Increasing Sustainable Bivalve Aquaculture Productivity Using Remote Non-Invasive Sensing and Upweller Technologies

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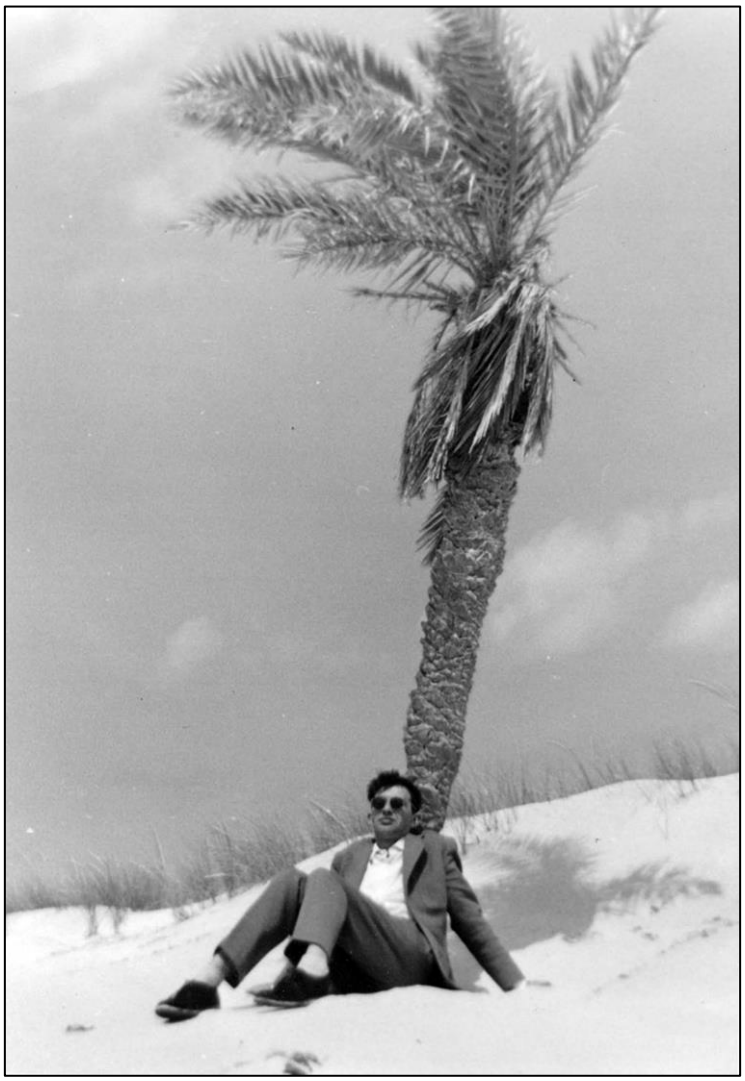
This thesis is dedicated to the memory of my grandfather, a remarkable man whose thirst for knowledge and talent for communication inspired all who met him.



Malcolm Holding

11th May 1932 – 7th April 2023

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I have not accomplished this thesis without oceans of help from many people I'm very lucky to know, and to all of whom I owe many, many, thanks…

To Paul Harding, Graham Baker and Sly Berecz at Colchester Oyster Fishery, I simply couldn't have done this project without you. From teaching me the ropes before it all began, to allowing me to work on-site throughout, the assistance from all of you has been invaluable.

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And lastly, to Lucy. Who wasn't there at the beginning, but who made it possible to reach the end.

My thanks to you all.

Summary

The work and findings described by this thesis aim to develop technologies and approaches relevant to bivalve aquaculture, focusing on non-invasive sensing to monitor bivalve shellfish, primarily the Pacific oyster (*Magallana gigas*). Following the introduction, Chapter 2 presents an overview of the Non-Invasive Oyster Sensor (NOSy), a sensor developed at the University of Essex that records bivalve openness (gape). NOSy was conceived to automatically detect spawning as an aid to oyster growers and has proved useful in field and laboratory, work which underpins three chapters in this thesis. NOSy remains under development, and has potential for use in aquaculture, monitoring and research.

Chapter 3 assesses the role of salinity in driving estuarine oyster behaviour. We replicated an estuarine tidal salinity cycle and recorded the gape of oysters exposed to it. Behaviours during the experiment did not resemble those in the estuary, suggesting that salinity alone does not drive estuarine oyster behaviour. We also discuss the challenges of controlling salinity in a laboratory, and suggest it is an under-studied area.

Chapter 4 discusses land-based systems for young oyster growing. Land-based systems have the potential to improve growth, condition and survival while reducing labour and maintenance costs. We trialled a system over three summers, with promising results. Reduction of localised densities improved growth rate and uniformity. Cost forecasts suggest that adoption of land-based growing systems could result in substantial savings.

Chapter 5 presents gaping records from an area where Blue mussels (*Mytilus edulis*) have become non-harvestable in recent years due to contamination. We used NOSy to assess gaping patterns of the mussel population to evaluate how their behaviours affect their vulnerability to contamination. Mussels in the bay closed over low tide as a response to extremely low salinity, inferring protection from contamination by limiting the mussel's exposure.

Declaration and Contributor Credits

I declare that this thesis is my own work. It would not have been possible however without funding from the Natural Environment Research Council and the ARIES Doctoral Training Partnership [grant number NE/S007334/1]. I would also like to acknowledge the following contributions from collaborators and colleagues.

- Chapter 2: Hafiz Ahmed (University of Bangor) provided guidance on the processing and analysis of sensor data. Marc Balsalobre (University of Essex) assisted with developing the initial sensor data processing protocols in R.
- Chapter 3: Dr Michael Steinke (University of Essex) conceptualised the initial experimental methodology. Marc Balsalobre (University of Essex) provided assistance with experimentation and data processing for the 2020 experiment.
- Chapter 4: Graham Baker (Colchester Oyster Fishery) initially suggested that land-based systems may be of advantage to the fishery. Szilvester Berecz (Colchester Oyster Fishery) assisted with constructing the system. Paul Harding (Colchester Oyster Fishery) allowed us to use the fishery's power supply and space on their quayside. John Green (University of Essex) conducted water sample analysis for samples taken in 2021 and 2022.
- Chapter 5: Dr Heather Moore, Dr Matthew Service and Christian Wilson (Agri-Food and Biosciences Institute, Northern Ireland) and Dr Michael Steinke (University of Essex) provided assistance in set-up and operation of the sensor unit at the study site.

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Introduction

1 Global and Fishery Background

1.1 Global Aquaculture

The global human population currently stands at over 7.7 billion, and the United Nations Department for Economics and Social Affairs forecasts that it will reach 8.5 billion by 2030 (DESA, 2022). The global per capita intake of fish and seafood has more than doubled since the 1960s (from 9 kg in 1961 to 20.2 kg in 2020), and now represents approximately 17 percent of global animal protein consumption (FAO, 2022). Fisheries and aquaculture will therefore play a key role in feeding this growing population. Ensuring that this role is carried out sustainably is a key challenge for global development, as highlighted by the UN 2030 Agenda for Sustainable Development (UN, 2015).

The importance of the aquaculture sector for meeting increased demand grows year on year. Whereas wild capture fishery production has remained effectively static since the late 1980s, aquaculture is one of the fastest-growing sectors of food-production and has contributed the majority of seafood for human consumption since 2014 (FAO, 2022). In 2020 the aquaculture sector produced 122.6 million tonnes, including algae and ornamentals, with a total farm gate value of $281.5 billion (FAO, 2022). Table 1.1 provides a breakdown of the animal species produced. The non-fed aquaculture subsector produced 24.3 million tonnes in 2020, of which 8.2 million tonnes were filter-feeding finfish and the rest aquatic invertebrates. However, the relative contribution of the subsector has declined markedly, from 43.9 percent in 2000 to 27.8 percent in 2020 (FAO, 2022).

Table 1.1 Global breakdown of food fish aquaculture production (in thousands of tonnes) in 2020 (FAO, 2022)

Category	Africa	Americas	Asia	Europe	Oceania	World
Finfish	2 236	2 420	50 000	2 674	101	57 461
Crustacea	8	1 266	9 951	4	9	11 237
Molluscs	6	688	16 351	578	116	17 740
Other aquatic animals	0	0	1 052	7	3	1 062
Total	**2 250**	**4 375**	**77 384**	**3 262**	**229**	**87 501**

The growth of aquaculture seen in recent years is forecast to continue (Figure 1.1), and by 2030 it is expected that two thirds of human fish consumption for food will be aquaculture derived (World Bank, 2013). This expansion presents an opportunity for sustainable food production, contributing to global health and nutrition in line with the UN Sustainable Development Goals (UN, 2015) as well as regional and national development frameworks, such as the EU-led multiannual national development plans for aquaculture (DEFRA, 2015). Growing of bivalves, molluscs that are encased between two hard shells, is likely to be a key component of this growth, and is the focus of this thesis.

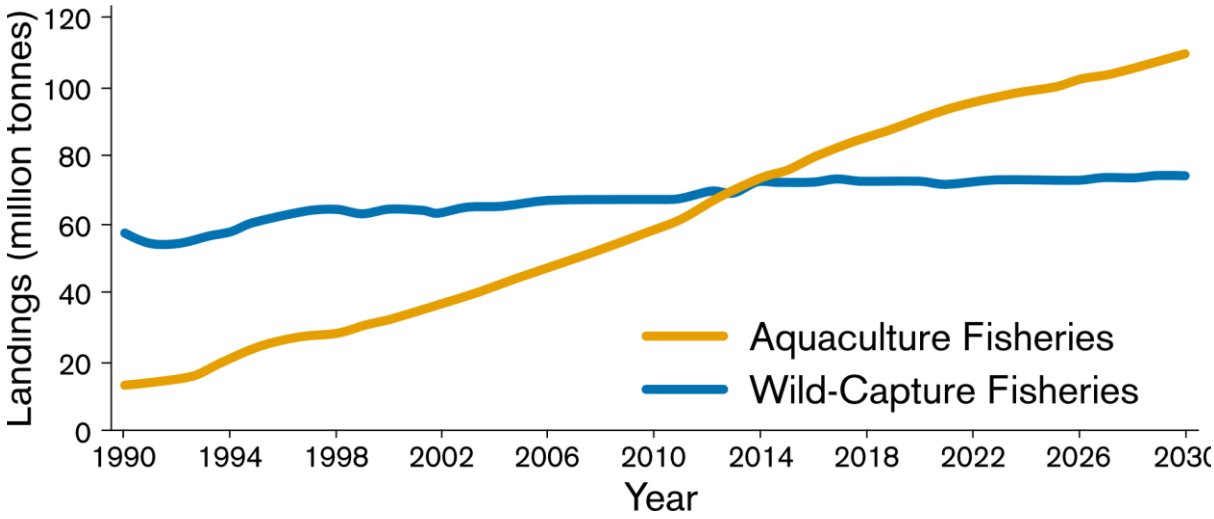

Figure 1.1: Wild-capture (blue line) and aquaculture (orange line) fishery landings tonnages since 1990, forecast to 2030. Modified from FAO (2020).

1.2 Bivalve Aquaculture

Bivalve aquaculture takes place virtually exclusively in coastal and nearshore waters, where growing operations are often relatively small-scale and provide employment in areas where opportunities for locals may be limited (Krause *et al*., 2019; Lucas and Southgate, 2012). The industry also supplies a nutritionally valuable food to the human supply chain, bivalves are an excellent source of vitamins and minerals, high in protein and low in carbohydrates and fat (Wright *et al*., 2018).

Many different methodologies are employed by the industry, from large-scale mussel culture operations using sophisticated anchored dropper rope systems (Figure 1.2A) to the managed intertidal seabed or ‘layings’ where Essex oystermen farm (Figure 1.2B). Despite the wide range of approaches, all bivalve aquaculture (other than some hatchery processes) has an important commonality; reliance on the local environment to provide a sufficient quality and quantity of food to sustain growth of the stock (Lucas and Southgate, 2012).

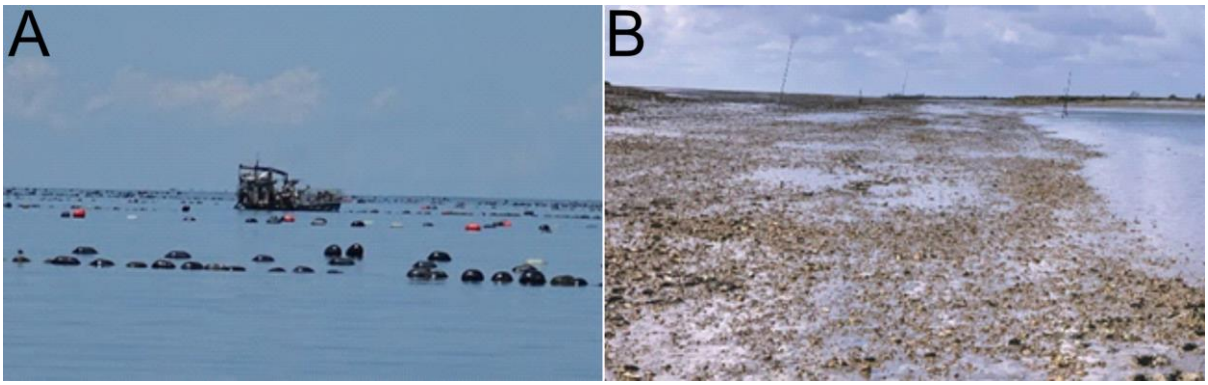

Figure 1.2: Images of example bivalve cultivation methods. (**A**) New Zealand Greenlip Mussel (*Perna canaliculus*) cultivation in the Hauraki Gulf, New Zealand on a longline system. (**B**) Pacific oyster (*Magallana* (*Crassostrea*) *gigas*) cultivation on intertidal ‘layings’, managed areas of extensive seabed culture, on the Mersea Island coast, Essex.

The use of naturally occurring food, rather than the manufactured feeds common to finfish and crustacean aquaculture, results in an innately low impact on the local and global environment (Coen *et al*., 2007; van der Schatte Olivier *et al*., 2018). Non-fed aquaculture therefore has the potential to provide economic and environmental benefits not seen in other forms of aquatic food production, including nitrogen drawdown and provision of habitat for other species (Barrett *et al*., 2022).

Bivalve populations, both commercial and natural, are associated with a wide range of ecosystem services including phytoplankton bloom control, refugia provision and seston filtration (Coen *et al*., 2007). Other authors have shown that bivalve populations aid in: carbon sequestration (Filgueira *et al*., 2015), water quality improvement and nutrient cycle control (Carmichael *et al*., 2012b; Clements and Comeau, 2019b; Lindahl *et al*., 2005; Zu

Ermgassen *et al*., 2013), pathogen filtration (van der Schatte Olivier *et al*., 2018), shoreline stabilisation (La Peyre *et al*., 2015) and linking primary production and epibenthic consumers (Beukema *et al*., 2010).

Despite its relatively low impact, there is inevitably the chance of negative effects of bivalve culture (Forrest *et al*., 2009). In the case of European oyster culture there have been issues associated with non-native species introductions (reviewed in Herbert *et al*., 2016). Harvest methods, particularly dredging, have the potential to physically disrupt and damage the seabed, leading to reduced biodiversity, altered species composition and reduced sediment grain size (Thurstan *et al*., 2013; reviewed in Wilber and Clarke, 2001). In extreme cases, where excessive stocking densities are achieved in a restricted flow area, as happened around Prince Edward Island, Canada, the water column can be stripped of food, negatively impacting on the ecology of the area (Cranford *et al*., 2003; Jiang *et al*., 2019). Nevertheless, bivalve culture is one of the most sustainable industrial forms of animal protein production (Shumway *et al*., 2003).

1.3 Oyster Aquaculture

This thesis focuses on bivalve aquaculture, particularly the culture of the Pacific oyster, *Magallana* (*Crassostrea*) *gigas* (Figure 1.3). The primary area of focus is the River Colne, an estuarine system on the Essex coast of the UK, where the industrial partner to this project, Colchester Oyster Fishery (COF) are based (Figure 1.4). COF are an oyster fishery and seafood wholesaler based on Mersea Island, just off the south Essex coast. The company is the current leaseholder of the historic Colchester fishery, which was bestowed to Colchester by Royal Charter from Richard I in 1189, meaning that it has exclusive rights to the shellfishery in the River Colne (Baggs *et al*., 1994).

Figure 1.3: An adult Pacific oyster (*Magallana* (*Crassostrea*) *gigas*) viewed from the dorsal aspect. The hinge is to the right of the image.

The British oyster fishery is historically important: Archaeological records date back several thousand years, fishing rights to areas of the Colne are recorded from 1189, and over 3 million oysters were landed from the Colne annually during the late 19th century (Baggs *et al*., 1994; Goodsall, 1965; Laing *et al*., 2006). The historic British oyster fishery was of the Native oyster (*Ostrea edulis*), but a combination of overfishing, invasive species, environmental challenges and novel pathogenic threats (particularly the haplosporidian parasite *Bonamia ostreae*) decimated stocks, collapsing the fishery in the mid-20th century (Axiak *et al*., 1995; Laing *et al*., 2006, 2014).

In recent years efforts have been made to restore *O. edulis* populations around the European coast (Pogoda *et al*., 2019). Given the level of stock loss that occurred in the 19th and 20th centuries this will not be a rapid process, but partnerships between academia,

industry and other stakeholders in the shape of organisations such as the Essex Native Oyster Restoration Initiative (ENORI, 2023) and the Native Oyster Network UK & Ireland (Native Oyster Network, 2023), are facilitating the process.

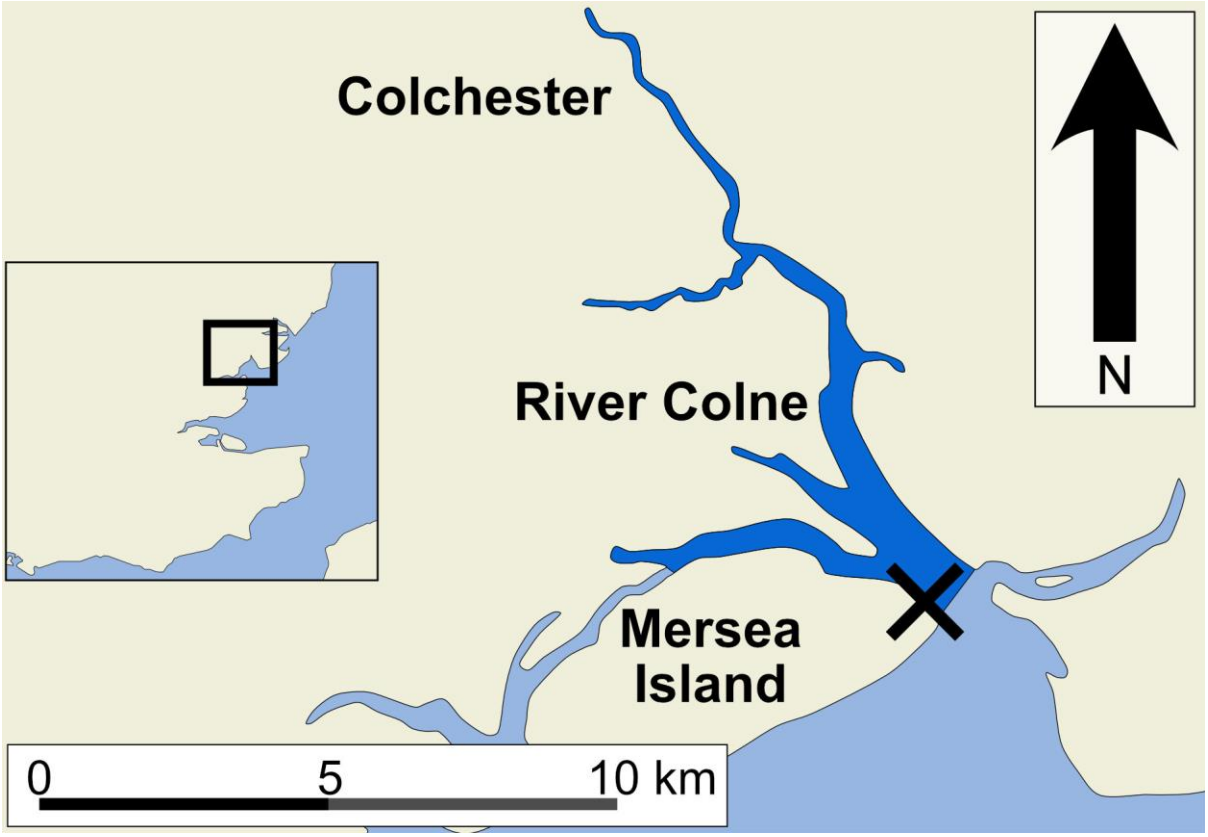

Figure 1.4: Map of the River Colne and surrounding areas. The extents of the River Colne are highlighted in dark blue. The location of Colchester Oyster Fishery, the CASE partner of this PhD project is indicated by the black cross (51.803N, 0.989E).

Following the functional extinction of the Native oyster, *M. gigas* was introduced to the British coast in an effort to re-establish a commercially viable oyster fishery, with the first large-scale efforts taking place in 1965 and 1972 (Spencer *et al*., 1994; Syvret *et al*., 2008; Utting and Spencer, 1992). The species has a number of advantages over *O. edulis* from a commercial perspective, it is faster growing, simpler to produce spat and resistant to endemic parasites (Humphreys *et al*., 2014). At the time, it was thought that the species would be unable to spawn in the comparatively cool coastal waters of the UK, reducing the chance of naturalisation and causing growers to rely on hatcheries to supply their stock

(Utting and Spencer, 1992). However, likely assisted by warming of marine waters in recent decades, the species has now naturalised in many areas of the British coastline and dominates the UK oyster market, as well as many others globally (FAO, 2019; Lallias *et al*., 2015; Ruesink *et al*., 2005).

Whilst there is no doubt that oyster culture is a relatively sustainable method of food production, as discussed in section 1.2, the potential negative impacts *M. gigas* as an invasive non-native species are considerable, wide ranging and complex. *M. gigas* culture was initially only permitted under a general licence excepting the species from the prohibition on non-native species release in British law (e.g. the Wildlife and Countryside Act (1981)). The species has the potential to create extensive hard shell reefs due to the preference of *M. gigas* larvae to settle on or near other oysters, acting as an 'ecosystem engineer' and considerably altering the environment in which it settles (Gutiérrez *et al*., 2003; Herbert *et al*., 2016). The impacts are reviewed by Herbert *et al.* (2016), with the primary issues raised including: biodiversity loss; alterations to community structure and interruptions to ecosystem processes.

There is a clear friction between the socioeconomic benefits of a successful *M. gigas* fishery and its environmental impacts, particularly when the species encroaches on marine protected areas (Humphreys *et al*., 2014). However, whilst the species is spreading around the British coastline, and will likely do so at an increasing rate under warming conditions, the evidence to date suggest that the negative effects remain relatively local (Herbert *et al*., 2016).

Humphreys *et al.* (2014) assessed the economic contribution of *M. gigas* fisheries to the UK economy taking added value into account, rather than the more usual first sale price. These figures suggest that the overall value of the British fishery was over £13 million in 2011/12, since when the sector's landings have nearly doubled (FAO, 2021).

The relative scarcity and perceived better taste of *O. edulis* means that they command a higher market price than *M. gigas*, and as such remain valuable as a harvest (P Harding, Colchester Oyster Fishery, personal communication). At Colchester Oyster Fishery (COF), the CASE partner for this project, *O. edulis* harvests of opportunity are organised as part of day-to-day harvest operations. Specific areas of the fishery are also dedicated to *O. edulis*, with targeted harvests taking place when the need arises (P Harding, Colchester Oyster Fishery, personal communication). There is scope for the methodologies developed in this study to also apply to *O. edulis* stocks.

2 Biology, Behaviours and Environmental Responses of *Magallana gigas*

2.1 Phylogeny

M. gigas is a 'true' or edible oyster. This taxa of oysters sit within the family Ostreidae, class Bivalvia, phylum Mollusca (Horton *et al*., 2019). A number of other species are commonly referred to as oysters but these exist outside of this taxonomic grouping (Bayne, 2017a). *M gigas* sits within the Crassostreinae subfamily, as does the Eastern oyster (*Crassostrea virginica*), which is also referenced throughout this thesis. Members of this taxa are broadcast spawners. In comparison the Ostreinae subfamily, of which *O. edulis* is a member, brood their larvae. A simplified phylogenetic tree illustrating this is provided in Figure 2.1.

There is an ongoing debate around the classification of *M. gigas*, which was historically referred to as *Crassostrea gigas*. Genetic taxonomic analysis led to suggestions of this reclassification, and subsequent alterations have been made to the WoRMS database (Horton *et al*., 2019; Salvi *et al*., 2014; Salvi and Mariottini, 2017). Strongly dissenting views are held by many in the field and discussions remain open (Bayne *et al*., 2017). We will follow the current classification on WoRMS and use *Magallana gigas* throughout.

