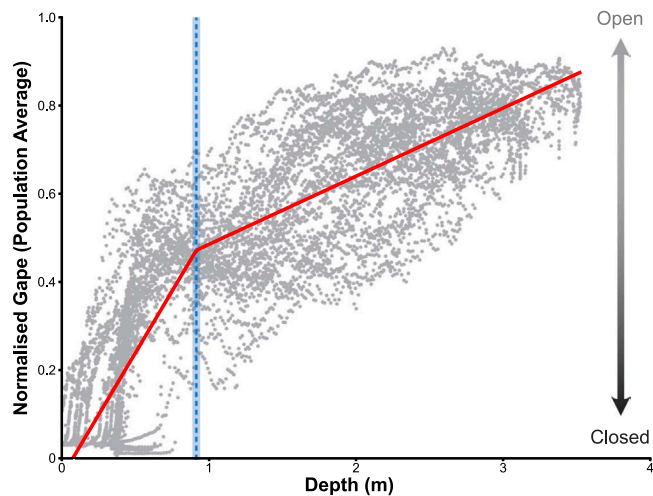


Fig. 5. Population-averaged gape of *Mytilus edulis* throughout the deployment at all tidal heights. Results of the inflection-point regressions are summarised, the solid red line indicates the two separate calculated depth/gape relationships and the dashed blue line the inflection-point with a shaded $\pm 5\%$ confidence interval.

Table 2

Summary of the Gape vs Depth inflection-point analysis. The presence of an inflection-point demonstrates that a change in gaping behaviour occurred at that depth. Significant analyses are highlighted in **bold**. DNC = did not converge, in these instances no significant inflection-point was identified.

Mussel ID	Breakpoint (m)	P Value	Number of observations
Average	0.92	0.021	11941
M1	0.81	< 0.001	11941
M2	DNC	DNC	11941
M3	0.13	0.926	11941
M4	1.83	0.826	11941
M5	0.70	< 0.001	10372
M6	0.79	0.063	11941
M7	0.98	0.106	11941
M8	0.82	0.002	11941
M9	1.30	0.195	11941
M10	0.35	0.026	11941
M11	0.85	0.019	11941
M12	0.77	< 0.001	9908
M13	0.91	< 0.001	11341
M14	1.80	0.446	11941
M15	0.85	0.001	11941

shallower relationship between the variables. Eight of the 15 individuals also had a significant inflection-point in the relationship of their gaping behaviour with depth. These ranged from 0.35 to 0.91 m, averaging 0.76 m (Table 2).

Bivalve molluscs are osmoconformers (Krogh, 1939). *M. edulis* have a long-term salinity tolerance of between 5 and 35 and can tolerate fully fresh water for a short time (Barrett et al., 2022; Westerbom et al., 2002). Tolerance to low salinity is primarily enabled by valve closure, which protects the organism from harmful conditions, including the ingress of freshwater (Riisgård and Larsen, 2015; Solan and Whiteley, 2016).

There are several factors, including temperature, food availability, pollutants and harmful algae, which can influence bivalve gaping. We suggest that the observed relationship between depth and gape is driven largely by the salinity variations (ranging in our experiment from 1.8–33.6) that occur in Dundrum Bay. Salinity was well correlated with depth (Spearman's rank, $r = 0.83$, $p < 0.01$) over the NOSy deployment (Figs. 2A and 2B). The significant inflection point in population-averaged behaviour at 0.92 m (Fig. 5) corresponded to an average salinity of 24.3, while the significant inflection points of individuals

corresponded to average salinities of between 12.6 and 24.3.

When population gape data were separated into salinity bands the gaping-behaviour patterns were clearly different between salinities below and above 25, mussels remain significantly less open at intermediate salinities far above their published tolerance (Fig. 6). This was supported by the calculated inflection-point (0.92 m, 24.3 salinity), suggesting that salinity is a significant influence on the behaviour of *M. edulis* in Dundrum Bay. Further work is required to ascertain the influence of other factors, possibly including post-feeding (satiation) induced closure and adaptation to, or stress caused by, the regular tidal changes in the bay.

This study demonstrated the suitability of the NOSy system for gape-data collection in the field. The data provided insight into the behavioural patterns of *M. edulis* over a tidal cycle covering extremes of salinity. We have shown that gaping behaviours of *M. edulis* in Dundrum Bay are significantly different above and below a salinity of 25, despite this species being tolerant to lower salinities in other areas. This could be evidence of an adaptive behavioural response in the population that reduces exposure to the near freshwater conditions that occur during low tide. Alternatively, it may indicate that tidal salinity variations in the bay result in high levels of background osmotic stress in the animals on the bed, decreasing their tolerance of salinities below 25.