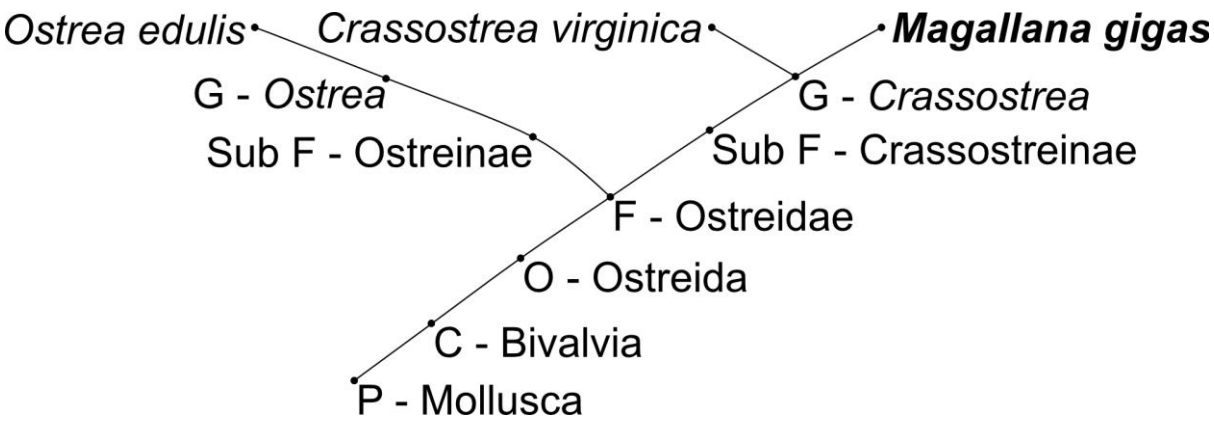

Figure 2.1: Schematic phylogeny illustrating the relationships between *M. gigas*, *C virginica* and *O. edulis*, the three oyster species most important to this thesis. After Horton *et al*. (2019).

2.2 Growth and Survival

Growth and survival of oysters has a substantial genetic component (Gutierrez *et al*., 2018), and is affected by a range of external factors, salinity and disease are of particular importance and are discussed separately below. Food availability and composition have a significant impact on the growth and survival of larval and settled bivalves (Hendriks *et al*., 2003; Marshall *et al*., 2010). Plentiful and high quality food results in high growth and survival rates throughout an oyster's lifespan, whereas insufficient or nutrient deficient foods have been shown to result in poor body condition, low growth rates and mortality (Fan *et al*., 2021; His *et al*., 1989).

Hypoxic conditions can also have significant impacts on growth and survival in bivalves. In larval Bay scallops (*Argopecten irradians*) and Hard-shell clams (*Mercenaria mercenaria*) low oxygen reduces growth by <50% (Gobler *et al*., 2014). Stevens and Gobler (2018) studied the effect of hypoxia on recently settled *C. virginica*, showing significantly reduced shell growth and tissue weights as a result of low oxygen conditions. The ability of *C. virginica* to feed is reduced under hypoxic conditions due to a protective closing response, likely contributing to a reduction in growth rates (Porter and Breitburg, 2016)

Whilst larger scale environmental factors, such as nutrient levels and eutrophication events, will impact on food availability and oxygen levels, local population density can have a substantial further impact. Large numbers of bivalves in a location can significantly increase the likelihood of reduced food availability, resulting in reduced growth in the centre of large farms, as well as impacting other organisms in the areas (Cranford *et al*., 2003; Jiang *et al*., 2019, M Moy, North Island Mussels Ltd, personal communication). High density can also alter competition regimes in the population, exacerbating the influence of dominant individuals (Begon *et al*., 2012).

In bivalve hatcheries, stocking density can have a significant impact on growth and survival, both of which are reduced above a threshold density, which is variable between species and hatchery practices (Ramos *et al*., 2021; Yan *et al*., 2006). Ramos *et al.* (2021) suggest a range of potential mechanisms for this, including reduced food availability due to competition between individuals. Similar issues arise in culture operations: for example the growth, survival and immune function of Noble scallop (*Chlamys nobilis*) are all reduced by high stocking densities in lantern nets (Liu *et al*., 2019).

Culture operations are not purely concerned about growth rates. The appearance and shape of animals, which can be significantly affected by culture practices, can also impact the market price of a product (Marshall and Dunham, 2013; Thomas *et al*., 2019). Growers of *M. gigas* in the intertidal zone of the Essex coast regularly ‘knock back’ oysters as they grow, by dragging chain netting over the stock. Oysters that undergo this treatment develop a deeper ventral valve in comparison to oysters that are left untouched during the growing process, and as a result are considered a more desirable product (G Baker, Colchester Oyster Fishery, personal communication).

Marshall and Dunham (2013) showed that high stocking density of *M. gigas* in a suspended (floating basket) culture system resulted in a reduced shell depth, and therefore reduced consumer desirability, of the oysters. Lower growth and survival were also seen at high

densities. The authors trialled the addition of clay or lava rock alongside the oysters, which resulted in reduced biofouling on the oysters in the system, reducing processing costs and further increasing the desirability of the product, with potential for an increased market value.

2.3 Response to Salinity

Salinity is the primary environmental factor influencing the structural and functional characteristics of estuarine organisms (Telesh and Khlebovich, 2010). Estuarine organisms are exposed to variations in salinity over every tidal cycle, with longer-term variations also occurring as a result of seasonal changes in precipitation patterns (Telesh and Khlebovich, 2010).

Oysters are euryhaline, tolerant of a wide salinity range, and broadly osmoconforming, unable to osmotically regulate their intracellular fluid (Shumway, 1977). The physiological optimum of *M. gigas* is between 22 and 35 salinity (Shatkin *et al*., 1997). Variations in external salinity result in an osmotic gradient across the cell membranes, rapid salinity changes and strong gradients lead to stress and in extreme situations mortality (La Peyre *et al*., 2013). *M. gigas* are able to manage the effects of changing external salinities to some extent, by regulating cell volume via amino acid concentration, but it is the limits of this ability that determine their physiological tolerance range (Bayne, 2017b).

The exact physiological mechanisms by which non-optimal salinities impact marine bivalves are not fully understood (Casas *et al*., 2018). Loss of metabolic enzyme activity and lysosomal membrane destabilisation have both been observed in bivalves exposed to low salinity (Ballantyne and Berges, 1991; Méthé *et al*., 2015; Moore *et al*., 1980). Intracellular ion and acid base regulation, as well as cellular metabolism, are also negatively impacted (Ballantyne and Berges, 1991; Moore *et al*., 1980).

Management techniques used in response to the Deepwater Horizon oil spill in 2010 led to low salinities (<3) throughout August in the Breton Sound off the coast of Louisiana, USA (La

Peyre *et al*., 2013). Typical salinities in the sound during this period ranged from 5 to 8, dependent on location, well outside the optimal range of the local oyster species, *C. virginica* (Casas *et al*., 2018; Dean and Paparo, 1983; La Peyre *et al*., 2013). The following year, significant flooding of the Mississippi River caused a similar magnitude, but shorter duration, low salinity event in the spring. La Peyre *et al*. (2013) studied the effects of these events on the *C. virginica* population in the sound. Recruitment, survival and growth in 2010 were significantly reduced, which the authors suggest was a result of the event's extended duration and high water temperatures (>25 °C) at the time.

The 2011 event had minimal impact on mortality and growth, although recruitment was again low, possibly as a result of the poor season in 2010 and declining stocks in the area. Levels of osmolarity (solute concentration) in oyster plasma did not match surrounding waters during the 2010 event, suggesting that the oysters closed their valves for an extended period during the event. By responding in this way the oysters would have reduced the impact of salinity shock, but at the cost of reduced feeding and gas/waste exchange opportunities, and possible asphyxiation. The authors suggest that this may have had a role in the high mortality. During the shorter 2011 event, oysters in the area appeared to osmoconform throughout (La Peyre *et al*., 2013).

Verdelhos *et al.* (2015) showed that two estuarine bivalves, *Scrobicularia plana* and *Cerastoderma edule* are similarly impacted by low salinities. Study animals exposed to a range of salinities (0 to 35) for 120 hours show 100% mortality below a critical salinity (5 and 10 for *S. plana* and *C. edule* respectively). No mortality occurs above respective salinities of 10 and 15. Activity levels, (quantified by assessing burrowing, siphon activity, foot muscle activity and response to mechanical stimuli) were also highly responsive to salinity levels, with peak activity occurring at a salinity of around 25, dropping off significantly with change in either direction for both species.

Salinity also has a significant impact on larval recruitment, survival and growth rates. The optimal salinity for *M. gigas* larvae is around 30, His *et al.* (1989) showed that growth rates of young (from fertilisation to 7 day old) larvae are significantly reduced below a salinity of 25 (Figure 2.2). Similar patterns are shown in other studies (His *et al*., 1989; Nell and Holliday, 1988; Pauley *et al*., 1988).

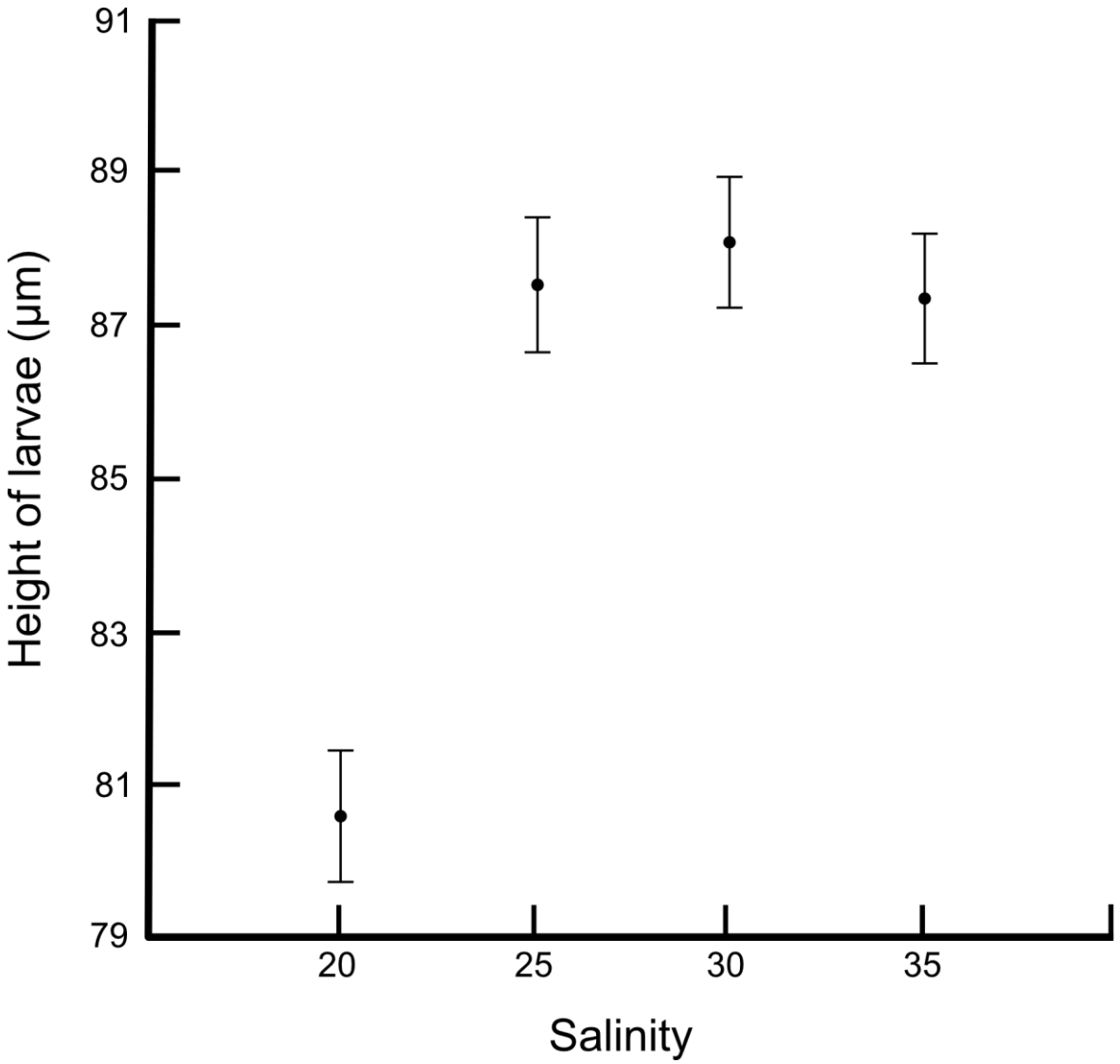

Figure 2.2. Mean size (height in µm ± 95% CI) of *M. gigas* larvae on the seventh day after fertilisation after exposure to 4 discrete salinity conditions. Modified from His *et al*. (1989)

2.4 Feeding Behaviours

Oysters filter large volumes of water when feeding, aggregating particles and providing an important pathway for particulate transfer from the water column to the benthos (Dame *et al*., 1984). Food resources that bivalves are able to exploit include: phytoplankton, resuspended benthic microalgae and bacteria (Cranford *et al*., 2005; Grant *et al*., 1997; Marín Leal *et al*., 2008).

Bivalve feeding behaviours are affected by a number of external factors. These include: the characteristics and quantity of the particulate matter in the water column (Bayne, 2002; Galimany *et al*., 2013; Higgins, 1980a, 1980b); algal blooms (Beninger and St-Jean, 1997; Comeau *et al*., 2012; Nagai *et al*., 2006); temperature and salinity (Galimany *et al*., 2017; Guzmán-Agüero *et al*., 2013).

Table 2.1 Parameters used to investigate and quantify feeding in bivalves and their definitions. (Galimany *et al*., 2017)

Parameter	**Definition**
Clearance rate	Volume of water cleared of suspended particles per unit time
Filtration rate	Proportion of particulate matter in the water retained on gills per unit time
Absorption efficiency	Proportion of organic matter removed from the water that is ingested
Particle rejection rate	Proportion of organic matter retained on gills that is then returned to the water

A number of studies have investigated the impact of salinity on various aspects of bivalve feeding, detailed in Table 2.1. The response of these parameters to salinity generally follows a typical environmental response curve, with rate and efficiency peaking at an optimal level (Casas *et al*., 2018; Galimany *et al*., 2017; Guzmán-Agüero *et al*., 2013).

Casas *et al*. (2018) used HFNI (high-frequency non-invasive) sensor technology to examine the impact of various constant salinities between 3 and 25 on the feeding behaviour of *C. virginica*, showing that the oysters spent more time with open valves, and had a greater amplitude of opening, at higher salinities. These differences were not significant, with only 4 individuals studied at each salinity, for only four hours following a feeding event. For oysters at salinities of between 6 and 25, time spent open during fed periods was lower (50-60% in

comparison to 93-95%) than shown by Higgins (1980a), possibly as a result of the lower feed concentrations used. Oysters in a salinity of 3 only opened for ~33% of the time, with smaller gape (degree of openness). This is a similar proportion to that shown in unfed oysters by Higgins (1980a), and possibly reflects basal metabolic requirements.

Galimany *et al*. (2017) showed that *C. virginica* in a shallow estuarine environment have a higher absorption efficiency in high salinity (~33) in comparison to mid salinity (~21) environments. Similarly, Casas *et al*. (2018) also found that clearance rates were positively correlated with salinity (within the range investigated), peaking at a salinity of 15 in winter conditions (17 ^{o}C) and at 15 and 25 in summer conditions (27 ^{o}C). This may, however, have been due to the organisms studied by Casas *et al.* having been taken from an environment with a relatively high salinity (~29) without a sufficient acclimation period, and the results therefore may be an acclimation artefact. Future work should ensure that a suitable acclimation process is planned into the methodology.

Higgins (1980a, 1980b) utilised strain gauges to record the openness (gape) of juvenile (~10g) *C. virginica*, showing that gaping behaviour is responsive to the levels of food available in the water. Under constant high feed (~5×10^4 cells ml^{-1}) conditions, the oysters were open for an average of 94.3% of the time, whereas unfed oysters were open 35.1% of the time. Oysters fed discontinuously (12 hours fed, 12 unfed) showed a similar pattern, open 95.7% of the time when fed and 60% when unfed, reacting within 30 minutes to the introduction of feed. Clearance rates during the experiment showed a significant variation, even during periods when valves were open, with higher clearance rates at the beginning of activity periods.

Gaping behaviours can also respond to the presence of non-food substances. Akoya pearl oysters (*Pinctada fucata*) respond to the presence of the toxic dinoflagellate *Heterocapsa circularisquama* (Dinophyceae, Myzozoa) by closing their valves (Nagai *et al*., 2006). Higher concentrations of *H. circularisquama* result in greater frequencies of closing, with prolonged

closures taking place at the highest experimental levels (100 cells ml^{-1}). The oysters returned to normal behaviour almost immediately once the dinoflagellate was removed. *C. virginica* clearance rates decrease in 'brown tide' conditions (harmful levels of the alga *Aureoumbra lagunensis*; Pelagophycea, Ochrophyta) (Galimany *et al*., 2017; Gobler *et al*., 2013). *A. lagunensis* is non-toxic, however the cells are covered in a mucous layer which may serve to reduce palatability or inhibit cilial transport (Galimany *et al*., 2017; Liu and Buskey, 2000).

2.5 Spawning Behaviour

M. gigas is a protandric sequential hermaphrodite (Amemiya, 1929). Young Crassostreinid oysters are predominantly male, with sex-change occurring as the oyster grows. Unlike oysters of the Ostreinae subfamily, this change is not typically reversed (Bayne, 2017c). The net result of this is an increasing percentage of females as cohorts age, from <30% in young oysters to >80% towards the end of their approximately 10-year lifespan (Dinamani, 1974; Lango-Reynoso *et al*., 2006). Variation in these ratios is driven by a number of factors including genetic differences, food availability and temperature (Guo *et al*., 1998; Quayle, 1988; Santerre *et al*., 2013).

M. gigas spawning is a seasonal event and tends to occur with a high degree of synchrony throughout a population (Bernard *et al*., 2016). The timing of spawning is predominantly driven by temperature (Helm *et al*., 2004; Lango-Reynoso *et al*., 2006; Mann, 1979; Robins *et al*., 2017; Syvret *et al*., 2008). Spawning condition is reached after between 350 and 600 cumulative degree days above a 'biological zero' (b^0) temperature, below which gonadal development ceases (Helm *et al*., 2004; Robins *et al*., 2017; Syvret *et al*., 2008). For *M. gigas* b^0 is between 8 °C and 12 °C, and was more precisely identified as 10.55°C by Mann (1979).

Lango-Reynoso *et al*. (2006) describe the seasonal pattern of *M. gigas* spawning on the French Atlantic coast. In this pattern, gamete generation takes place after summer spawning and the gametes remain dormant throughout winter. As temperatures rise through 13 °C and up to >21°C the oocytes grow and mature, after which spawning takes place. Oocytes not expelled during spawning are reabsorbed once temperatures fall below ~12 °C in the autumn. Other investigations show similar patterns with minor local differences in trigger temperatures. Bimodal spawning, where two events occur in the season, is not uncommon under poorer conditions (Dutertre *et al*., 2009; Enríquez-Díaz *et al*., 2008; Ubertini *et al*., 2017)

Other factors that influence the timing and magnitude of oyster spawning events include: phytoplankton abundance and community assemblage (Enríquez-Díaz *et al*., 2008; Ubertini *et al*., 2017); turbidity (Dutertre *et al*., 2009); photoperiod (Fabioux *et al*., 2005) and lunar phase (Ubertini *et al*., 2017). Aranda *et al*. (2014) show that *C. virginica* respond to environmental conditions by employing either a seasonal and synchronous or continuous and asynchronous spawning strategy. They hypothesise that the former strategy occurs in good conditions, whereas the latter is a more opportunistic strategy suited to poorer (e.g. food-limited) conditions.

Post-spawning larval duration, closely linked to survival, is also a function of temperature, while nutrition plays a further role (Helm and Millican, 1977; Syvret *et al*., 2008). Pauley *et al.* (1988) and Syvret *et al.* (2008) present a range of degree days to first recruitment in *M. gigas* larvae, ranging from 224 to 350, although Syvret *et al.* (2008) question the higher value. Low temperatures have a significant impact on overall recruitment, with values of below 15 °C resulting in a marked decrease in settlement.

2.6 Disease

The exposed nature of most culture sites and potential for rapid spread of pathogens in the aquatic environment means that prediction, management and control of infectious diseases in an aquaculture setting is challenging (King *et al*., 2019; McCallum *et al*., 2003). Disease threats are considered to be greater at lower latitudes due to the associated higher temperatures, therefore warming waters have the potential to increase the magnitude of the threats (Harvell *et al*., 2002; Leung and Bates, 2013). The vulnerability of an oyster stock to pathogenic threats is a result of a complex suite of biotic and abiotic factors, summarised in Figure 2.3.

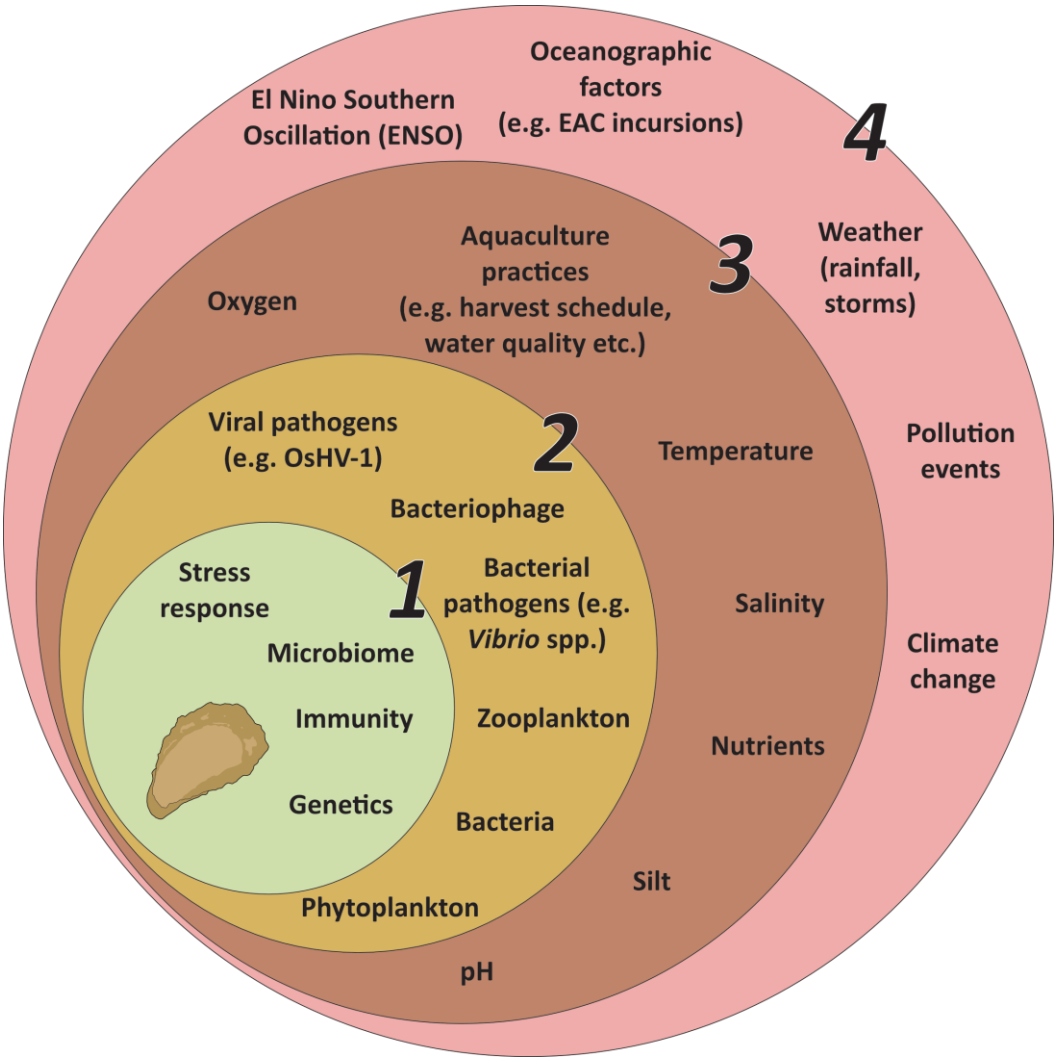

Figure 2.3 King *et al*.'s (2019) summary of the synergistic factors causing oyster disease. (**1**) The host's innate reactions and ability to combat disease. (**2**) The biotic elements of the environment. (**3**) Local scale abiotic factors that influence both 1 and 2. (**4**) Large/medium scale environmental changes which impact all other factors.

King *et al.* (2019) summarise the primary disease threats to cultured *M. gigas* (Table 2.2). Of most concern to European culture operations are Vibriosis and Ostreid herpesvirus-1 (OsHV-1).

Vibriosis is a bacterial disease, caused by a number of different *Vibrio* species (King *et al.* 2019). Whilst the condition primarily affects larvae and spat it has been shown to induce mortality in adults under experimental conditions (Go *et al*., 2017). *Vibrio* infections are also commonly found alongside OsHV-1, with some authors suggesting a degree of synergy between the two (De Lorgeril *et al*., 2018). *Vibrio* species are ubiquitous in the aquatic environment (Hsieh *et al*., 2008). Infection therefore tends to be opportunistic, as in the suggested synergy with OsHV-1, occurring only when the oyster immune system is suppressed as a result of environmental stress or disease challenge (De Lorgeril *et al*., 2018; King *et al*., 2019). King *et al.* (2019) suggest that the bacteria may occur naturally in the oyster microbiome, causing a problem only under stress.

Given the ubiquity of *Vibrio* species, management and prevention of vibriosis is challenging for oyster culture operations. Monitoring of conditions, forecasting of likely outbreaks and removing oysters if possible may aid in prevention, as will ensuring that the overall health of the stock is high enough to resist infections (King *et al*., 2019)

OsHV-1 is the most commercially significant viral disease of oysters. It has been connected with multiple large mortality events internationally, with increasing levels of incidence under modern temperature regimes and the emergence of new varieties (Friedman *et al*., 2005; Mortensen *et al*., 2016; Renault *et al*., 2014; Segarra *et al*., 2010). Other viruses that can affect oysters include irido-like viruses which cause gill necrosis and oyster velar virus disease which effects which can infect hatchery reared spat (Renault and Novoa, 2004)

Outbreaks of OsHV-1 occur above 18 °C for most strains of the disease (Petton *et al*., 2015; Renault *et al*., 2014). In the last decade a novel strain (OsHV-1 µVar) has become more

widespread, outbreaks of which can occur at 16 °C (Petton *et al*., 2013). The disease disproportionately affects spat and young oysters, which is possibly a function of size not age (de Kantzow *et al*., 2017; Dégremont, 2013; Hick *et al*., 2018).

The route of transmission and reservoirs of OsHV-1 are not fully understood. It is currently thought that previously infected individuals may act as reservoirs, possibly long after they appear to be recovered (Evans *et al*., 2017; Whittington *et al*., 2018). Whittington *et al.* (2015b) suggest that transmission between oysters relies on a particulate vector as very fine (5 µm) filtration and the use of settling tanks to reduce particulate content significantly reduces mortality in 2.5 to 6.5 month old spat. Epidemiological analysis of outbreaks in Australia suggest that plankton is the vector (Paul-Pont *et al*., 2013).

Relatively successful mitigation of OsHV-1 has been accomplished in a number of ways. Resistant strains have been developed that show significant reductions in mortality, which is promising for operations relying on hatchery produced spat (Dégremont, 2011, 2013; Dégremont *et al*., 2010). In an enclosed or semi-enclosed system, such as a hatchery, the above mentioned impact of suitable water filtration can also offer a degree of protection (Whittington *et al*., 2015b).

Petton *et al.* (2013) show that exposure to low temperatures (13 °C) for 40 days can mitigate the effect of OsHV-1, although this may prove challenging to achieve in a commercial culture operation. Perhaps more realistic is the suggestion that a reduction in handling, particularly of juvenile oysters, during periods of greatest OsHV-1 threat, may result in reduced stress and therefore greater immune function (de Kantzow *et al*., 2017; Peeler *et al*., 2012). Peeler *et al.* (2012) suggest that a reduction in immersion time, and therefore potential exposure to the pathogen, may aid in mortality reduction, although this would also result in reductions in feeding and therefore growth.

Table 2.2: Summary of the major disease threats to cultured *M. gigas*, the affected life stage, pathology and geographical distribution. After King *et al.* (2019)

Disease/pathogen (agent)	**Affected life stage**	**Pathology**	**Geographical distribution**	**Mortality range (%)**
Denman Island disease/*Mikrocytos mackini* (Protozoan)	Adult	Green pustules, ulcers and abscesses on oyster tissues	USA Northwest Coast and Canadian Southwest Coast	17-53
Nocardiosis/*Nocardia crassostreae* (Bacterium)	Adult	Green pustules and lesions on oyster tissues	USA Northwest Coast and Canadian Southwest Coast	47-50
Vibriosis (Bacillary necrosis)/*Vibrio* spp. (Bacterium)	Larvae, spat	Abnormal swimming, necrosis, lesions	Worldwide	79-100
Pacific Oyster Mortality Syndrome/ OsHV-1 and OsHV-1 µ variant (Virus)	Larvae, spat	Lesions and cells with viral inclusions and hypertrophied nuclei. Reduced feeding and impaired swimming in larvae	USA East Coast, Australia, New Zealand, France, Sweden and Norway	40-100
Summer Mortality/Unknown or multifactorial	All stages	Ill defined, characterised by high level mortalities during the warmer months	USA, France, Australia, Japan, Germany, Ireland, Sweden and Norway	30-100

3 Colchester Oyster Fishery

3.1 Harvesting Procedures

Of interest to this review are the processes in use at COF. The fishery has exclusive rights to the bed of the Colne Estuary on the South Essex coast (Figure 1.4). Oysters are grown to harvest size on ‘layings’, marked areas of the seabed that are managed to ensure a suitable environment for culture of market quality oysters. This involves regular turning over of the seabed, either by dredging or harrowing. This serves to remove fine sediment and ‘knock back’ the oysters, encouraging thicker shell growth and a deeper shape, which are desirable qualities for the consumer.

Harvesting on the farm takes place either by dredging or hand picking at low tide. Dredging is a non-discriminate method, necessitating that the crew sort the catch on deck, selecting suitably sized oysters (and other organisms present in the estuary such as the Hard-shell Clam (*Mercenaria mercenaria*) and Palourde/Manilla Clam (*Ruditapes philippinarum*) and returning the rest of the material to the laying.

3.2 Larval Retention

The oyster population in the estuary spawns naturally each summer. Maximising retention of the resultant larvae reduces COF’s outlay on seed. In order to do so, the layings are prepared with ‘cultch’, shell and gravel material which attracts the larval oysters when they are ready to settle (Laing and Bopp, 2019). Optimal timing of cultching is key, too late and the larvae will settle outside the layings or perish, too early and the cultch will be covered up or washed away (P Harding, Colchester Oyster Fishery, personal communication, Luckenbach *et al*., 1999; Morales-Alamo and Mann, 1990).

Correct timing of cultching is particularly key in *M. gigas* culture given the seasonal pattern of spawning. In the past, environmental cues, such as blossoming of chestnut trees, have been used to predict when best to lay cultch, (F Gouzer, personal communication). However, the

use of indirect cues was likely never to have been particularly accurate, and as global temperature regimes become more variable under climate change will only become less so. Monitoring of the gonadal condition of the stock and larval counts of local water can inform the decision, although these require lethal sampling in the first instance and are not species specific in the second without advanced methods being employed. The use of technology, as discussed in Chapter 3, to replace these methods would allow culture operations to gather more accurate, species specific, information whilst avoiding the time costs and stock loss currently experienced.

3.3 Spat Ongrowing

The majority (>90%) of oysters sold by COF are originally sourced from hatcheries as small ‘seed’ oysters. This represents a significant capital cost, so it is key that growth rates and survival are maximised. Growth uniformity is also an important factor, seed stocks with uneven growth patterns result in significantly more work for farm crew (G Baker, Colchester Oyster Fishery, personal communication).

COF’s standard procedure for ongrowing of bought in oysters is to house the seed in large cages that are placed around the Colne and Pyefleet, in which the oysters grow until they are a suitable size (>2 g) to be distributed on the fishery’s layings (Figure 3.1A). In some locations large barrels are used to float the cages, but the majority are sunk to the riverbed. Mesh bags, made from the largest possible mesh for the size of oyster, are used to house the oysters (Figure 3.1B). Where necessary, the seed are spread out between bags after a period of growing. This allows transfer to a larger mesh if appropriate and offers an opportunity to assess the seed, but is labour intensive and often requires that the seed are returned to shore for processing. This method has been used for many years and is broadly effective, but has several inherent drawbacks.

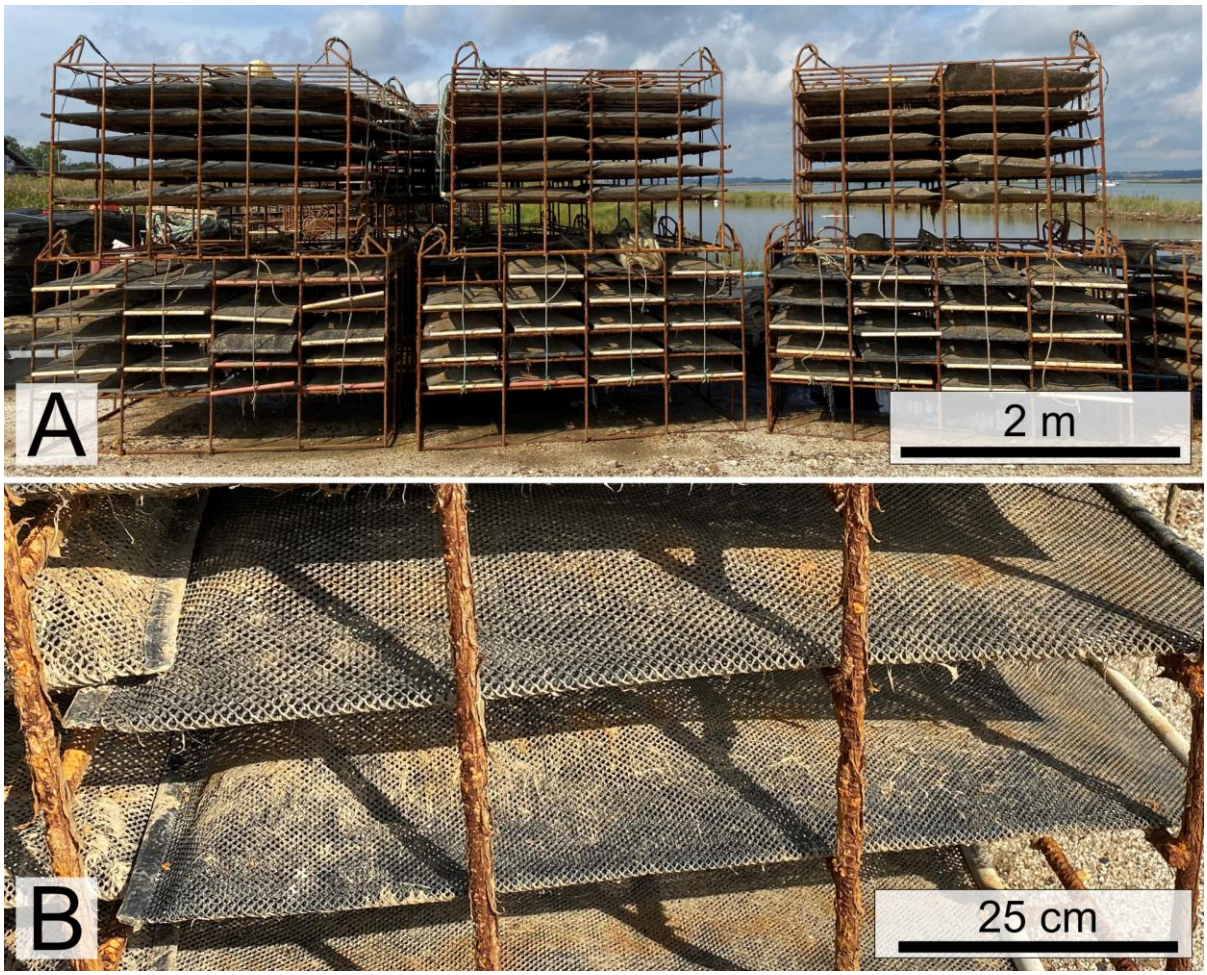

Figure 3.1 The cages (**A**) and mesh bags (**B**) used as part of the current seed ongrowing protocols at COF. The cages are constructed of steel 'rebar' by staff at the fishery. Bags made of two different sized mesh are shown in image B, these are selected to maximise mesh size, resulting in higher water flows, whilst not allowing oysters to fall out.

High water quality and consistent food supply are required to optimise seed growth rates. However, the mesh sacks are prone to clogging with pseudofeces, settled sediment and fouling organisms, which substantially reduces water flow, and therefore food supply, through the bags and crowds the oysters. This limits growth, often forcing them to grow into the mesh, deforms shells and can even suffocate them. Using larger mesh sizes mitigates this to a degree, but limits the minimum size of seed that can be bought in without losing it through the mesh. It is possible to minimise the impact of clogging with regular cleaning, but typically it becomes limiting once oysters reach 3 to 4 grams (G Baker, Colchester Oyster Fishery, personal communication).

Cleaning requires a large boat with a deck-mounted crane to lift the cages, each of which contains over 50 bags of oysters when fully stocked. Several crew are required to remove and clean the bags, a process that takes a full day when seed stocks are high, reducing harvest opportunities substantially. A high pressure hose is used to wash the accumulated sediment off the oysters, which likely induces a substantial handling stress in the oysters. In peak season cleaning should be done once a week, although operational pressures regularly prevent the crew from doing so.

Placing the cages in the estuary also exposes the oysters to threats associated with the natural environment, including pathogenic, environmental and predatory challenges. Similarly to the clogging issue, this limits the minimum size of the seed that can be used, as larger (>1 g, 10-15mm long) seed are typically more resilient to these threats (Hick *et al*., 2018; Paul-Pont *et al*., 2013).

Larger seed have a higher unit cost, so stocking the cages is a compromise between limiting capital outlay, maximising growth and survival and reducing labour costs where possible. Seed supply is also not totally reliable, as hatchery issues can result in seed arriving that is not precisely as ordered (P Harding, Colchester Oyster Fishery, personal communication).

Land-based systems offer an alternative to the cages as currently used, offering protection from some natural threats, reducing the time and energy costs of maintenance and increasing ease of access. Chapter 4 tests the feasibility of using such systems in a commercial bivalve aquaculture setting.

3.4 Depuration

The relatively non-discriminate feeding behaviour and typically raw consumption of oysters means that they present a greater risk of illness than most other food, although it is the gut contents, rather than the flesh itself, that present risk to the consumer (Leal Diego *et al*., 2013; Lee *et al*., 2008; Martinez *et al*., 2009). A list of the potentially pathogenic organisms

that can be transmitted to the consumer is provided in Lee and Younger (2002), primary threats include the noroviruses and *Escherichia coli*. United Nations Food and Agriculture Organisation (FAO) guidelines are that relatively simple purification procedures, commonly referred to as depuration, are sufficient to reduce this risk to an acceptable level (Lee *et al*., 2008). In areas where shellfish is harvested from pristine or near pristine waters there is no need for any depuration (Lee *et al*., 2008).

There are three classifications of UK waters where shellfish are harvested; A, B and C, which are classified firstly by levels of *E. coli* in the live flesh of the shellfish. Shellfish from Class A waters can be harvested for direct human consumption. Those from Classes B and C require either relaying in a cleaner area, cooking or depuration (The Water Environment (Water Framework Directive) (England and Wales) Regulations, 2017). The area in which COF grow their oysters is classified as Class B, and therefore they are required to undergo depuration.

Depuration typically involves keeping harvested shellfish in recirculating water at a suitable temperature and salinity for a defined period of time (42 hours under current UK regulations), during which the water passes through ultra-violet (UV) or ozone filtration (e.g. **Error! Reference source not found.**). The filtration system sterilises the water passing through it, removing the pathogens and rendering the harvest safe for human consumption (Lee *et al*., 2008). The process relies on the shellfish being active for a sufficient proportion of the time they are in the system for any potentially harmful substances or organisms in their gut to be expelled and filtered from the water. In the UK the specifics of the depuration process are set out in the Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998 (The Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations, 1998)

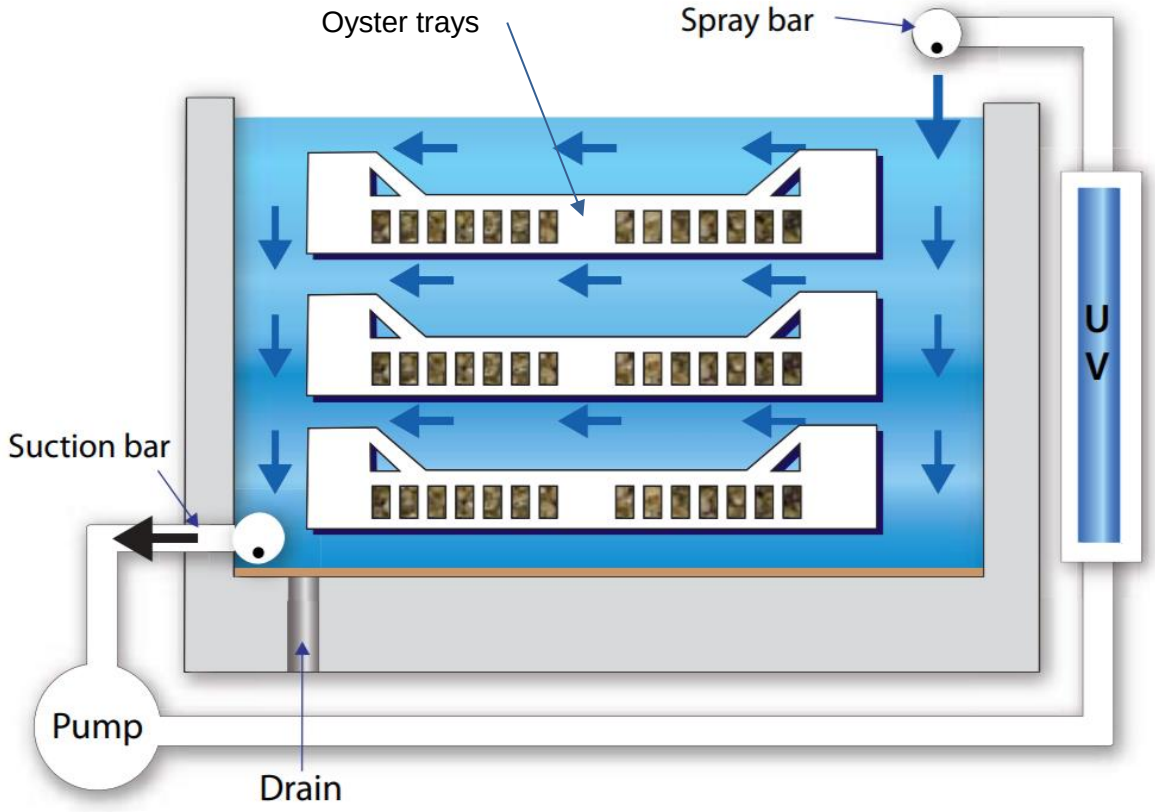

Figure 3.2: The FAO example depuration unit, in which a suitable biomass of shellfish are stored on trays for purification. Clean water is introduced at the start of each cycle, and continually recirculated through an ultraviolet filter, neutralising any pathogenic threats from the wild-grown harvest (Lee *et al*., 2008).

4 Animal Monitoring and Sensor Technology

4.1 Monitoring in Aquaculture

Monitoring of animal stocks is a crucial part of the farming process (Segner *et al*., 2012). Well designed and implemented monitoring programs allow producers to make informed decisions about a myriad of factors that can influence the health, wellbeing and quality of their stock, ultimately having a significant impact on the output of an operation. In aquaculture, however, there are significant impediments to this. The animals spend a large proportion of their lives out of sight under the surface of the water, sometimes in the benthos. Accessing the culture site can be expensive, often requiring use of a boat and is challenging in poor weather. Furthermore, the flesh of bivalves is encased between hard closable valves, as a result of which bivalve stock monitoring has historically required direct disturbance of animals (Andrewartha *et al*., 2015; Lucas and Beninger, 1985)

Even non-invasive sampling events can result in a degree of handling stress, and repeated measurements of individuals or populations have potential to impact on the accuracy of the data (Segner *et al*., 2012). In many instances, such as checking bivalve gonadal development to assess spawning condition, lethal sampling is required. Growth monitoring of rope-grown mussel stocks involves the removal of stock from the ropes, after which they are unable to reattach and are therefore lost as a product (M Moy, North Island Mussels Ltd, personal communication). Spawning condition analysis of *M. gigas* requires opening of the animals to allow visual inspection of the gonads. Monitoring is also a time-consuming process, presenting a cost to producers in both labour hours and lost stock. Consequently, a compromise is necessitated between gathering of useful information from a representative number of animals and time points, and the costs of doing so.

In recent years remote sensing technology has become an increasingly common tool for monitoring of animal stocks, in both agri- and aquacultural settings (Chopra *et al*., 2020; Kramer and Foekema, 2001; Rutten *et al*., 2013). Gape sensors monitor the distance

between valves as a proxy for bivalve openness and are the most commonly used in the study and monitoring of bivalves. Their use, known as valvometry, has become increasingly widespread in recent years, and inferences about a wide range of behaviours can be made from the data (Section 4.3).

In valvometry studies, sensor attachment is the only part of the process requiring disturbance of the subject animal and the devices are typically small enough that once attached they present no impediment to an animal's behaviour. The systems are capable of recording at relatively high frequencies (up to 10 Hz) over extended (multiple months up to years) deployments with remote data transmission (Ballesta-Artero *et al*., 2017; Sow *et al*., 2011). Use of sensors therefore eliminates handling-associated stresses and provides a long term, high frequency dataset for each attached animal, and as such they are often referred to as High Frequency, Non-Invasive (HFNI) systems.

4.2 Gape Sensing Approaches

There are a range of methods that can be used to record gaping, some of which are summarised here. Comprehensive histories are available in Kramer and Foekema (2001) and Fox (2022).

Accounts of bivalve gape sensing studies date back to the early 20th century, when smoked glazed paper was used to trace the openness of bivalves (Marceau, 1909). A similar approach was used by Galtsoff (1961) who attached *C. virginica* to a kymograph, a mechanical device with a rotating drum that records movement over time, to record spawning behaviours. Higgins (1980a, 1980b) studied *C. virginica* gaping behaviours by gluing animals down and fixing a strain gauge to their dorsal valves which allowed their gaping behaviour to be electronically recorded.

More modern equipment began to be developed in the late 20th century. One of the first of these systems mentioned in the literature was the Dreissena-Monitor which was first used

circa 1992 to monitor the behaviour of Zebra mussels (*Dreissena polymorpha*). (Borcherding, 2006) The system uses reed switches and magnets attached to up to 42 individual mussels split between two flow channels, one with natural, potentially contaminated, water and the other with water of a known high quality. An on-board computer classifies each mussel as open or closed every second, and a running average of the number of open mussels in each channel is calculated. The system compares the two channels, with alarms raised if the difference between them is above a previously determined threshold value.

The reduction of gaping behaviour to a binary value suits the purpose of systems like the Dreissena-Monitor but does not allow for examination of more nuanced behaviours. The MosselMonitor, and Valvomètre are two systems, both first used in the early 2000s, that utilise electromagnetic coils glued to a bivalve to semi-continuously record the distance between valves. A high frequency current passed through one coil generates a magnetic field, which in turn generates a current in the second coil of proportional strength to the gape of the subject (Kramer and Foekema, 2001; Tran *et al*., 2003).

Use of Hall-Effect sensors is another approach in gape sensor design, one which was taken when designing the NOSy (Non-Invasive Oyster Sensor) system, a primary data collection tool for this thesis that is examined in detail in Chapter 2. Hall-Effect sensors are a form of variable resistor in which the voltage that passes through is a function of the local magnetic field strength (Ramsden, 2006). Hall-Effect based systems therefore allow for the use of passive magnets on one valve of the subject animal. Similar sensors were in use in the 1990s to examine bird pecking (Allan, 1992) and systems of this kind began to be used in bivalve study in the early 2000s (Moody, 2003). Measurement frequency and data resolution are comparable between the two approaches to magnetic sensor design, but Hall-Effect sensors are the most commonly used approach in the field of valvometry (Fox, 2022).

Non-magnetic sensing approaches include fibre-optic sensors (Frank *et al*., 2007), 3D-printed strain gauges (Wu *et al*., 2022), accelerometers (e.g. Valve-Trek, TechnoSmArt, Rome, Italy) and video recording (de Vargas Guterres *et al*., 2020). These approaches may become more widespread in future, but as it stands their use is substantially less common.

4.3 Uses of Gape Data

Gape data can be used to study a diverse range of bivalve behaviours, an illustrative but not exhaustive list of examples is provided in Table 4.1.

Table 4.1: Example uses of valvometric data from the literature, detailing the study species and research topic.