The quality of the data and our analysis suggest that the NOSy sensor unit is suitable for monitoring bivalve behaviours for a diverse range of purposes. Behaviours recorded during this study, i.e. closure over low-tide periods, are likely to provide some protection from the high concentrations of freshwater run-off-derived contaminants that can occur at these times. However, the ongoing food-safety problems with *M. edulis* in Dundrum Bay indicate that the population is exposed to sufficiently high contaminant concentrations over the rest of the tidal cycle to outweigh this protection. The contamination may originate in freshwater runoff, but further work is required to understand why mussels in the bay remain unsuitable for harvest despite high levels of tidal water exchange.

Remote, non-invasive, gape sensing has the potential to be a valuable tool in shellfish monitoring. This study has shown that even a relatively short deployment can provide a detailed insight into a population's behaviours. We have shown that, as a result of response to low-salinities, *M. edulis* in Dundrum Bay are least exposed to their environment when contamination concentrations are highest. This finding in turn raised concerns around persistent contamination in a well-flushed bay. The widespread use of similar sensors in shellfish aquaculture should be considered as a useful tool to gain similar insights across the sector, particularly where stocks are grown in potentially contaminated waters.

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CRediT authorship contribution statement

Alex Shakspeare: Methodology, Software, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Heather Moore:** Conceptualization, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Matt Service:** Conceptualization, Investigation, Writing – review & editing. **Christian Wilson:** Investigation. **Hafiz Ahmed:** Software, Funding acquisition. **Tom C. Cameron:** Conceptualization, Investigation, Writing – review & editing, Supervision, Funding acquisition. **Michael Steinke:** Conceptualization, Investigation, Supervision, Project administration, Funding acquisition, Writing - review & editing.

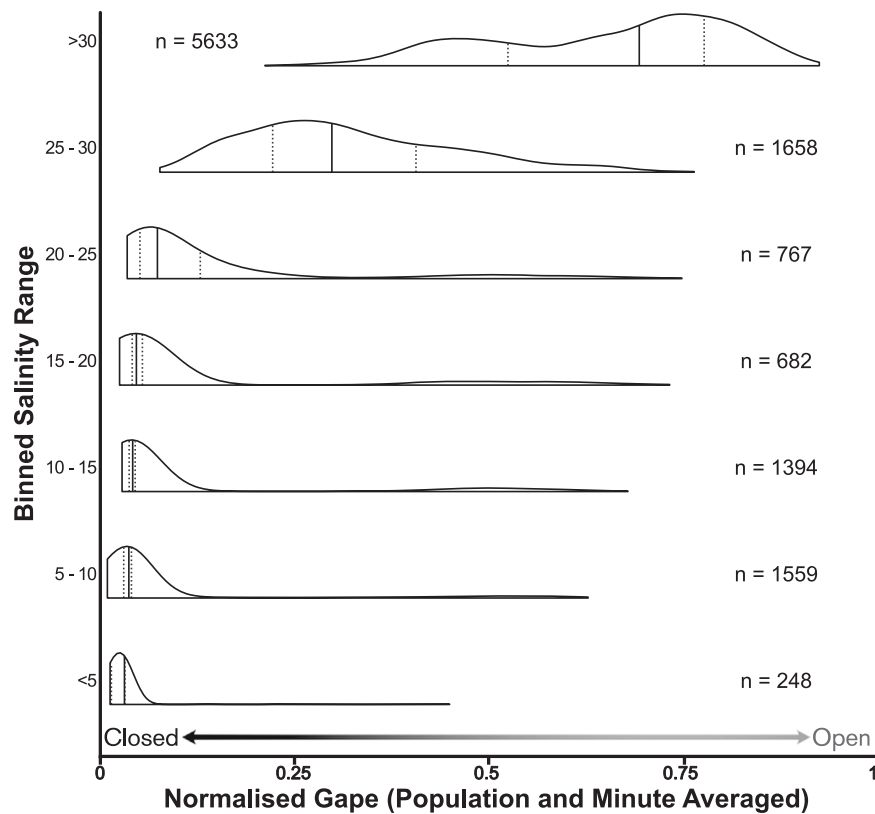


Fig. 6. Population average gape frequency distributions split by bins of 0.5 salinity. Vertical lines indicate the mean (solid line) and quartiles (dashed lines) for each depth bin. Values for n indicate the number of minutes spent at salinities within each bin over the full deployment.

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.

Data availability

Data are available in the Mendeley Data Repository, referenced in the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.aqrep.2023.101719](https://doi.org/10.1016/j.aqrep.2023.101719).

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