Study Species	Research Topics	Authors
Akoya pearl oyster (*Pinctada fucata*)	Detection of harmful algal species.	Nagai *et al*., 2006
Asiatic clam (*Corbicula fluminea*)	Use of bivalves as a biosensor for cadmium detection	Tran *et al*., 2003
Blue mussel (*Mytilus edulis*)	Copper exposure impacts on gaping behaviour.	Curtis *et al*., 2000
	Annual growth patterns	Tran *et al*., 2020
	Feeding behaviour and predation risk.	Robson *et al*., 2010
	Oil dispersant impact quantification	Durier *et al*., 2021
	Detection of harmful algal blooms	Durier *et al*., 2022
Eastern oyster (*Crassostrea virginica*)	Salinity and seasonal impacts of clearance rates	Casas *et al*., 2018
	Siltation impacts on gaping behaviours.	Poirier *et al*., 2021
	Feeding responses to food availability levels.	Higgins, 1980a, 1980b
	Hypoxic response	Porter and Breitburg, 2016
Giant clam (*Hippopus hippopus*)	Long-term growth monitoring in relation to temperature regime.	Schwartzmann *et al*., 2011

Study Species	Research Topics	Authors
Icelandic scallop (*Chlamys icelandica*)	Diurnal behaviour patterns.	Berge *et al*., 2015
	Annual growth patterns.	Tran *et al*., 2020
Mediterranean mussel (*Mytilus galloprovincialis*)	Circadian gaping behaviours on a long-line mussel farm	Comeau *et al*., 2018
	Predator response behaviours	Clements *et al*., 2020
Ocean quahog (*Arctica islandica*)	Environmental regulation of gaping activity.	Ballesta-Artero *et al*., 2017
Pacific oyster (*Magallana* (*Crassostrea*) *gigas*)	Bio-monitoring in an aquaculture setting.	Andrewartha *et al*., 2015
	Automated spawning detection	Ahmed *et al*., 2016
	Quantification of stress impacts	Ahmed *et al*., 2017
	Water quality assessment	Sow *et al*., 2011
	Assessment of the synergistic impacts of heatwaves and toxic algae	Funesto, 2023
	Investigation of submarine noise impacts	Charifi *et al*., 2017
Razor clam (*Ensis leei*)	Siltation impacts on growth dynamics.	Witbaard *et al*., 2012
Sea scallop (*Placopecten magellanicus*)	Feeding responses to flocculation and sedimentation	Cranford *et al*., 2005
Zebra mussels (*Dreissena polymorpha*)	Water quality monitoring	Borcherding, 2006

4.4 Bivalves in Sensor Networks

Biological early warning systems (BEWS) utilise an animal's ability to sense and react to potentially hazardous environmental changes. BEWS are a familiar concept, perhaps the most well-known illustration being the canary in the coal mine. Vereycken and Aldridge (2022) suggest that bivalves are almost uniquely well suited for use in aquatic BEWS due to their sessile nature, widespread and high abundance and high bioaccumulation ability. In effect, they can function as broad spectrum, highly sensitive, water quality sensors. Typically speaking, prolonged and synchronised closures in a population indicate a potential water quality concern (Barile *et al*., 2016).

The Dreissena-Monitor (section 4.2) was developed as one such system, and as of 2006 was deployed at 13 sites on the Rhine and Elbe in Germany. Several municipalities employ an array of freshwater bivalves, typically clams or mussels, as a first line of defence against poor water quality (McGuire, 2019; Micu, 2023). One example is the Gruba Kaśka (Fat Kathy) water pumping system in Warsaw, Poland. This system pumps water from the Wisla, a river that flows through an area with a history of heavy industry, and is therefore at particular risk of heavy metal contamination. Eight clams are attached to a sensing system (Figure 4.1) in the water intake of the pumping system and when four or more close the pump is shut off, preventing ingress of contaminated water to the municipal supply (Pelka, 2019; Micu, 2023).

Andrewartha *et al.* (2015) tailor the BEWS concept for aquaculture. They suggest that the deployment of sensors onto a small number of 'sentinel animals' within a stock can provide information about the stock's well-being and environmental responses, allowing informed decisions on stock management to be made. Similar networks have been suggested as a tool for in situ monitoring of wild populations, where the potential for long-term remote assessments of bivalve populations could substantially increase the resolution of available water quality data (Andrade *et al*., 2016)

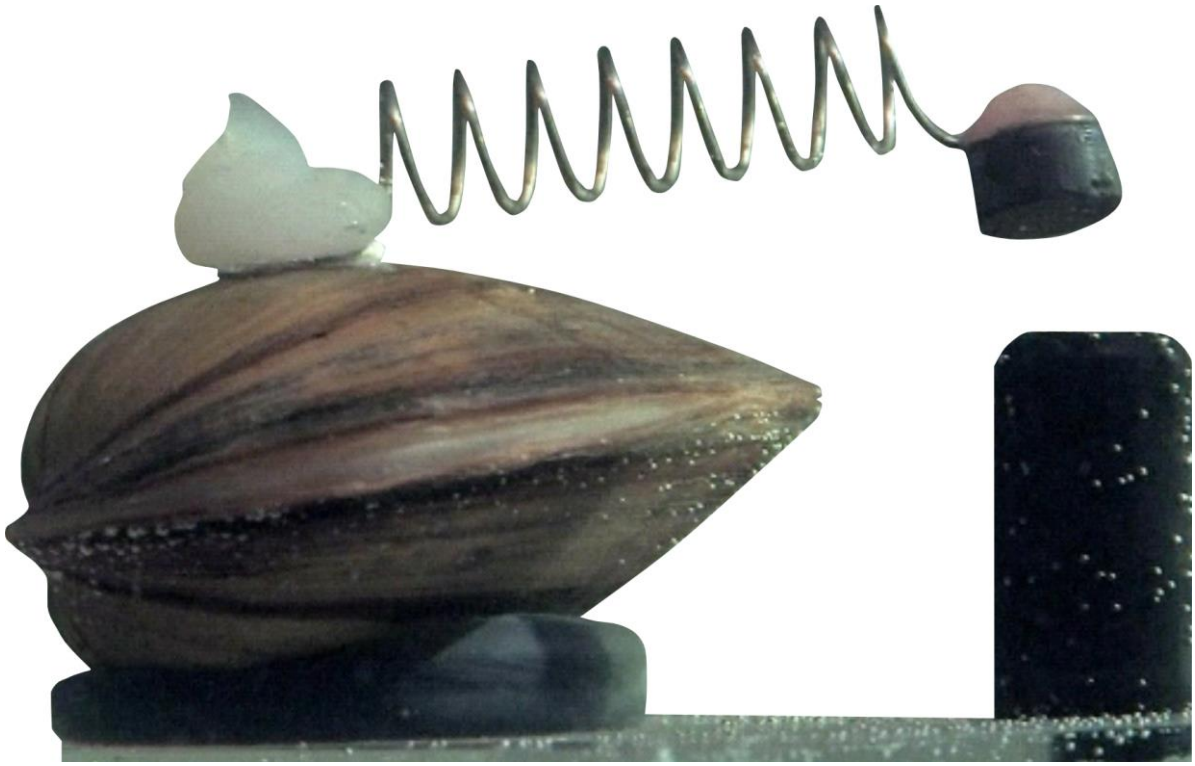

Figure 4.1: A freshwater clam attached to a magnet as used in the ‘Fat Kathy’ municipal water pumping system. An array of eight clams are attached at any one time and situated in the water intake. If more than four clams close at any one time it is assumed that this indicates excessive toxin levels in the water and the pump is switched off whilst further investigations take place (Micu, 2023).

The utility of bio-sensor networks is dependent on the quality of the sensors and ability to interpret the data. As discussed above, sensor technology has advanced considerably in recent decades, but the challenge of precisely characterising gaping behaviours as a function of their cause is considerable. Some behaviours, such as spawning in *M. gigas* and the response of the freshwater clam *Corbicula fluminea* to mercury, are highly characteristic and relatively simple to detect (Ahmed *et al*., 2016; Tran *et al*., 2007), but responses to contamination or stress in bivalves are often less obvious. The use of bivalves in municipal water systems for example uses a precautionary interpretation of the data, closing supply down when data suggests there may be an issue without requiring a precise identification of the nature of the problem.

With further work it may be possible to refine analysis protocols to a point where the precise cause of behavioural perturbation can be identified. Substantial efforts have been made to identify bivalve gaping responses to many potential stressors, including but not limited to; toxic algae (Bertolini *et al*., 2022; Durier *et al*., 2022; Funesto, 2023), heavy metals (Tran *et al*., 2007, 2003) and heatwaves (Clements *et al*., 2018; Funesto, 2023). Vereycken and Aldridge (2022) state that significant further work in this field is required. Truly high quality BEWS systems are likely to involve the integration of other sensing technologies, such as cardiac monitors, and use a multi-specific array of bivalves. Nevertheless, there is a high degree of potential for the use of bivalves as biomonitors, and valvometry will play an important role.

5 Climatic/Environmental Changes and Challenges

Aquaculture relies on suitable environmental conditions for success. Bivalve aquaculture, dependent on the environment for food supply and with sessile stock, is particularly vulnerable to deviation from these conditions. Short term challenges, such as algal blooms, and more chronic issues such as climate change present notable potential problems for successful culture operations (Galappaththi *et al*., 2020).

The most recent Inter-Governmental Panel on Climate Change summary report predicts that the global mean surface temperature will rise by between 1.4 °C and 4.4 °C by the end of the 21st century (IPCC, 2023). An increase in the frequency of terrestrial and marine heatwaves, greater numbers of extreme storm events and considerable alterations in precipitation patterns will also occur in the same time frame (IPCC, 2023; Oliver *et al*., 2019). These changes, among others, have several potential direct impacts on bivalve aquaculture, summarised below.

5.1 Temperature Increases

All organisms have an optimal thermal condition, with a thermal tolerance range above and below this (Bayne, 2017d). Rising global temperatures and an increased frequency of heatwaves will therefore have major ecological impacts, particularly on organisms already inhabiting areas at the high end of their tolerance or which are adapted to a narrow thermal range (Pearson and Dawson, 2003; Walther *et al*., 2002).

Ferreira *et al*. (2008) suggest that *M. gigas* production will be comparatively resilient to the effects of climate change, particularly as many of the areas it has been introduced to are cooler than the species' native range. However under a 4 °C warming scenario their models still forecast reduced productivity, weight and size of farmed oysters. While the thermal tolerance range of farmed bivalve species is relatively wide, a sustained increase in sea temperatures will increase the vulnerability of stocks to extreme events such as heatwaves (Rodrigues *et al*., 2015). Heatwaves themselves can significantly alter the behaviour and reduce the fitness of bivalves (Funesto, 2023; Peruzza *et al*., 2023). For oysters cultured in the intertidal, air exposure at low tide further complicates the thermal regime, with factors such as evaporative cooling and radiative heating playing varying roles depending on organism size and the prevailing conditions (Helmuth, 1998).

5.2 Changes in Precipitation Patterns.

Climate change is forecast to dramatically alter global precipitation patterns. Summers will become drier, with more drought events, whilst storm events will increase in number and produce more intense precipitation, resulting in an increase in flooding (Trenberth, 2011). Higher peak flood discharges cause increases in turbidity, nutrient loads and contamination (Callaway *et al*., 2012). Flood events are frequently associated with bypassing of standard sewage treatment works, although bypass is becoming an increasingly common practice around the UK coast in the absence of extreme conditions (BBC, 2023). Nutrient peaks resulting from raw sewage discharge can cause a variety of harmful effects, including hypoxic/anoxic events, zoonotic disease outbreaks and habitat degradation (Howarth, 2008).

Bivalve aquaculture faces a further threat from sewage-related pollution as it is typically associated with peaks in diseases such as norovirus, requiring further purification of harvested stock or even fisheries closures in extreme cases (Miossec *et al*., 2000).

Flood events can cause rapid salinity changes in the estuarine environment (La Peyre *et al*., 2013; Telesh and Khlebovich, 2010). As previously discussed (section 2.2), this type of salinity change has the potential to induce stress, inhibiting growth and immune function of the stock and potentially causing mortality. Increasing rates of flooding may therefore create an additional pressure to estuarine aquaculture operations, placing further restraints on site and stock selection.

5.3 Pollution

There are a number of historic and current pollutants that impact estuarine ecosystems. Notable are agricultural runoff and persistent metallic pollutants from industrial and marine applications (Alzieu, 2000; Antizar-Ladislao, 2008; Carmichael *et al*., 2012a; Matthiessen, 2019, 2013).

Nutrient enrichment, primarily from agricultural runoff, can result in eutrophication events in coastal waters. The resultant increase in algal production, organic matter accumulation and increased nitrogen (N) concentrations in water and sediment can lead to widespread anoxic events, and major ecosystem damage (Carmichael *et al*., 2012a; Cloern, 2001; Desprez *et al*., 1992). Eutrophication events in the Bay of Somme in Northern France during the 1980s caused high mortality in local benthic bivalve communities, resulting in a collapse of the local cockle fishery (Desprez *et al*., 1992). However, in less extreme eutrophic events, the increased food supply can result in increased bivalve growth and, as mentioned in Section 1.2, bivalve feeding can result in high levels of nitrogen drawdown, reducing the impact of nutrient enrichment (Carmichael *et al*., 2012b; Kirby and Miller, 2005; Lindahl *et al*., 2005).

Persistently high runoff, or a high frequency of point events such as flooding, can result in previously productive shellfish waters becoming unsuitable for harvest. In some instances this is due to the stocks being unable to survive in contaminated areas, but this can also be as a result of the increased processing and purification costs associated with shellfish harvest from non-pristine waters (H Moore, Agri-Food and Biosciences Institute, personal communication).

5.4 Storm Damage

Estuarine environments are particularly vulnerable to marine flooding (Chaumillon *et al*., 2017). As such, the increase in storm activity that is forecast to result from climate change will have a disproportionate impact on some key areas for bivalve aquaculture, resulting in infrastructure damage, loss of stock from the seabed and increased stock stress (Callaway *et al*., 2012; Epstein, 2001; Mölter *et al*., 2016). Non local storm events can also cause damage via increased freshwater flows, as mentioned in the previous section (5.2) this has the potential to cause a number of issues in bivalve populations.

5.5 Disease and Harmful Algal Threats

Sea temperature increases will have an indirect impact on bivalve stocks through alterations in the abundance of, and susceptibility to, pathogenic threats. Turner *et al*. (2016) show a significant interactive effect between climate change effects and bivalve immune response in the Asian green mussel *Perna viridis*. Likely climate change scenarios and exposure to harmful organisms amplify the effects of both. The authors further show that exposure to two harmful organisms serves to reduce the impact of both, highlighting the complexity of immune-environment interactions, even in a controlled laboratory setting.

In general it is expected that the increased sea temperatures, altered salinity and variation in water chemistry associated with climate change will increase the prevalence of, and therefore infection risk from, bacterial and viral pathogens (reviewed in King *et al*., 2019). As

discussed in section 2.6 the mortality caused by OsHV-1 is temperature dependent, and is therefore likely to become a greater threat under warmer temperature regimes.

Strategies are available to combat the threat: selective breeding for OsHV-1 resistance has proven successful in reducing mortality (Dégremont, 2011; Dégremont *et al*., 2010, 2007); culture practices such as increasing emersion times have been shown to reduce the risk of infection at the cost of reduced growth (Evans *et al*., 2019; Peeler *et al*., 2012) and various water treatment methods can prevent juveniles in a hatchery environment being exposed to disease (Paul-Pont *et al*., 2013; Whittington *et al*., 2015b)

Harmful algal blooms (HABs) present an additional threat to bivalve populations. HABs consist of a variety of algal species, some of which produce toxic compounds that can affect both shellfish and human consumers, with effects on humans including paralysis, diarrhesis and amnesia (Castrec *et al*., 2019; Hallegraeff, 2004). It is expected that climate change, as well as water quality issues, will bring about a change in algal community composition and increased prevalence of harmful algal blooms around the British coast (Hinder *et al*., 2011; Wells *et al*., 2015). Management and monitoring of this issue is likely to be problematic around Britain due to the complex shoreline morphology and regular cloud cover that precludes effective satellite monitoring, biosensor networks may be a useful tool in this effort (Callaway *et al*., 2012; Vereycken and Aldridge, 2022).

6 Contribution to Scientific Knowledge

This overarching aim of this thesis was to develop technologies and approaches with potential to improve our understanding of bivalve biology, with a primary focus on oyster production methods. Cooperation with our industrial partner Colchester Oyster Fishery underpinned the project. There were three strands to the work contributing to this thesis, which will be separated into four data chapters (Chapters 2 – 5), each with specific aims that can be described as follows.

Chapter 2 first aims to provide a methodological overview of the NOSy sensor, detailing the principles of its construction and how the data it records is processed and analysed. Secondly a record of the work carried out with the sensor to date is provided alongside example results from field and laboratory work. Finally we aim to discuss the potential and challenges of the NOSy system, and future plans to ensure the system has utility across the aquaculture, monitoring and research sectors.

Chapter 3 builds on data recorded by a NOSy unit monitoring *M. gigas* in an estuarine environment, in which patterns of gaping behaviour were semi-synchronous with the tidal cycle. This laboratory study aimed to isolate one aspect of the estuarine tidal cycle, salinity, and examine its importance as a driver of oyster gaping behaviour.

Chapter 4 aimed to investigate the utility of a new method for on-growing of seed oysters purchased from a hatchery. Land-based systems were constructed and trialled over three summers, during which the system design and husbandry protocols were iteratively improved and a range of oyster sizes, stocking density and patterns were trialled.

Chapter 5 resulted from a collaboration with colleagues at the Northern-Irish Agri-Food and Biosciences Institute and focused on the gaping behaviours of Blue mussel (*Mytilus edulis*). Work for this chapter had two aims, the first was the trial of NOSy on a novel (to the sensor) species, enabling us to further develop our processes. Secondly we aimed to record the behavioural responses of *M. edulis* to their environment, and assess how these may affect their vulnerability to waterborne contamination. I was the primary analyst and author for this work, and am the first author on the associated publication in Aquaculture Reports.

Chapter 2

Development and Trial of the Non-Invasive Oyster Sensor

Summary

This chapter details the concepts and design underlying the Non-Invasive Oyster Sensor (NOSy), a valvometric sensor developed at the University of Essex. NOSy was initially designed as a method to automatically detect spawning of the Pacific oyster, a process which is accompanied by a characteristic pattern of valve movement. Precise knowledge of when spawning has taken place aids allows growers to maximise retention of the naturally spawned oysters, reducing outlay on oysters from hatcheries. NOSy has been extensively trialled in the field and laboratory, recording high quality data suitable for in-depth study of bivalve behaviour. Spawning has been detected in the laboratory, but automatic detection in the field is yet to be accomplished. A detailed methodology for use of the NOSy system is presented along with example data from trials, including from an induced spawning in the laboratory. We conclude with an account of the challenges encountered when developing NOSy, and a plan for future work to develop the system into a viable commercial product.

1 Introduction

Oyster spawning, as detailed in Chapter 1, Section 2.5, takes place with a relatively high degree of synchrony throughout the population (Bernard *et al*., 2016). Larval settlement patterns are complex, and controlled in large part by current regimes (Bayne, 2017c). However, larvae are able to exert significant influence on their final settlement location, a choice driven primarily by chemical stimuli (Coon *et al*., 1990). Calcium carbonate, the primary component of bivalve shell, is the main attractant to larval oysters (Soniat and Burton, 2005). Provision of suitable substrate, such as recycled shell, gravel or lime coated materiel, in areas where larvae are naturally produced can therefore significantly increase settlement rates, reducing long-term outlay on seed oysters from hatcheries (Soniat and Burton, 2005; Taylor Goelz *et al*., 2020).

As discussed in Chapter 1, Section 3.2, cultching, the deposition of shell material onto areas where a culture operation wishes to encourage larval settlement, is therefore a key process

in many oyster farm's annual calendars (Laing and Bopp, 2019). Timing of the process is vital for maximising settlement. Historically useful temperature cues, such as tree blossoming, are becoming less reliable as a result of climate change, and direct stock monitoring is labour intensive and wasteful. Water temperature monitoring can assist in estimation of when the oyster stock is likely to spawn, as detailed in Chapter 1, Section 2.5, but can only provide an estimate of spawning time.

In female Crassostreinae oysters there is a distinct pattern of valve movement (Figure 1.1) associated with gamete release, which takes place over a relatively short time period of 1 to 2 h (Ahmed *et al*., 2016; Galtsoff, 1961). The NOSy sensor was conceived to be able to detect this pattern by continually recording oyster openness and alert users to the fact that a confirmed spawning event has taken place.

Larval duration in the water column (discussed in Chapter 1, Section 2.5) is primarily a function of water temperature (Syvret *et al*., 2008). Typically it is in the order of 14 days, real-time confirmation of spawning therefore would provide ample time for an operation to lay cultch, with a low risk of it being smothered or washed away, maximising larval retention and reducing future outlay on seed oysters from a hatchery.

The Non-Invasive Oyster Sensor (NOSy) is a high frequency, non-invasive (HFNI) valvometric sensor developed at the University of Essex, in collaboration with Colchester Oyster Fishery, designed to record the gape of up to 16 bivalves simultaneously. The NOSy project was conceived with the principal aim of developing a system capable of detecting *M. gigas* spawning events and automatically alerting oyster culture operations in improving farm management protocols. Gape sensing data can also be used to study many other behaviours, detailed in Chapter 1, Section 4.3, the NOSy system is designed to be capable of providing data for these investigations as well.

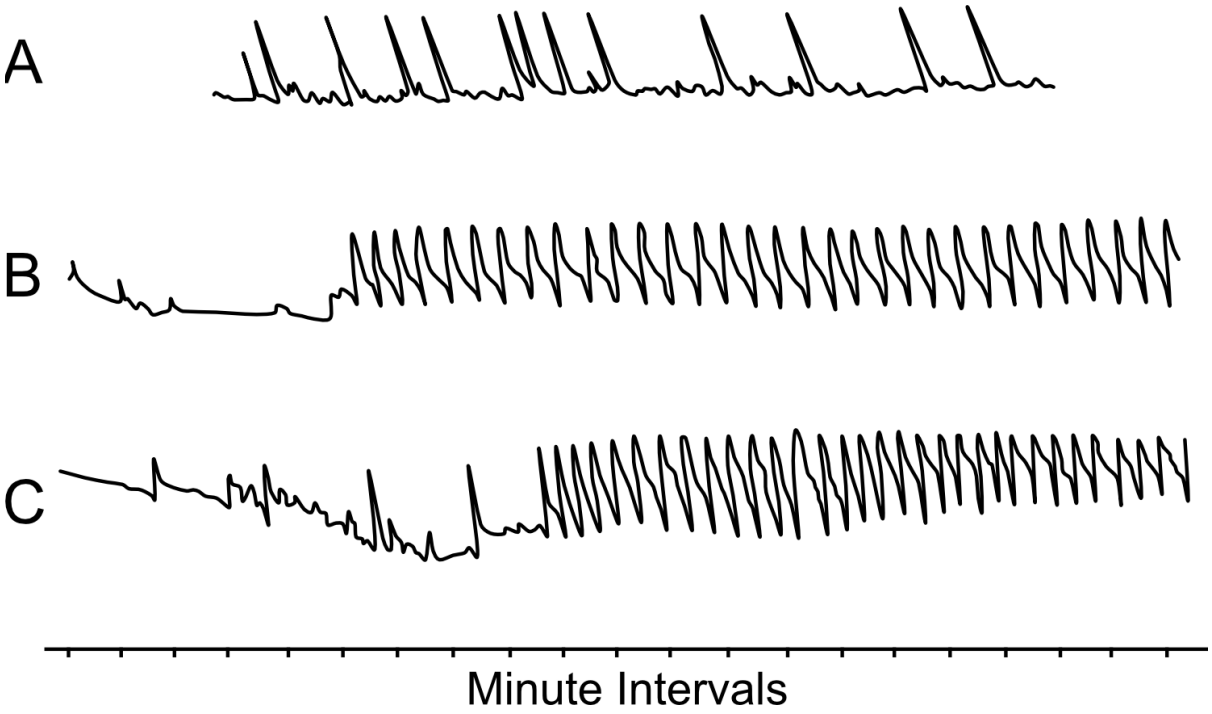

Figure 1.1. Relative gape readings taken with a kymograph, (a mechanical device with a rotating drum that records movement over time) of Crassostrea virginica during spawning. **A** Male oyster, **B** and **C** females. It is not clear from the source whether open or closed is indicated by an increase in position on the y-axis, but comparison with other sources suggest that it is closed. Modified from Galtsoff (1961)

2 Methods

2.1 Development of the NOSy System

The NOSy system uses passive permanent magnets and a Hall-Effect sensor, a form of variable resistor through which the voltage varies depending on the strength of the local magnetic field. These are each glued onto the subject animal on opposite valves and record gaping at 3.7 Hz. One unit can monitor up to 16 animals and is ruggedised for extended field deployments. The overall design is similar to that detailed in Nagai *et al.* (2006).

The first generation of prototype NOSy field units was a single unit constructed for use in summer 2019. This was housed in a 60 cm × 50 cm × 23 cm pelicase (Storm Case iM 2700, Pelican Products, Inc., Torrance, CA, USA), the top of which was fully covered with a solar panel. External waterproof plugs were used to attach sensor cables to this unit, which proved slightly prone to corrosion. Five ‘second-generation’ field units were constructed for

use in 2020. These were housed in slightly smaller (47 cm × 35 cm × 15 cm) generic waterproof boxes, with smaller solar panels and waterproof grommets that the sensor cables passed through to be attached to an internal circuit board via choc-blocks. There was no difference in the data output format between the two generations of instrument.

The inside of a second-generation is shown in Figure 2.1. Units are powered by a 12V battery (e.g. RS Pro 12V 13Ah, RS Pro, Corby, UK), supplemented by a solar panel on the unit case. Sensor management, power and data storage are carried out by a BeagleBone Black (BeagleBoard.org, Michigan, USA) and a custom-printed circuit board. The system generates just under 40MB of data per day. In principle the unit is capable of remote data transfer via cellular networks, but to date this has not been realised and a USB connection used instead, via the ‘Terminal’ program on a laptop with a Linux operating system.

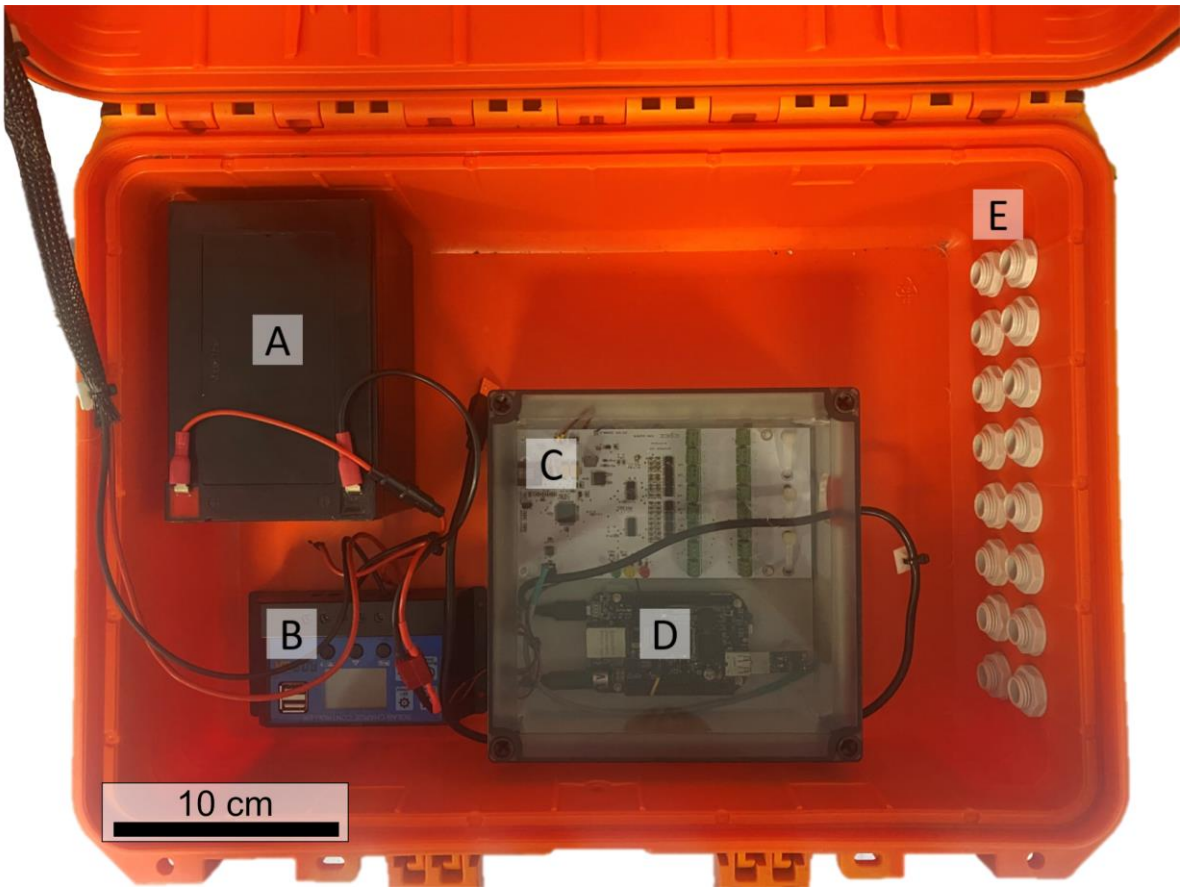

Figure 2.1: The inside of a second-generation NOSy unit. Shown are the 12 V battery and connectors (**A**), charge controller for the solar panel (**B**), custom-printed circuit board with choc-blocks (green) for sensor attachment (**C**), BeagleBone Black with USB connection for data download (**D**) and the waterproof grommets for sensor cables to pass through (**E**)

2.2 Sensor Construction and Magnet Preparation

An image of a sensor prepared for use is shown in Figure 2.2. Hall-Effect sensors (SS495A, Honeywell International Inc, Wabash, USA) were soldered onto < 5 m of 3-core 0.14 mm^2 signal and data transmission wire (PP002620 Multicomp Pro, Farnell UK, Leeds, UK). The wires were sheathed in custom silicon tubing (supplied by A Littlejohn, Cowbridge, UK), over the full 5 m length for field deployments, or ~1.5 m for laboratory use. The ends of the tubing were sealed using flexible silicon sealant (Elastosil E43, Wacker, Munich, Germany). To ensure that the sensor remained in place, ~1 cm from the end with its face as close to the wall of the tubing as possible, it was clamped whilst the sealant cured, using a clothes peg or

similar. At the non-sensor end of the wire ~10 cm was left un-sheathed for attachment to the choc-block in the control unit.

Figure 2.2: Close-up of a sensor prepared for use with a NOSy system. Note the flattened end that aids in obtaining a good signal strength. Air bubbles remain in the silicon sealant after injection and care must be taken when preparing sensors to ensure that these do not compromise the waterproof seal.

Neodymium magnets (e.g. 724-3875, Eclipse Magnetics, Sheffield, UK) were preferred for use with the NOSy sensor as they have a strong magnetic field for their size. Stronger fields resulted in a higher response from the sensor, and therefore reduced the signal-to-noise ratio. Larger magnets also have stronger fields but can be difficult to site effectively on the subject organism. Magnets were typically coated in a two-part epoxy resin (UHU+ Endfest, Bolton Adhesives, Milan, Italy) prior to deployment to protect them from corrosion.

2.3 Sensor Attachment

The collection of high quality data with NOSy relied on the selection of suitably shaped animals and an optimal sensor attachment site. Animals with particularly thick or uneven valve edges were challenging to effectively attach sensors to. Attachment sites generally needed cleaning, particularly when freshly collected from the field, typically using a soft brush, e.g. toothbrush. If necessary, the attachment site was cleaned with a rough brush or abrasive material, taking care not to harm or overly stress the animal.

Sensors were attached utilising the setup mode of the NOSy unit, which provided a live waterfall display of the sensor data. Using this it was possible to identify a point on the animal where the magnet and sensor can be glued, one on each valve, as close together as possible (without impeding the animal's ability to open). Sensors were attached with the

sensor unit facing towards the valve, and magnets orientated so that the output numbers fall with increasing proximity to the sensor. As the units are currently designed, a sensor free of magnetic influence gives a readout of approximately 1450 on the waterfall display. A sensor attachment site where a fully closed animal gives a reading of <1100 provides suitable data, but the difference between closed and open readings should be maximised to reduce data noise

For field deployments a UV-curing fibreglass resin (Solarez Ding Repair, Vista, CA, USA) was used. Sensor attachment was carried out in shaded areas as this resin cures in natural bright light (UV light) within minutes. For long duration deployments sensors are prepared for attachment by closing 2 small cable ties tightly around the cable, 2 to 4 cm from the sensor, providing a grab site that increases the strength of the adhesive bond (Figure 2.3).

For laboratory use a two part epoxy putty (Milliput, The Milliput Company, Gwynedd, UK) was used. This was preferred for two reasons, firstly the resin used for field deployments can release solvents into the water unless 100% cured, potentially harming anything in the tank, whereas the putty is aquarium safe. Secondly it was slightly easier to remove without accidentally damaging the oyster, allowing for sensors to be swapped between animals during short duration experiments. Historically Milliput was liable to release hormone analogs into the water, potentially affecting reproductive behaviours, but the modern formulation no longer appears to do so.

Whilst the adhesive cured a rubber band or similar was used to hold the sensor in place, this ensures that the signal strength remained high when the animal is deployed (Figure 2.3). Care was taken throughout the attachment process to ensure that the valves were not glued together. Typically, sensors were attached first and the adhesive left to cure sufficiently that the sensor was fully fixed in place. Magnets were then attached to the valve, utilising the live readout again to ensure maximal signal strength.

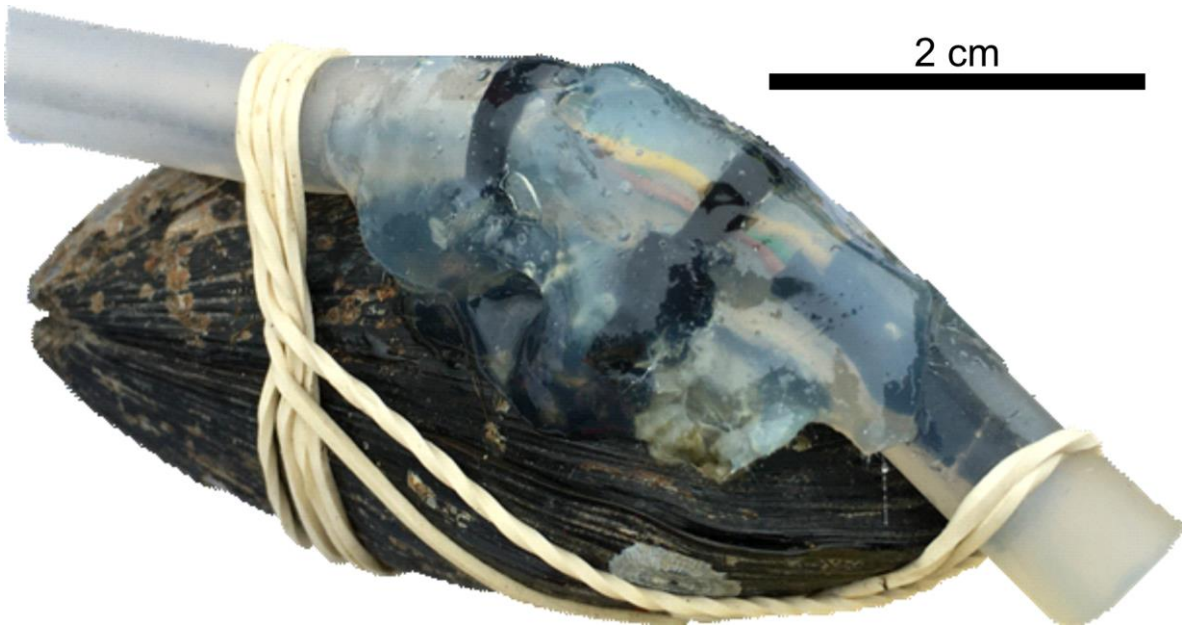

Figure 2.3: A Blue mussel during the sensor attachment process with UV-curing fibreglass reinforced resin. Note the small cable ties to provide extra grip to the adhesive, and rubber band to keep the sensor stationary whilst the adhesive cures.

2.4 Data Processing

Data processing protocols were developed alongside the units, utilising Python (v3.9) and several associated packages, as detailed below.

1. The output of the sensor unit is raw data in the form of 18 column text (*.txt) files, each 122 kb file covering approximately 4 min, an example is provided in Figure 2.4 below.

Column 1: Time/Date	Columns 2-17: Sensor Data	Column 18: Voltage

```
15-11-18-16/09/20,00886,01259,01180,00726,00927,00843,01192,01344,00930,01305,01328,01475,01037,00780,01351,01199,00012,
15-11-18-16/09/20,00886,01257,01181,00726,00926,00843,01194,01343,00927,01301,01328,01475,01037,00782,01349,01200,00012,
15-11-18-16/09/20,00887,01257,01185,00725,00928,00844,01192,01345,00929,01307,01326,01471,01039,00784,01352,01202,00012,
15-11-19-16/09/20,00885,01257,01182,00727,00927,00842,01194,01345,00930,01304,01327,01477,01034,00781,01350,01203,00012,
```

Figure 2.4. Exemplar ‘waterfall’ sensor data from a NOSy unit showing the raw output as seen during setup, including datetime values, readings from 16 sensors and the system voltage.

2. The files were concatenated using the Windows Command Prompt. The amount of data concatenated into a single file depends on the purpose of the study, typically a maximum of 10 days was included per file in order to keep file sizes manageable.
    a. Long term (>10 day) files require the use of high-performance computing to process in a reasonable time frame.
3. With the extant NOSy firmware it was necessary at this point to correct two things:
        i. Dates were stored in dd/mm/yy format, but the current firmware processed them as dd/mm/yyyy, meaning that data recorded in both 2020 and 2021 are stored as xx/xx/20.
        ii. The column separator between the date and first sensor was an underscore rather than the comma used for the rest of the columns
    a. Data from 01/01/2021 was stored as 01/01/20, with an incorrect separator after. The find and replace function was used on the concatenated file, in this instance replacing '"/20_" with "/21,".
4. Files were then imported into Python (v 3.9), and processed utilising the *NumPy* and *pandas* packages (Harris *et al*., 2020; McKinney, 2010). As a first step the time column was classified as a datetime object and set as an index.
5. A first check of the data was then carried out. The NOSy unit was liable to output spikes (as zeroes and erratically high values), thought to be as a result of power supply or sensor-connection issues (Figure 2.5A). Data from each sensor were plotted as a time series, at which point it was clear whether spikes were present. A filter was applied where necessary to set any spike values to nan, removing them from the analysis (Figure 2.5B). This process was not conducted by default, as a result of high variation in raw values and spikes between units and over time.
6. Once cleaned the data were time averaged to either the second or the minute, depending on the time resolution of the behaviour of interest. This accomplished two primary objectives, reducing the noisiness of the data, and minimising the file size, allowing faster processing and file sharing.

   a. The use of NOSy for growth monitoring does not require a time averaging stage, instead a weekly minimum value was used to track changes in sensor/magnet proximity.

7. The data were then normalised such that 0 represents the most closed and 1 the most open values for each individual, at which point they were ready for analysis.
   a. For data covering a relatively short (<10 day) time the normalisation function of the Python package *sklearn* (Pedregosa *et al*., 2011) was used.
   b. When data covered longer periods, there was a risk that the subject animal had grown sufficiently to skew the normalised values by increasing the minimum distance between sensor and magnet, which would appear as if the animal had ceased to close fully. In these instances, a custom function was used, which applied a rolling window of a week in length to the normalisation, eliminating the effects of growth. This was both computationally intensive and resulted in the loss of a week's data from the beginning of the dataset, hence the preference for *sklearn* for datasets of short duration.
8. Depending on the purpose of the monitoring project, behavioural indices were calculated, including 'time open' and 'closure rates'. For both indices a threshold value of 0.2 was set as the boundary between open and closed, as described by Tonk *et al.* (2023).
   a. For time open this value was used to classify each NOSy sample point as open or closed, and the percentage of time that the subject animal spent open was calculated.
   b. Closure rate was calculated by
      i. Applying an automatic detection, classifying when gape readings decrease from > 0.2 to < 0.2. These data were manually processed in order to avoid overestimating the number of events. e.g. when second averaged data are used closures within a minute of the previous are declassified.

ii. Normalising the number of closures to an average number of events per day over the time period of interest.

iii. Using this procedure closures were calculated and openings were the same values ± 1.

9. Automated detection of spawning behaviours is in development. Others have developed algorithms that identify data that indicates a high likelihood of a spawning event using similar sensors (Ahmed *et al*., 2016)

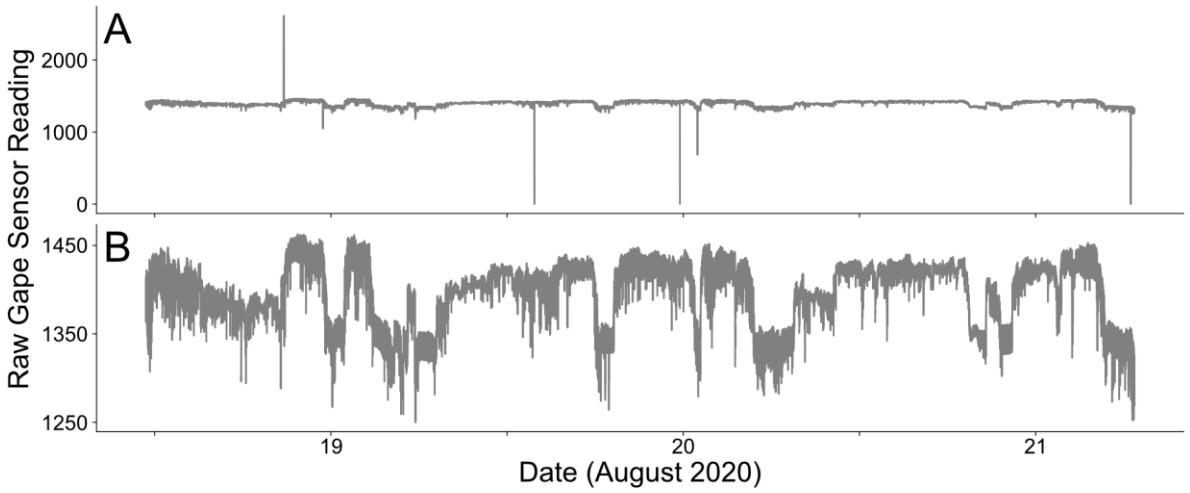

Figure 2.5. (**A**) Unprocessed NOSy data with clear erroneous high and low value data spikes resulting from power supply or sensor connection issues. (**B**) The same data after being passed through a de-spiking filter.

Some of our prototype units developed issues when exporting data. In some cases, this resulted in >16 columns of data being exported, and, in others, the sensor readings were often altered by an order of magnitude. These errors were inconsistent and required extensive manual processing to remove erroneous data. The memory on the units was also unreliable and appeared, in some cases, to fill even when files were deleted. Regular visits and data downloads were therefore required. However, this also provided opportunity to check battery charge levels and carry out replacements when needed.

2.5 NOSy Deployments

NOSy has been used extensively since 2019, in both the field and laboratory (Table 2.1).

Table 2.1: Details of deployments/experiment that have utilised the NOSy system since 2019. Rows in **bold** contributed to the research described in this thesis and the relevant chapter numbers are detailed.

Date	Deployment Location	Number of Units (Generation)	Details
Jun-Sep 2019	COF	1 (1)	Field trials: 16 *M. gigas*
Jan-Mar 2020	**University of Essex**	**1 (1)**	**Lab Experiment: Effect of salinity on *M. gigas* behaviour (Chapter 3)**
Jun-Sep 2020	COF	2 (2)	Field trials: 28 M. gigas, 4 *O. edulis*
Sep 2020	**Dundrum Bay, Northern Ireland**	**1 (2)**	**Field experiment: *M. edulis* monitoring for contamination vulnerability (Chapter 5)**
May 2021	AFBI, Belfast, Northern Ireland	1 (2)	Lab Experiment: *M. edulis* and *M. gigas* feeding trials
Jul-Sep 2021	**COF**	**2 (2)**	**Field trial: 16 *M. gigas* (units not deployed simultaneously).**
Jul & Jun 2022	University of Essex	1(2)	Lab Experiment: Investigation of the impact of harmful algae species on *M. gigas* behaviour.
Jul 2022	**University of Essex**	**1 (2)**	**Lab Experiment: Spawning induction of *M. gigas***
Jul 2022	University of Essex	1(1)	Lab Experiment: Investigation of the impact of harmful algae species on *M. gigas* behaviour.
Jul-Aug 2022	**COF**	**1 (2)**	**Field trial: *M. gigas* growth monitoring**
Feb-Mar 2023	**University of Essex**	**1 (1)**	**Lab Experiment: Effect of salinity on *M. gigas* behaviour (Chapter 3)**

2.6 Laboratory Spawning Induction

As part of the NOSy trial and development process a short-duration spawning induction experiment was carried out in July 2022. This study aimed to confirm that the NOSy unit, and accompanying data processing methodologies, were able to detect spawning behaviours in *M. gigas*.

COF staff reported on 06/07/22, that *M. gigas* on their layings were close to reaching spawning condition. 40 freshly dredged adult oysters were therefore brought into the laboratory at the University of Essex, where they were kept in a recirculating aquarium stack of three 30 L tanks (total system volume including sump was 150 L) filled with full-salinity natural seawater and maintained at 17°C. They were fed a commercial shellfish feed (Shellfish Diet 1800, Reed Mariculture, Campbell, CA, USA) at the rates recommended by the manufacturers. After a short (6 day) acclimation sensors were attached to 16 of the most suitably shaped oysters and logging commenced.

Data were processed as per section 2.4 above, with second-averaged NOSy data used due to the short duration movements of spawning behaviour.

2.7 Field Deployment Methodologies

The majority of NOSy field deployments took place on a floating raft in The Pyefleet, a creek off the Colne Estuary that forms part of COF's grounds (Figure 2.6). The raft provided a dry platform for mounting the units and a secure underwater area where oysters were attached within easy reach whilst remaining submerged at all states of the tide (Figure 2.7). Other NOSy field deployments have utilised similar floating platforms to mount the unit, as detailed in Chapter 5, Section 2.3.

For trials in the Pyefleet, temperature and light loggers (HOBO Pendant MX, Onset, Bourne, USA) were attached next to the oysters for all deployments. When available a sonde with conductivity and dissolved oxygen meters (YSI EXO1 Sonde, Xylem, Yellow Springs, USA) was also attached to the raft.

A comprehensive temperature record for the Colne and Pyefleet was collected from 28/01/20 to 02/10/20. As detailed in Chapter 1, Section 2.5 these data were used to calculate average cumulative temperature degree days (above b^0) for the estuary to estimate when spawning was likely to be imminent.

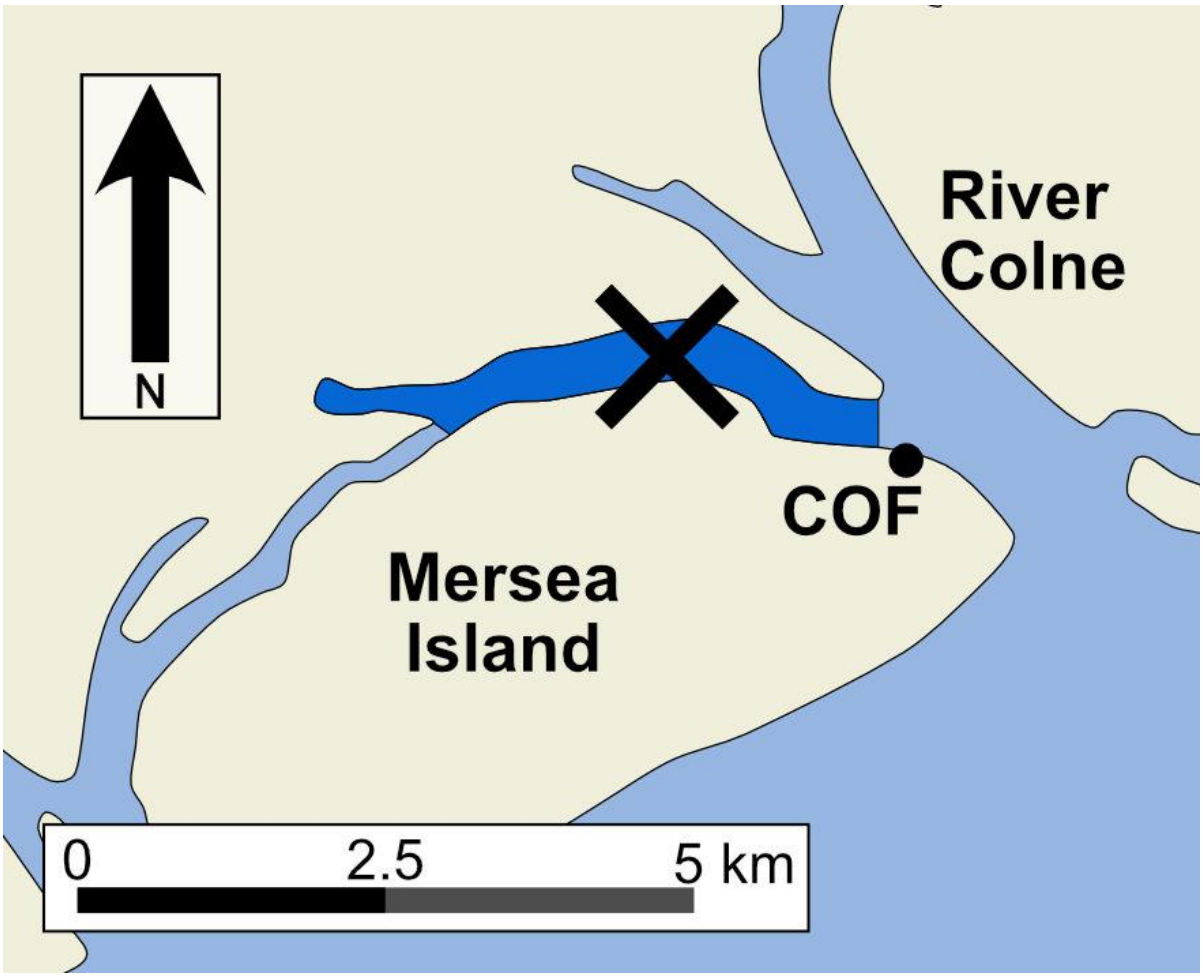

Figure 2.6. Location of the NOSy field trials at COF (51.810N, 0.959E). The location of the raft used to mount the sensor and oysters is indicated by the black cross and Pyefleet Creek is highlighted in dark blue.

Regular visits were necessary during deployments to ensure the units remained powered and fully functional. During these visits NOSy, HOBO and sonde data were downloaded, solar panels were cleaned and batteries swapped out when necessary. Failed sensors or dead oysters were replaced where needed.

Data from all field deployments were processed as per section 2.4 above, with second-averaged NOSy data used due to the short duration movements of spawning behaviour.

Figure 2.7. Two NOSy units deployed on the raft at COF, with the unit on the right open for data download. Note the metal ‘cage’ structure visible above the water behind the walkway which was used as an attachment site for the study animals.

2.8 Spawning Condition Monitoring

Spawning condition monitoring was carried out as a method of verifying the general spawning condition of the *M. gigas* population in the area. Between 13/07 and 10/09/20 13 samples of between 12 and 26 *M. gigas* from around Mersea Island, 7 of which were from the Colne or Pyefleet, were monitored for their spawning condition. These oysters were shucked and visually inspected for the presence of ripe gonadal tissue, oysters were measured and classified as un-spawned or spawned, based on guide images (Figure 2.8)

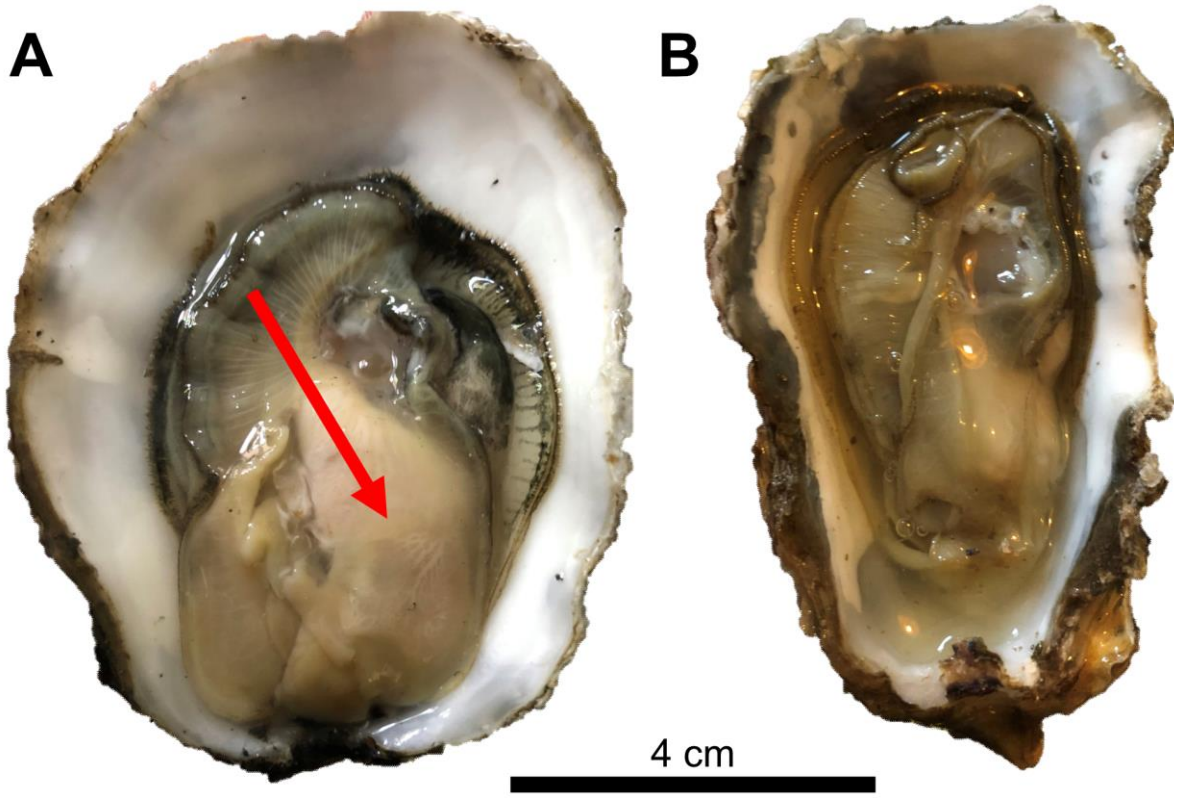

Figure 2.8. Guide images for *M. gigas* spawning condition analysis. (**A**) An unspawned oyster with ripe gonadal tissue highlighted with red arrow. (**B**) A post-spawning, or non gonadally developed, oyster.

3 Results

3.1 Laboratory Experiments

Spawning was successfully induced overnight on the 12/07/22. It was detected in 4 oysters, demonstrating that the NOSy unit and data processing protocols are suitable for spawning detection (Figure 3.1). Microscopic analysis of water samples confirmed the presence of newly fertilised bivalve larvae. The spawning events lasted between 1 and 2 hours and the observed gaping behaviours were very similar in nature to those captured by other investigators (Figure 1.1) (Ahmed *et al*., 2016; Galtsoff, 1961).

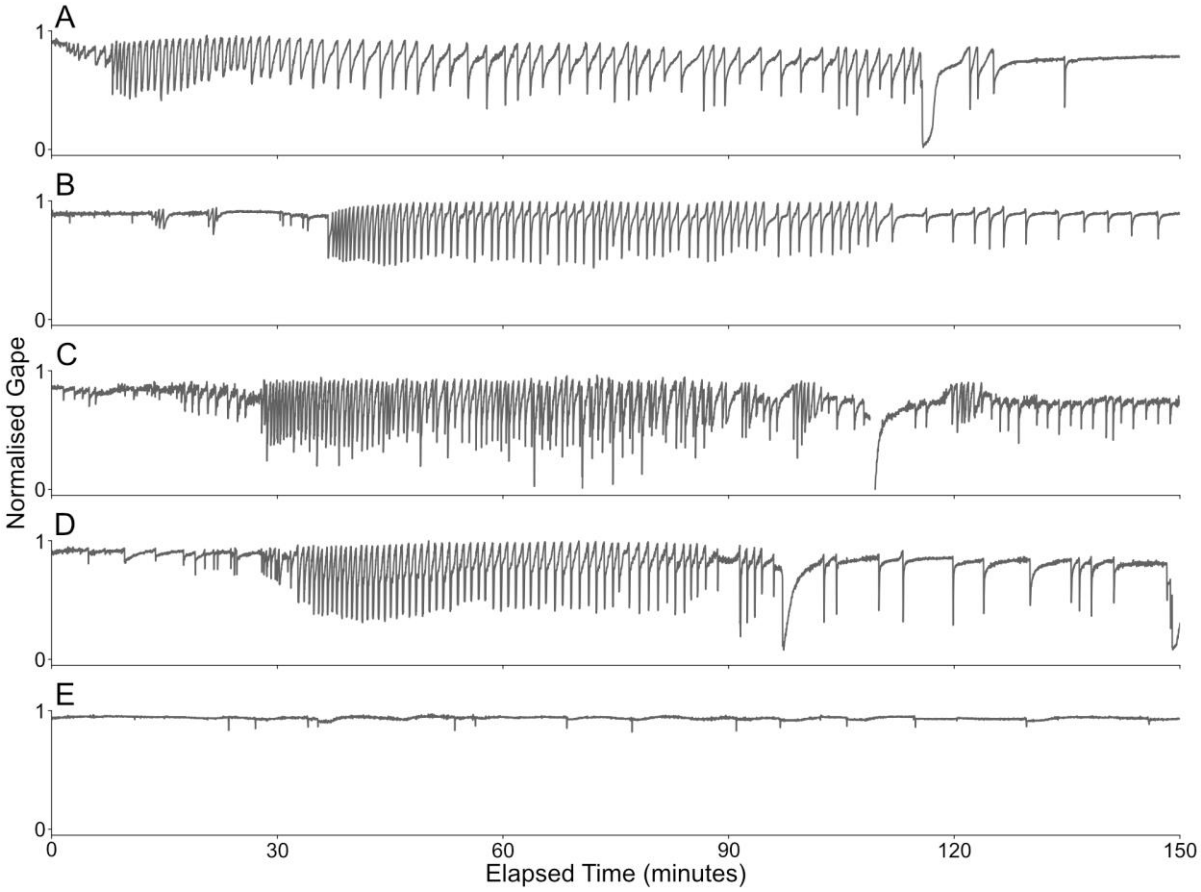

Figure 3.1. Normalised gape time series from five *M. gigas* kept in the laboratory on the night of 12/07/22, times were not concurrent for each event, but all took place within three hours of each other. A normalised gape value of 1 indicates maximum opening and 0 indicates minimum opening. **A-D**: Spawning behaviour traces from four different oysters **E**: Non-spawning behaviour, from a separate oyster at the same time as the trace in panel **D**.

A repeated pattern of a rapid closure followed by re-opening over ~1 min was clear in 10-minute subsamples from the middle of each spawning window (Figure 3.2). All 4 oysters returned to virtually full gape, but minimum gape is variable, ranging from 0.24 to 0.55 (excluding the single nearly full closure (0.03) in panel C at 64 minutes).

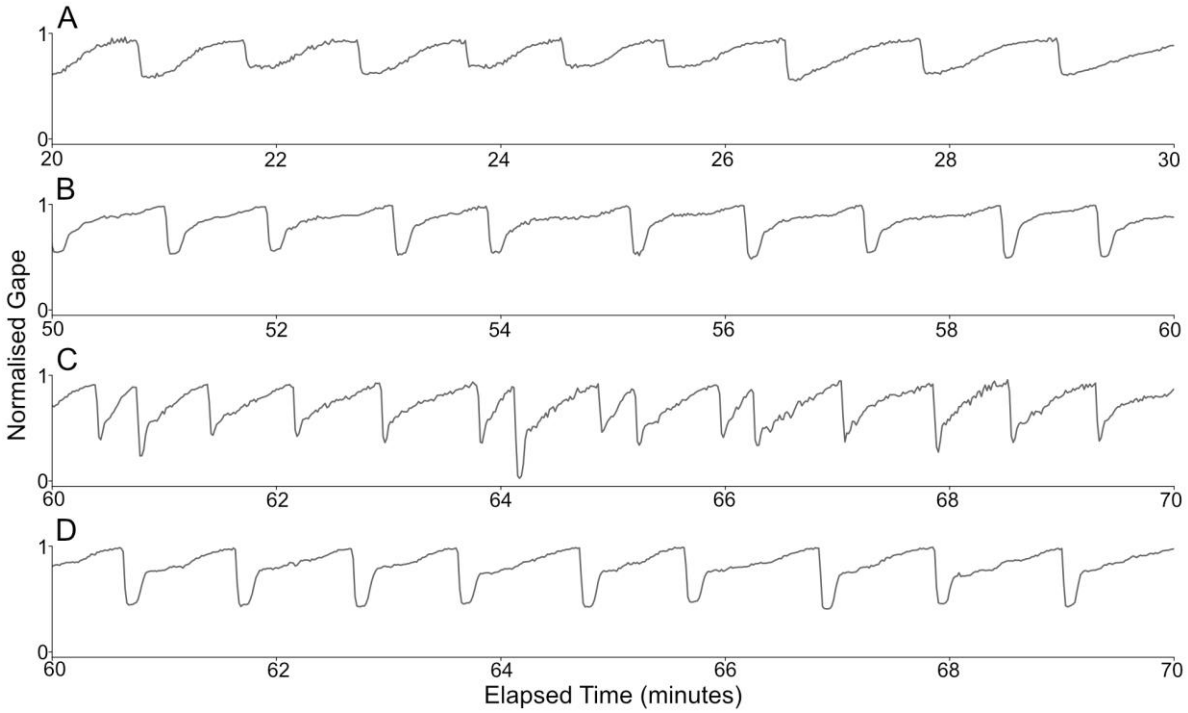

Figure 3.2. 10 minute time series of gape from the same oysters shown in Figure 3.1 during the time period in which they were spawning. A normalised gape value of 1 indicates maximum opening and 0 indicates minimum opening. These traces show the consistent ~1 min closure/re-opening behaviour characteristic of spawning in *M. gigas*.

3.2 Field Trials

3.2.1 Behavioural Analysis

The most-prolonged deployment of NOSy units was during summer 2020, with two units deployed for 78 days. In the summer of 2021, one unit was deployed for 73 days. Full time series from these deployments are not provided here, but example data from relevant windows are.

Attempts to capture and identify spawning behaviour in *M. gigas* in the field were unsuccessful. Power supply issues presented a major challenge, limiting data coverage to 70% of the deployment in 2020 and 83% in 2021.

Data were visually inspected for apparent spawning behaviours, but given the length of deployments and relatively short duration of spawning this is not the preferred method, and

may have missed any events that did take place. The use of automated detection via specific algorithms (e.g. Ahmed *et al.*, 2016) could provide a useful future avenue to progress with the development of fully autonomous systems that alert bivalve likely spawning events are detected.

The data that were gathered were however of consistently high quality, and once processed were suitable for analysis of gaping behaviours. An example of the data that were collected, from July 2020, is provided alongside tidal height data in Figure 3.3. These plots show patterns in gaping that appeared to be semi-synchronous with the tidal cycle, closing on or around most high tides. Figure 3.3 details the behaviour of 5 oysters over an 8-day period, as well as the average gape from all oysters connected to the NOSy unit at the time, but this pattern was consistent throughout most NOSy field trials.

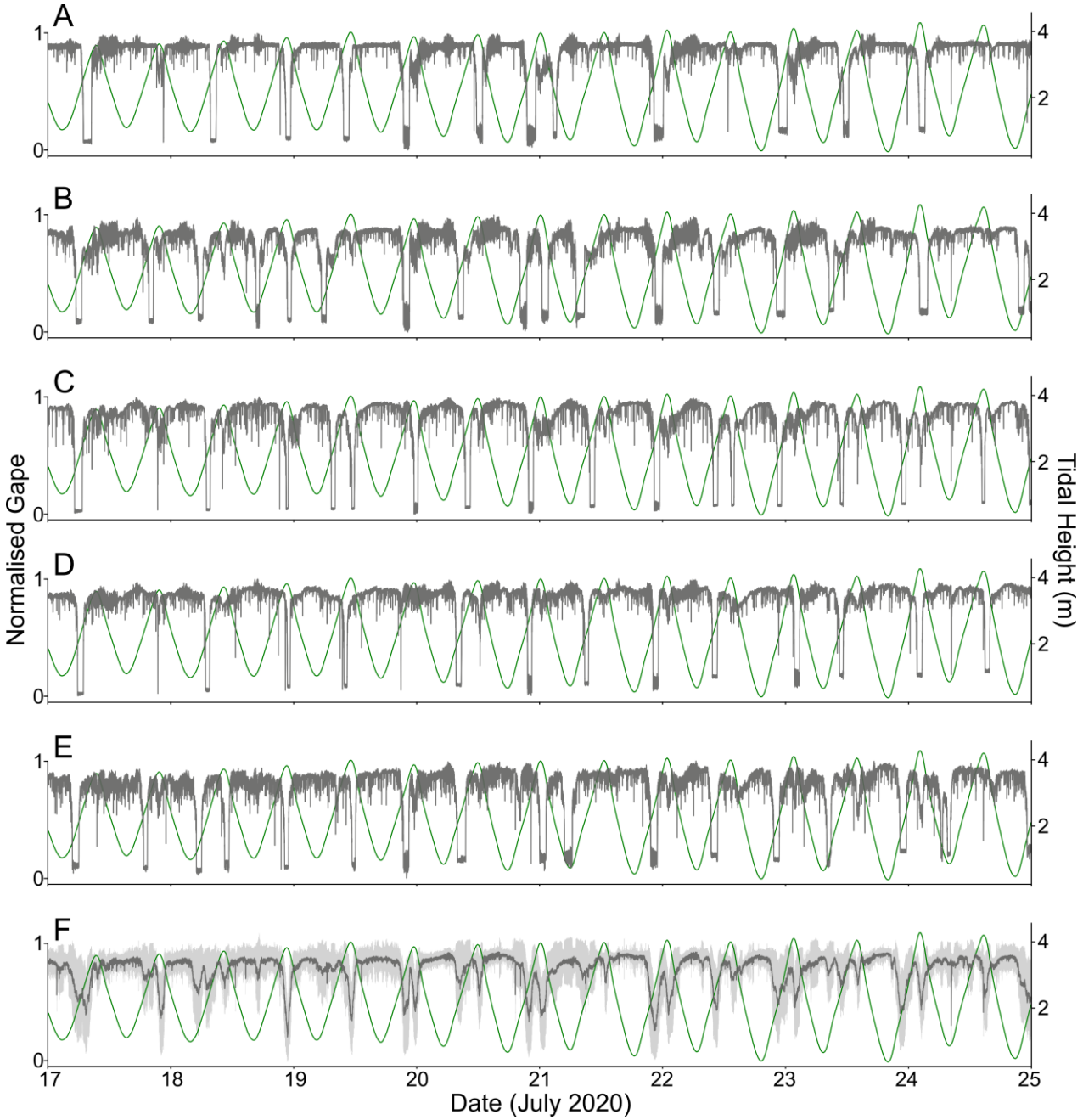

Figure 3.3. Exemplar data from NOSy field deployments showing eight days of normalised gape data from 5 *M. gigas* attached to a NOSy unit in July 2020. A normalised gape value of 1 indicates maximum opening and 0 indicates minimum opening. Panels **A**-**E** show data from five individual oysters, panel **F** is the averaged data (± SD) from all live oysters attached to the unit (n=16). Tidal height (green line) for the area is also shown in all panels.

3.2.2 Species Characterisations

Species comparisons, both graphical (Figure 3.4) and using behavioural indices (Table 3.1) showed clear differences between *M. gigas* and *O. edulis*. Both species had a similar average gape, 0.81 and 0.89 for *M. gigas* and *O. edulis* respectively. Closure rate in *M. gigas* was nearly 3.5 times greater than in *O. edulis*, and time open was 7.5 percentage points lower.

Table 3.1 Behavioural index comparison between *M. gigas* and *O. edulis* for the individuals detailed in Figure 3.4 (n=3). Data collected from the River Colne in July 2020

Behavioural Index	***M. gigas* (average ± SD)**	***O. edulis* (average ± SD)**
Time Open (%)	91.7 (± 1.6)	99.2 (± 0.5)
Closure Rate (Events d^{-1})	5.3 (± 1.8)	1.6 (± 0.4)
Average Gape	0.81 (± 0.06)	0.89 (± 0.01)

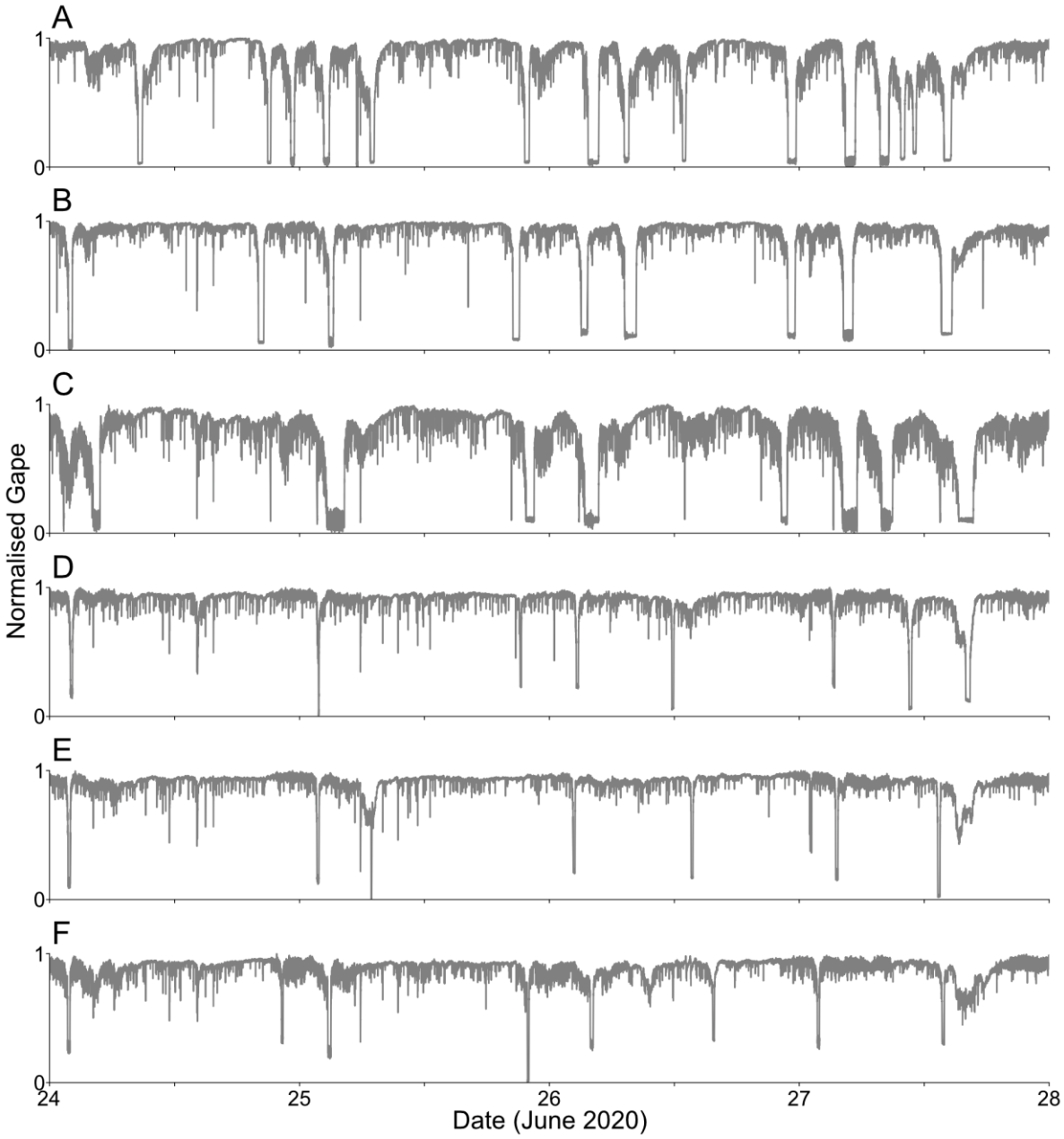

Figure 3.4. Four days of normalised gape data from June 2020 for three *M. gigas* (**A**-**C**) and three *O. edulis* (**D**-**F**). A normalised gape value of 1 indicates maximum opening and 0 indicates minimum opening.

3.2.3 Growth Tracking

The use of NOSy for growth tracking purposes has not been fully developed. However, example weekly minimum raw readings from an *M. gigas* monitored throughout the 2020 field trials (Figure 3.5) increased from 1196 to 1388 (16%) over 78 days. This is not linearly relative to growth, nor is it an absolute quantification of it, but indicates that the gap between sensor and magnet when the oyster is fully closed substantially increased over the summer.

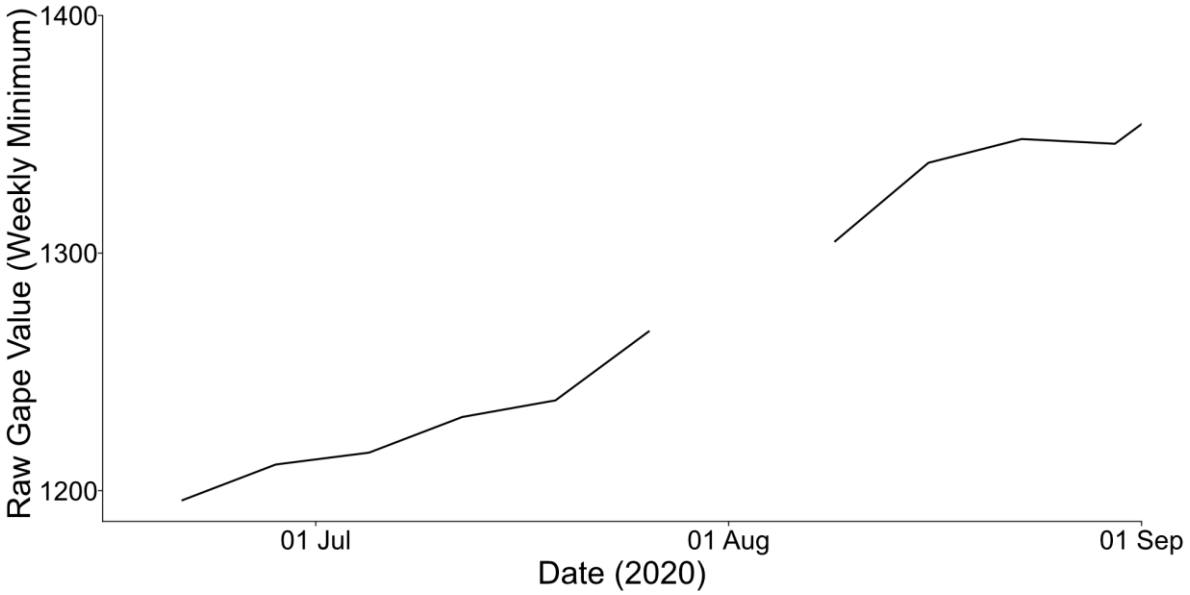

Figure 3.5.Three months of weekly minimum raw gape values from an example *M. gigas* in 2020. The increase in minimum values indicates that a degree of growth has taken place over the deployment, as the sensor and magnet become progressively further apart when the oyster was fully closed. The gap in the data is due to power failure in the NOSy unit that the sensor was attached to.

3.2.4 Temperature and Spawning Condition Monitoring

Corrected (above b^0) cumulative degree days (as described in Chapter 2, Section 2.5) calculated from average daily river temperatures in 2020 reached over 350 on 24/06/20 and over 600 on the 25/07/20 (Figure 3.6). Spawning was therefore theoretically likely to have taken place between these two dates, and settlement 14 to 21 days later.

The later dates of spawning estimated from the degree day calculations somewhat align with the spawning condition analysis (Figure 3.6), which showed that prior to the readings taken on 27/07/20 less than 10% of sampled oysters had spawned, a proportion which increased as the summer went on, reaching 79% by 9/9/20.

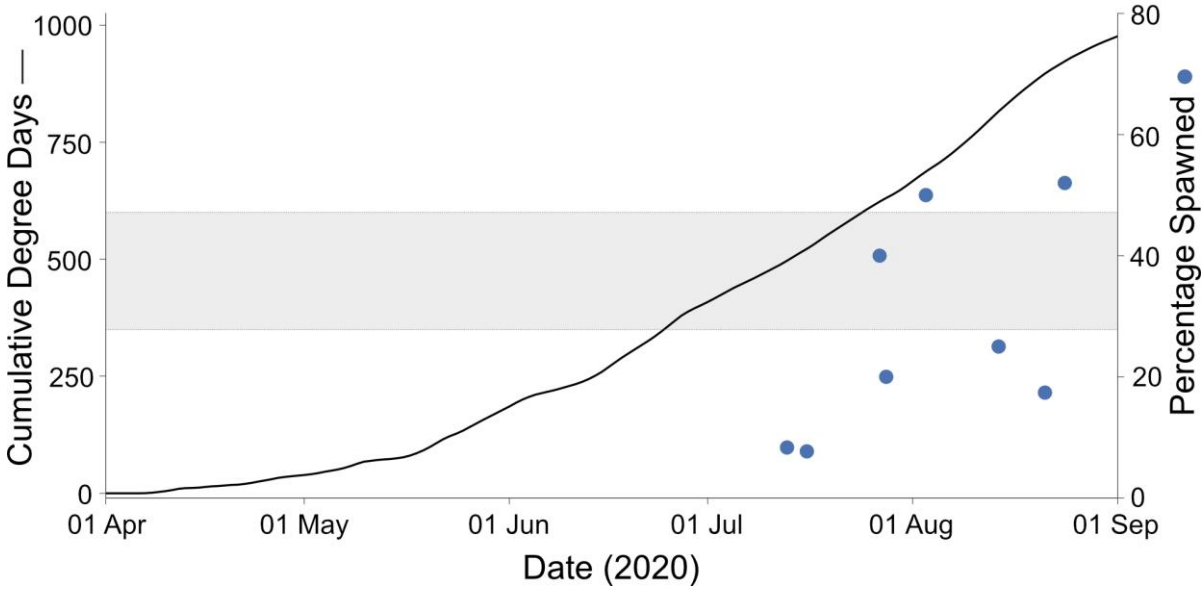

Figure 3.6. Combined plot showing corrected cumulative degree days (above b^0 of 10.55°C) calculated from Colne and Pyefleet average temperatures in summer 2020. The grey region indicates the published value for cumulative degree days when spawning is likely. Secondly, results of the *M. gigas* spawning condition analysis carried out on oysters collected from the Colne and Pyefleet from 13/07 to 10/09/20 are shown, indicating the percentage of the collected sample which had spawned at the time of collection.

4 Discussion

Since 2019 NOSy units have been extensively trialled, collecting large amounts of high quality data. The ability of the unit to detect spawning, the primary aim when developing NOSy, has been successfully demonstrated, proving the potential of the technology to meet

its aims. Use of NOSy in the laboratory underpins the work in Chapter 3, as well as other research at the University of Essex (Funesto, 2023). Other work with NOSy in the field has resulted in a soon to be published study as presented in Chapter 5, and the potential of the unit for growth measurement via monitoring of minimum gape has also been demonstrated.

However, attempts to detect and automatically alert users of spawning events in the wild, were unsuccessful. In 2020 river temperature and spawning condition monitoring results show that the *M. gigas* population in the area spawned. In 2021 the monitoring of the river was less comprehensive but reports from staff at COF confirmed that spawning took place. It is very likely that at least some of the *M. gigas* monitored in 2020 and 2021 would have spawned, but no evidence of this has been found in the data.

Due to power supply issues, there were 30% and 15% coverage losses in 2020 and 2021 respectively, and, therefore, a possibility that the oysters being monitored spawned but data were not captured. Consistent, and ongoing, efforts were made to improve the unit's reliability and to reduce power consumption. The next technological development step is ongoing, with the view to bring the units into commercial use.

Data access and quality became issues of concern with some second-generation units denying access. Three of the units developed issues with the quality of exported data, as discussed in Section 2.4. At least one second-generation unit has a severely diminished storage capacity, filling up within days of data deletion when there should be sufficient storage for over 400 days of data. Given the prototype nature of the NOSy system, these issues are not surprising but there is a considerable level of improvement required before the system would be suitable for commercial use.

HFNI valvometry sensors can be based on a range of technologies, as discussed in Chapter 1, Section 4.3. The use of passive, unpowered magnets underpins the potential for NOSy in long-term and remote deployments. In contrast to the use of powered electromagnets in

some comparable systems (Andrade *et al*., 2016; Clements and Comeau, 2019a; Tran *et al*., 2003) NOSy is simpler and has reduced power requirements in the sensors. For example, the system used by the research group at the University of Bordeaux, discussed in detail by Andrade *et al.* (2016) uses up to 1 Watt of power, and would therefore use a fully charged 13 amp-hour battery in 13 hours when measuring 16 animals at 0.6 Hz each. The same battery sustains a NOSy system for several days without charging, monitoring each individual at 3.7 Hz. With the addition of external power sources and batteries this duration can be substantially extended. Powered magnets have the advantage of being able to be 'tuned', which may enable a signal of higher quality, although with careful sensor attachment we have not yet found sensor signal quality to be an issue of concern.

Minimum raw gape values can be used to infer growth, a concept also discussed by Andrade *et al.* (2016) (Figure 3.5). The use of Hall-Effect sensors as a distance/proximity measurement tool is well established (Jezný and Čurilla, 2013). However, procedures to turn NOSy readings into absolute measurements will be complex to develop. Hall-Effect sensor readings reflect the strength of a magnetic field, which is both non-linear and directional (Weir *et al*., 2020). Distance calculation is therefore complicated by several factors, including the initial distance/orientation between sensor and magnet, the changing orientation as gape increases and the change in minimum distance with growth. Models have been successfully developed to quantify Great scallop (*Pecten maximus*) gaping distance using magnetic gape sensors (Guarini *et al*., 2020), suggesting that growth monitoring using the NOSy system is certainly feasible.

Future generations of NOSy could be designed with increased and higher quality on-board power-storage capacity. A wider range of power generation options could be explored, including tidal and wind-turbine units that will reduce the need for regular visits to check and replace batteries. Remote transmission of data would also substantially increase the user friendliness of the units, although this is a relatively high power-consumption activity. A

crucial step in development is therefore the addition of sufficient processing power to the units to make them able to recognise when behaviours of interest have occurred before triggering communication, as this is the only point at which data transfer would actually be needed.

The focus of automatic detection will initially be spawning, the potential for which was demonstrated by Ahmed *et al*. (2016). In principle it is possible, once baseline 'normal' behaviours have been established for a species or population, to design a system that automatically detects any deviation (Andrade *et al*., 2016; Vereycken and Aldridge, 2022). Other forms of sensors, such as the heartbeat monitors developed by Electric Blue (2020) and Miller *et al*. (2022), can be combined with valvometry to increase data richness and accuracy of behavioural monitoring, e.g. the MusselMonitor (Kramer and Foekema, 2001).

Sensors that can detect abnormal behaviour have potential within and outside the aquaculture industry, particularly given their potential for long-term and remote deployments. The wide-ranging sensitivity of bivalves to perturbations in the environment, such as harmful algal species, heatwaves and the presence of heavy metals (Durier *et al*., 2022; Funesto, 2023; Nagai *et al*., 2006) means that they can, in effect, function as broad-spectrum sensors, aligning with the sentinel animal and biological early warning system concepts discussed in Chapter 1, Section 4.4.

Morbidity and mortality in bivalves are accompanied, in most cases, by increased gape due to failure of the adductor muscle and are, therefore, clear in a gape sensor signal (Figure 4.1). Bivalve gaping behaviours associated with the onset of diseases, such as OsHV-1 and vibriosis, are under-studied, but with sufficient development sensors may be able to warn of disease outbreaks before mass-mortality occurs. For operations that are able to re-site their stock, such as trestle-based oyster farms, early warning systems could enable growers to move stock into safer areas until more normal conditions return. Even farms where stock

cannot be moved could utilise such knowledge, either to reduce operations around highly stressed populations or to target harvest at healthier stocks.

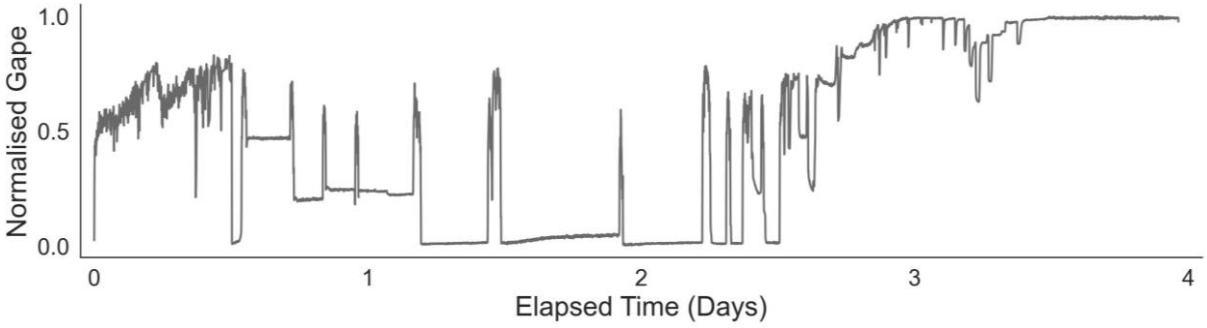

Figure 4.1: *M. gigas* gaping behaviour plot from laboratory work in 2023, showing a morbidity signal after ~2.5 days followed by mortality after ~3.5 days. Note that when dead the oyster's gape was greater than at any point at which was alive, due to the total relaxation of the adductor mussel.

5 Conclusion

We have demonstrated the potential for the NOSy system for use in bivalve culture operations, as a research tool and as part of sensor networks. We have shown that the system generates high-resolution signals capable of providing insight into bivalve behaviours. The basic principles of the sensor are strong, and the use of unpowered magnets in particular will reduce the challenges of long-term deployments. We believe that the planned additions of improved battery capacity, field power generation and on-board processing power will enhance the units to a point where they are a viable product for use across bivalve aquaculture, as part of large-scale biosensor networks and in research.

Chapter 3

The Role of Salinity as a Driver of Pacific Oyster (*Magallana gigas)* Gaping Behaviours

Abstract

Recorded gaping behaviours of *Magallana* (*Crassotrea*) *gigas* in an estuarine system were semi-synchronous with the tidal cycle. In order to establish the role of salinity as an exogenous control on the observed behaviours we exposed *M. gigas* to varying salinity regimes that replicated estuarine cycles in a laboratory, monitoring gape throughout. We were unable to replicate the behaviours seen in wild oysters and conclude that salinity is not the primary driver of the observed gape patterns. Controlled replication of tidal salinity cycles whilst maintaining all other conditions in a constant state, was a considerable challenge, and we suggest that development of such methodologies would prove useful in further study of estuarine bivalve behaviours.

1 Introduction

Estuaries are complex and dynamic environments where many biologically important parameters change over the tidal cycle. These include but are not limited to: water temperature, seston quality and quantity, predator activity, water chemistry and salinity (Bayne, 2017b; Dutertre *et al*., 2009; Guzmán-Agüero *et al*., 2013; Hawkins *et al*., 1998; Malham *et al*., 2009). Of these parameters, salinity is possibly the most significant in an estuarine environment, playing a large role in determining the structure and function of the ecosystem (Bayne, 2017b; Grabowski *et al*., 2004; Telesh and Khlebovich, 2010; Verdelhos *et al*., 2015).

Oysters are euryhaline osmoconformers, requiring substantial metabolic activity to combat osmotic pressures and maintain cellular volume during periods of salinity change (Bayne, 2017b; Shumway, 1977; Sokolov and Sokolova, 2019). Outside of an optimal salinity range, 22-35 for adult *Magallana* (*Crassotrea*) *gigas* (*M. gigas*), oysters are able to survive but with reduced growth, feeding and reproductive efficiency (Pollack *et al*., 2011; Shatkin *et al*., 1997). Oysters are also capable of closing their valves fully under salinity stress, a behaviour

that likely offers protection from extremes in salinity variation (Shumway, 1977; Shumway and Youngson, 1979).

M. gigas monitored during NOSy sensor development trials exhibited semi-synchronous patterns of gaping behaviours that appeared related to tidal cycles (Chapter 2, Figure 3.3). Typically, oysters closed during a flooding or at high tide and were most fully opened during periods of low water. Tidally entrained rhythms of gaping behaviour have been demonstrated in wild *M. gigas* populations elsewhere, with increased valve opening occurring over high water periods (Tran *et al*., 2011). However, laboratory trials assessing the influence of salinity on rhythmicity in oyster, or other bivalve, behaviour are lacking, and where it has been studied static salinity conditions have been used (Casas *et al*., 2018).

In this experiment we aimed to establish whether the gaping patterns observed in the field resulted from a response to salinity variation. We exposed *M. gigas* in the laboratory to a salinity variation that replicated a complete natural tidal cycle, whilst all other conditions remained constant. Identical gape-sensing equipment to the field trials was attached to oysters, allowing them to be monitored over multiple simulated tidal cycles.

2 Materials and Methods

2.1 Experimental Summary

Two separate experiments were carried out for this study, the first in 2020 and the second in 2023. Each consisted of three runs, or blocks, with separate oysters used in each. Four treatments with varying salinity regimes were used in each experiment, each with three replicates per run.

2.2 Experimental Animals

Oysters for both experiments were supplied by Colchester Oyster Fishery. Two batches of 24 were collected on 27/01/20 and 24/01/20, and a single batch of 42 on 25/01/23. All

oysters were graded to ‘AA’ size (~90-110 mm height) and of hatchery origin, i.e. not locally seeded.

On arrival oysters were stored in a three-tank recirculating stack system (total volume 150 L, tank size 30 L). In 2020 this was filled with artificial seawater at 30 salinity and 12°C, which was as close to the river temperature of 7°C as possible. In 2023 the stack was filled with natural seawater, diluted to 27 salinity and chilled to 7°C, the temperature in the river at the time. Temperatures were increased by 1.5 – 2°C per day until the tanks reached an ambient temperature, 16.5°C in 2020 and 19°C in 2023.

In 2020 oysters were then haphazardously sorted into three 30 L tanks of filtered artificial seawater made from RO water and aquarium salt (H2Ocean Salt, D-D The Aquarium Solution, Ilford, UK) at salinities of 26, 30 and 34, where they were acclimated to the experimental condition they would be used in. Oyster IDs were designated using run number, treatment code, individual ID and year. e.g. R1EA_20 designated an oyster from run 1, experimental treatment, replicate ‘A’ and 2020.

Acclimation periods were between 35 and 42 days in 2020, and between 26 and 40 days in 2023, dependent on which run the oysters were used in. During this period oysters were fed regularly, daily by hand in 2020 and using a dosing pump at half hour intervals in 2023. A commercial shellfish feed (Shellfish Diet 1800, Reed Mariculture, Campbell, CA, USA) was fed at just over recommended levels throughout the acclimation period.

Sensors and magnets were attached to the oysters using Milliput epoxy (The Milliput Company, Gwynedd, UK). These were attached on arrival for oysters used in the first run of each experiment, and between runs to oysters in subsequent runs. Following attachment, oysters were returned to acclimation tanks. The attachment process took around 3 h, during which the oysters were out the water to allow the adhesive to cure

2.3 Salinity Regimes and Control Protocols

Salinities were cycled between 26 and <34 for both experiments, depending on the treatment's salinity regime. These values were chosen to reflect the maximum and minimum values logged at the NOSy trial site during summer 2020.

2.3.1 2020

Oysters were housed in 1 L chambers, three in each experimental treatment (Table 2.1). Salinity variation in the experimental treatment was continual throughout each run. Flow to all chambers was controlled by a 16-channel peristaltic pump (205U/CA16, Watson Marlow Fluid Technology Solutions, Falmouth, UK), ensuring that the flow rate in all chambers was identical and even throughout the experimental run at 2.76 mL min^{-1} to 2.77 mL min^{-1}.

Table 2.1: Details for the treatments used in the 2020 experiment. n = 3 in each treatment for each of the three experimental runs.

Treatment	**Treatment ID**	**Salinity Range/Salinity**
Experimental	E	26 – 34
Low-salinity control	LC	26
Mid-salinity control	MC	30
High-salinity control	HC	34

Replication of a tidal salinity cycle in the experimental chambers was achieved using a 'Victorian Plumbing' system (Figure 2.1A). Four vessels of different salinities were connected with a network of fluid bridges, air bridges and siphons. Experimental chambers started with a salinity of 26 in all chambers. They were initially fed from vessel D, linked to C by a fluid bridge, resulting in a gradual increase in salinity in the outflowing water over the 6 h it took to empty the two vessels. Mixing in vessel D was aided by bubbling. The control chambers were fed by separate header tanks of the appropriate salinities. Figure 2.1B details the salinity curve produced by the system, as measured in an experimental chamber.

Vessels A and B were prevented from emptying during this period as they were gastight. Once vessels C and D were empty, air ingress to B was made possible by an air bridge at

the base of vessel D, triggering the siphon between vessels B and D. A and B were connected by fluid and air bridges, resulting in a gradual lowering of the salinity as these vessels emptied into D and from there into the experimental chambers. Mixing in vessel C was aided by a low-speed magnetic stirrer.

Each refill of the system replicated a single tidal cycle, from 26 salinity to 34 and back to 26 over 12 hours. The system was restarted every 12 hours for the duration of each experimental run. Artificial seawater was prepared at each required salinity, allowed to mix for over 6 hours and retested prior to use.

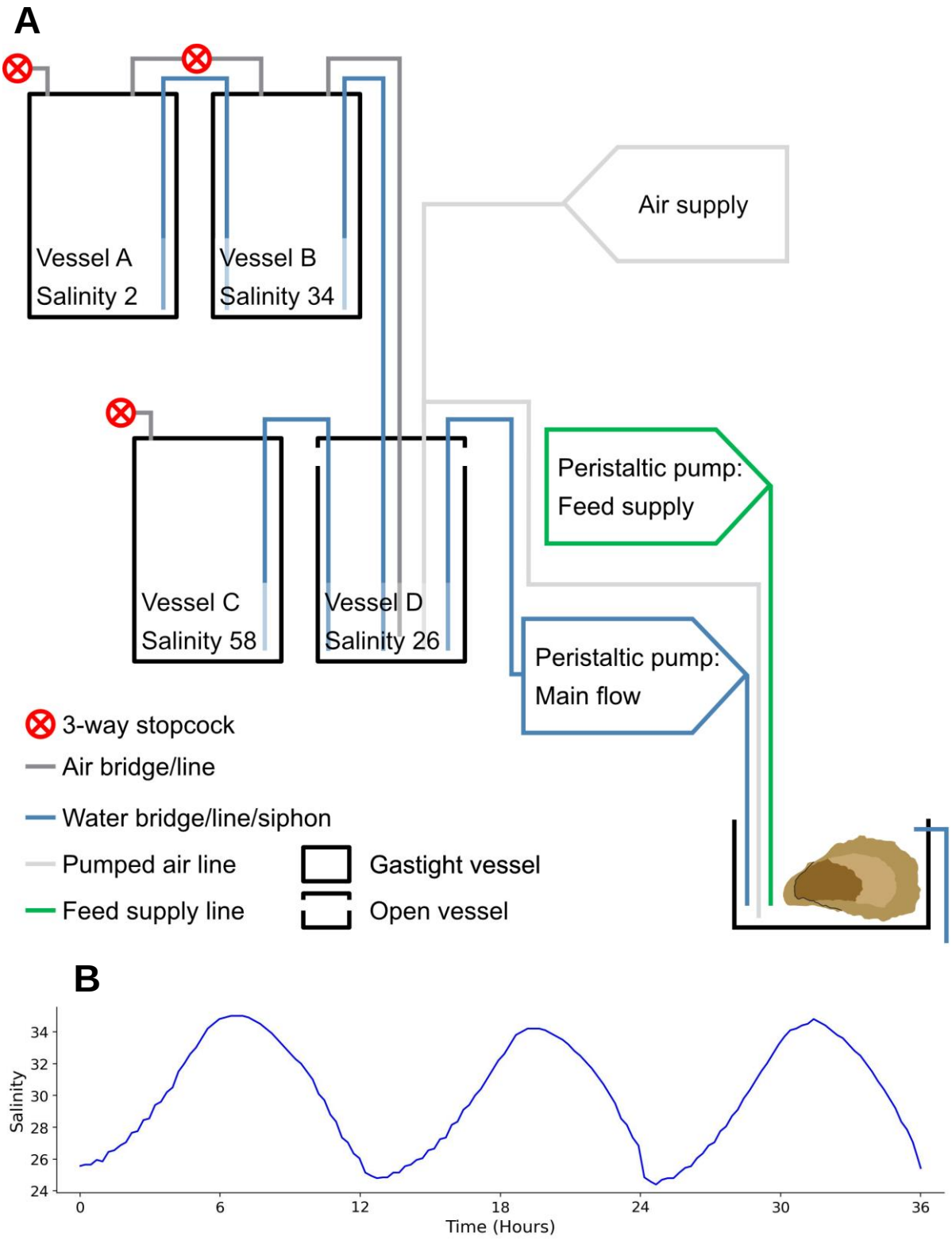

Figure 2.1. (**A)** Schematic of the 'Victorian Plumbing' system developed for the 2020 experiment. Vessel IDs (A-D) and the starting salinities for each repetition of the 12 h cycle are shown in each vessel. (**B)** An example 36 h salinity curve produced by the system.

2.3.2 2023

Oysters were housed as in 2020, with altered salinity regime treatments (Table 2.2) Salinity variation in 2023 was for 12 h per day and oysters were kept at a salinity of 26 overnight. Flow to all chambers was controlled by the same pump as 2020 at a flow rate of 1ml.min^{-1} throughout. Separate header tanks were used for each experimental chamber.

Table 2.2 Details for the treatments used in the 2023 experiment. n = 3 in each treatment for each of the three experimental runs.

Treatment	**Treatment ID**	**Salinity Range**
Control	C	26
Low-variation experimental	L	26 – 28.6
Mid-variation experimental	M	26 – 31.3
High-variation experimental	H	26 – 34

Salinity control for the chambers with variation was achieved by the continuous addition of high-salinity water (34.5, 43.6 and 52.5 for the L, M and H treatments respectively) for 6 h, followed by low-salinity water (19.8, 14 and 7.5 for the L, M and H treatments respectively) for the following 6 h, approximating the salinity variation of a tidal cycle. Overnight, a single header tank with 26 salinity water was used. All experiments began with an initial salinity of 26 in the chambers.

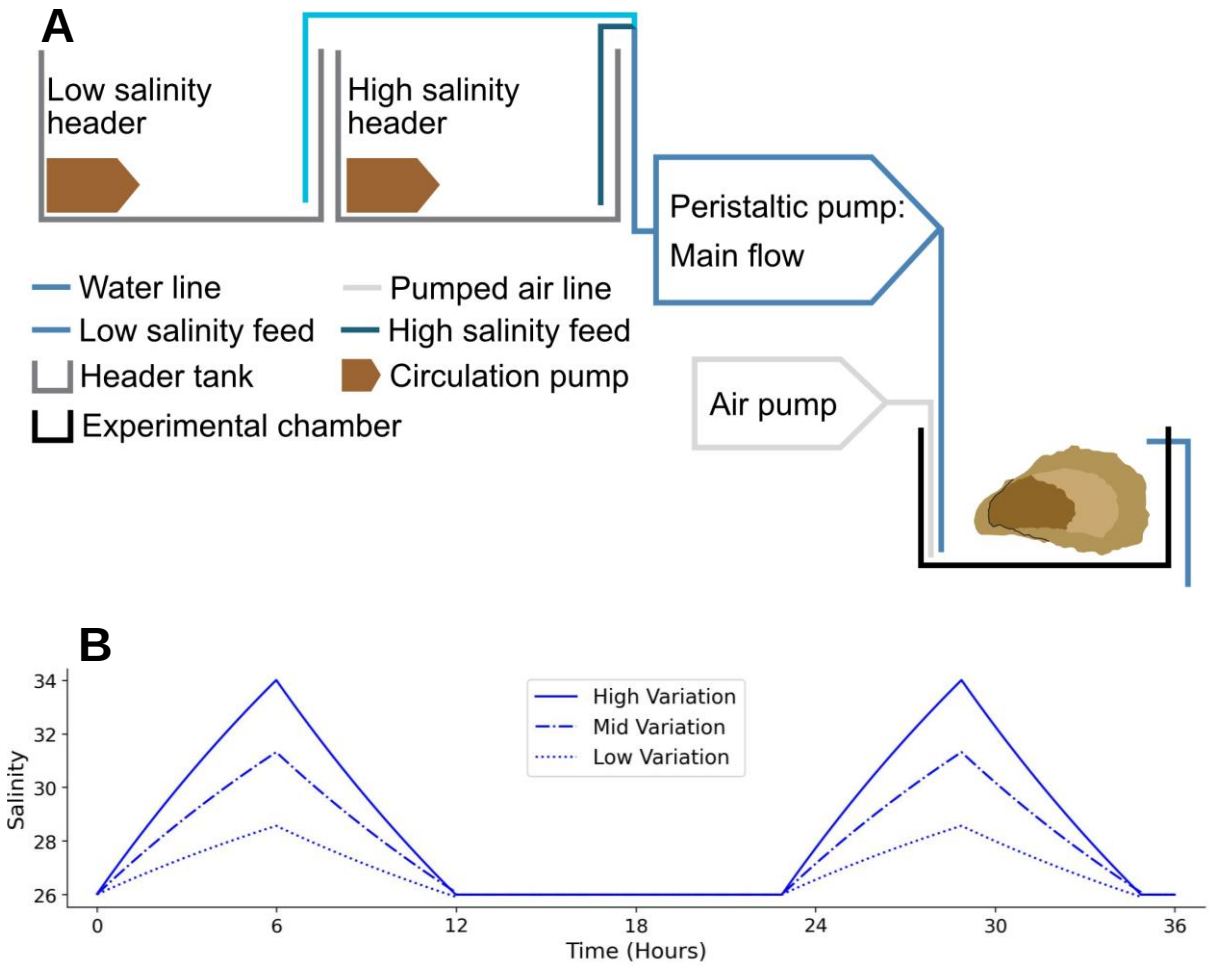

Figure 2.2 (**A**) Schematic of the salinity control system used for the 2023 experiment. Water supply was switched between the high and low-salinity header tanks after 6 h, with differing salinities (as detailed in Section 2.3.2) dependent on the treatment. (**B**) An example 36 h salinity curve produced by the system.

2.4 Experimental Set-up

Oysters were fed using a commercial shellfish feed throughout the experiments at slightly over the recommended levels to ensure that food limitation did not occur. In 2020 food was provided from a single separate header tank, in which the feed was stored in artificial seawater (30 salinity) at a 250-fold dilution and fed at a slow drip of 0.1 mL min^{-1} directly into each chamber by a separate, but identical, peristaltic pump to the one used for primary flow management. In 2023 all header tanks were dosed with feed.

Twelve separate 1 L tanks were used as experimental chambers. These had an overflow drilled to ensure a constant level of 1 L of water throughout the experiment and drained into a sump tank fitted with a pump for emptying as required. Fluid and feed ingress to the chambers was mounted at the opposite end to the overflow, with mixing aided by gentle bubbling.

In 2020 the experiment was carried out in a fully dark room in which there was minimal disturbance. Red light was used when entering the room for experimental maintenance. In 2023 this space was unavailable, and a larger laboratory was used. In order to maintain constant conditions the light remained on 24/7, with the oysters kept under an opaque tarpaulin throughout each experimental run. In both years experiments were conducted at the same ambient temperatures as the acclimation period, 16.5 and 19 °C in 2020 and 2023 respectively.

Three experimental runs were successfully completed in each year. In 2020 each commenced at 1900 h and ran for 5 d, in 2023 each commenced at 0700 h and ran for 4 d.

2.5 Sensing Equipment

2.5.1 Salinity

Salinity data in 2020 were measured for oysters E1 and E2 at 15 min intervals using a desktop laboratory meter (HQ440D desktop meter, Hach, Colorado, USA) equipped with two conductivity probes (Intellical CDC401, Hach, Colorado, USA). Salinity for E3 oysters was measured at 5 minute intervals using a handheld multiparameter water quality meter (YSI ProDSS equipped with ProDSS conductivity and temperature sensor).

2.6 Gape Sensing and Data Normalisation

The NOSy unit was used in both years, procedures for setup and data normalisation were as described in Chapter 2, Section 2.4. Second averaged data were used for these analysis as the relatively short run durations meant that data files would not become large enough to be computationally limiting.

2.7 Data Treatment, Plotting and Statistical Analyses

Excel 2016, Python (v3.9) and the *pandas* (McKinney, 2010) and *NumPy* (Harris *et al*., 2020) packages were used for all data processing. Plotting utilised the *matplotlib* (Hunter, 2007) and *Seaborn* packages (Waskom, 2021)

2.7.1 Oyster Mortality, Sensor Failure and Non-Experimental Hours

In instances of sensor failure and oyster mortality all data for that oyster were excluded from the analysis in order to ensure that potentially unreliable data did not influence the analysis. In instances where an oyster was dead at the end of an experimental run it was not possible to confirm that sensor failure had not occurred prior to mortality, so death and sensor failure were treated as indistinguishable events. Additionally, data collected overnight during 2023, when all oysters were kept in a control salinity, were excluded from the analysis.

2.7.2 Behavioural Indices

Two behavioural indices were used to classify oyster behaviour, the percentage of time spent open and closure rate as closure events per day. These were calculated for each individual oyster using 0.2 gape as the threshold for closure, as described in Chapter 2, Section 2.4.

2.7.3 Processing of Salinity Data

Salinity data from 2020 were linearly interpolated between sampled data points (detailed in section 2.5.1) to calculate equivalent data points for the minute averaged gape data. In instances where sensor failure occurred, salinity data from the following cycle were substituted. n.b. during day 3 of Run 1 in 2020 the sump tank flooded the experimental chambers, resulting in all chambers staying at a salinity of ~30 for ~10 hours.

2023 salinity data were estimated for each minute using modelled data based on flow rates, volumes and salinity of incoming water.

All gape data were classified as from either increasing or decreasing salinities, based on when they were logged within the simulated tidal cycle. Increasing data modelled the flood tide, and decreasing modelled the ebb.

2.7.4 Plotting

Gaping plots were presented as individual line plots for each oyster, with relevant salinity data also shown on the plots. Behavioural index results were presented as standard boxplots, showing the median value, quartiles and full range in the box and whiskers. Outliers, as calculated by default *Seaborn* procedure, were presented as shaded diamonds, these were not excluded from the statistical analysis. Mean values (white dots) and sample sizes were also shown.

2.7.5 Statistical Analysis

Due to the high sensor failure/mortality rates in some treatments, resulting in small and uneven sample sizes, a non-parametric approach to analysis was taken.

Other than for comparison of behavioural indices between the two experiments data from the 2020 and 2023 experiments were treated entirely separately. Selected results are presented in the main text, a full tabular breakdown of all statistical testing is shown in Appendix 1.

2.7.5.1 Comparisons between years, treatment and runs

Time spent open and closure rate was calculated for all oysters for the duration of each experimental run. Kruskal-Wallis tests were then carried out between years, treatments and runs using the *Pingouin* Python package (Vallat, 2018).

2.7.5.2 Comparison between salinity directions

Data were split by salinity direction, time spent open and closure rates were then calculated for each oyster for each salinity direction. Analysis was then carried out as per section 2.7.5.1.

2.7.5.3 Comparison between binned salinities

Only data from experimental oysters in 2020 (R*x*E*x*_20) and the high salinity variation in 2023 (R*x*H*x*_23) were used for this analysis. Salinities were classified into 4 bins, <28, 28-30, 30-32 and >32. Time spent open and closure rates were then calculated for each oyster/salinity bin. Analysis was then carried out as per section 2.7.5.1.

3 Results

In total 13 oysters were excluded from the results, due to either sensor failure or mortality, and summarised in Table 3.1. Three were excluded in 2020, and 10 in 2023, a full list is provided in Appendix 2.

Table 3.1: Summary of oysters excluded from the final analysis of this experiment. All treatments began with 3 oysters per run (9 in total per condition)

Year	Condition	Number of Oysters Excluded
2020	Experimental	2
	Low salinity control	1
2023	Low-variation	2
	Mid-variation	4
	High-variation	4

3.1 Gape and Salinity Data

Example normalised gape plots, also detailing salinity, from each year are shown in Figure 3.1. Equivalent plots for every oyster are provided in Appendix 3.

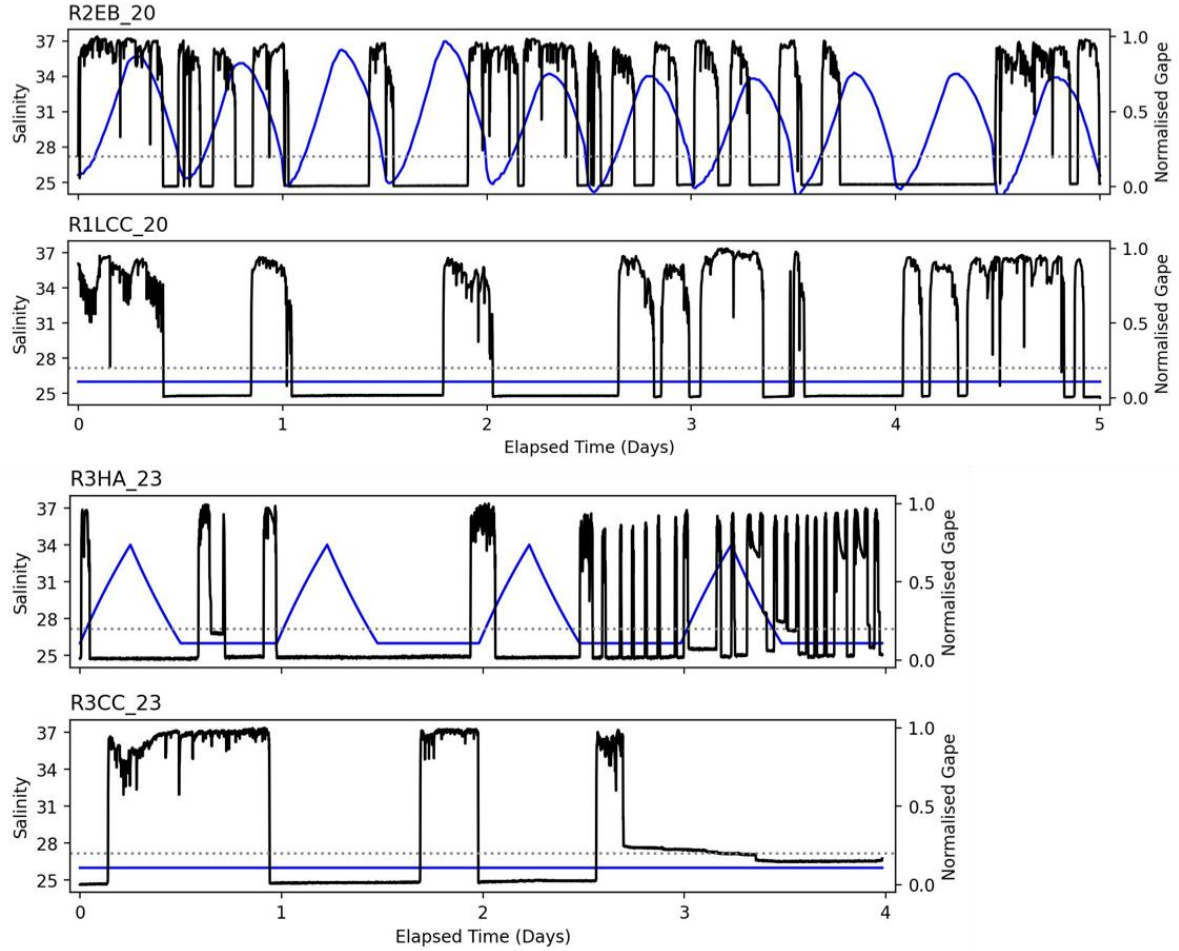

Figure 3.1: Example plots showing salinity (blue line) and normalised gape (black line) over the duration of an experiment for oysters from a 2020 experimental run (R2EB_20), a 2020 low-salinity control run (R1LCC_20), a 2023 high-variation run (R3HA_23) and a 2023 control run (R3CC_23). The grey dotted line indicates the gape level below which oysters were considered closed.

Behavioural indices for the oysters in Figure 3.1 are provided in Table 3.2.

Table 3.2. Example behavioural index results for the oysters included in Figure 3.1. These are included as illustrative data to contextualise the plots and indicate the processing procedures used throughout.

Oyster	Experimental Condition	Time open (%)	Closure rate (closures d^{-1})
R2EB_20	Experimental	57.2	5.7
R1LCC_20	Low-salinity control	65.8	3
R3HA_23	High-variation	16.1	6
R3CC_23	Control	28.1	0.5

3.2 Behavioural Indices

3.2.1 Differences Between Years

Oyster behaviour across all treatments was significantly different between the two experiments for both behavioural indices (Figure 3.2). Oysters spent more time open (Kruskal-Wallis, $H_1 = 6.12$, $p = 0.01$) and had a higher closure rate (Kruskal-Wallis, $H_1 = 5.97$, $p = 0.01$) in 2020.

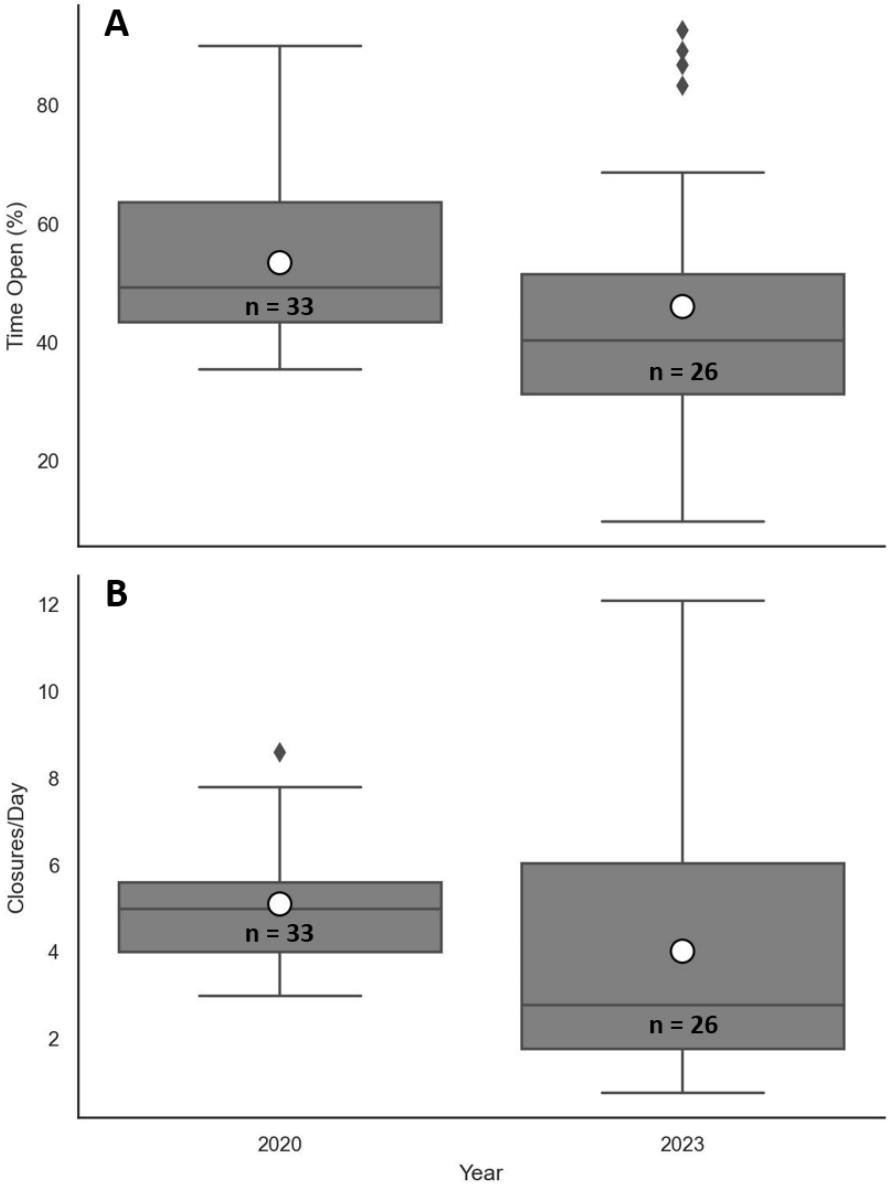

Figure 3.2: Boxplots showing time open (**A**) and closure rate (**B**) for all oysters in experiments conducted in 2020 and 2023. Median, quartiles and full range are shown by the box and whiskers, outliers by shaded diamonds and the mean values by white circles.

3.2.2 Effect of Experimental Condition

In 2020 there was no significant difference found in either behavioural index between conditions (Figure 3.3). The difference in median time open was not statistically significant (Kruskal-Wallis, $H_3 = 7.48$, $p = 0.06$), however experimental oysters spent the most time open (median = 67%). Median closure rates were within 1.3 closures d^{-1} between conditions.

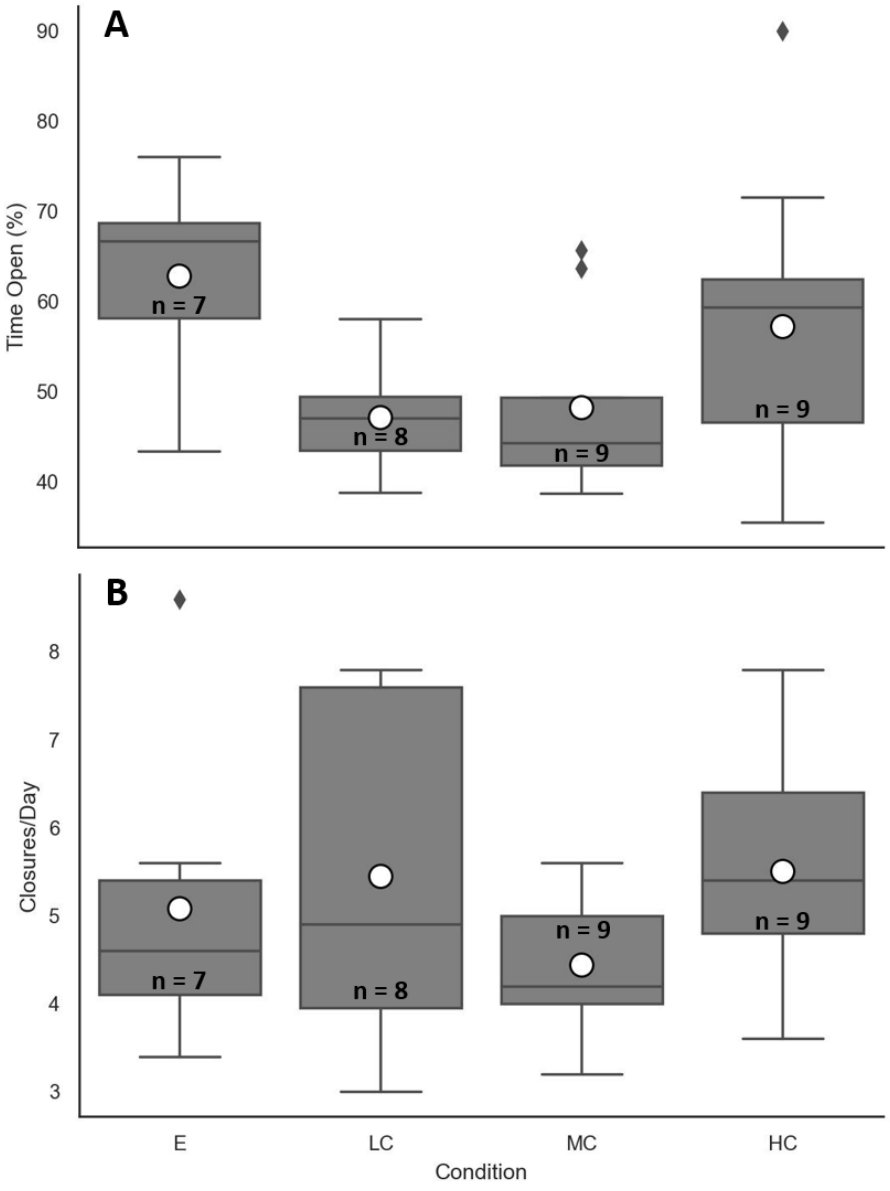

Figure 3.3 Boxplots showing time open (**A**) and closure rate (**B**) in each experimental condition in 2020. The conditions are E – experimental, LC – low salinity control, MC – mid salinity control and HC – high salinity control. Median, quartiles and full range are shown by the box and whiskers, outliers by shaded diamonds and the mean values by white circles.

In 2023 there was no significant difference found in either behavioural index between conditions (Figure 3.4). The difference in median time open was not significant (Kruskal-Wallis, $H_3 = 6.9$, $p = 0.08$), although high variation oysters spent 10 percentage points less time open than mid variation oysters, the next least open group. Median closure rates were within 1.3 closures d^{-1} between conditions.

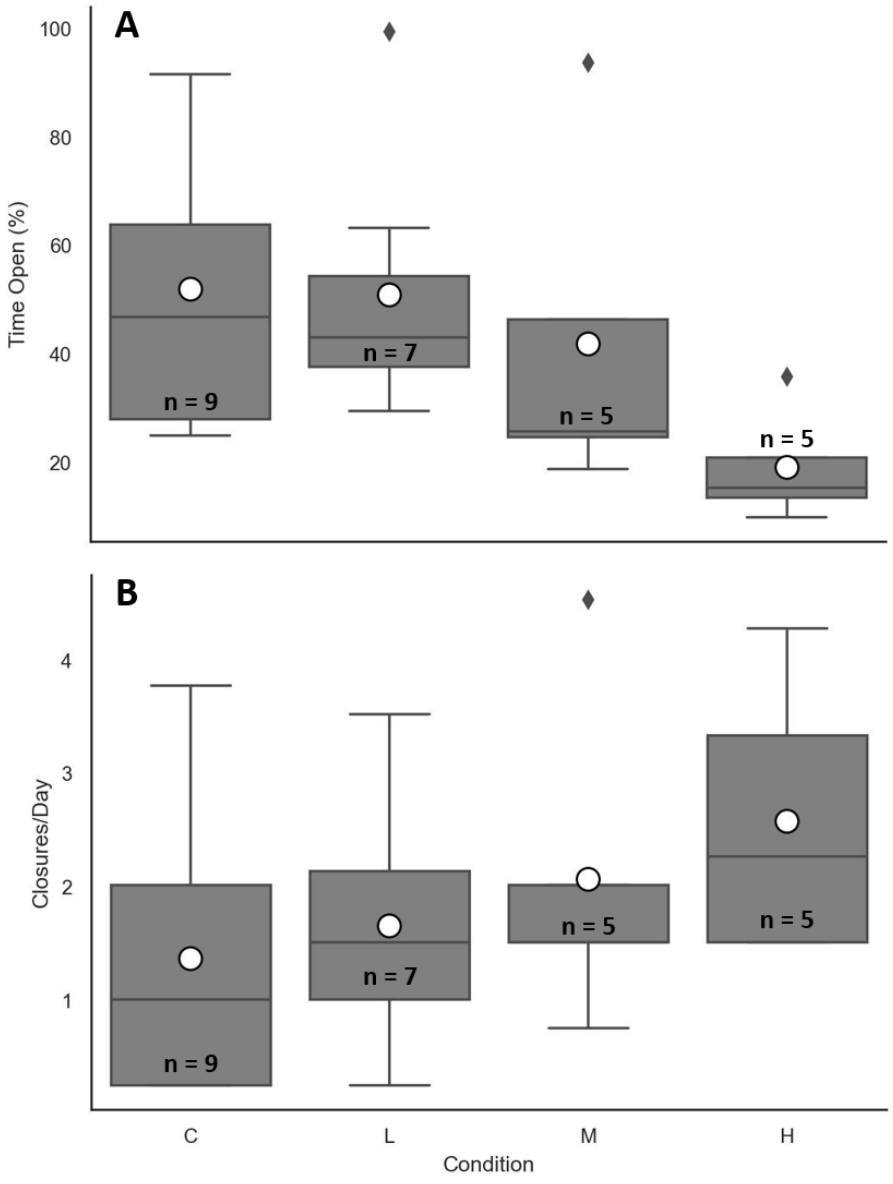

Figure 3.4 Boxplots showing time open (**A**) and closure rate (**B**) in each experimental condition in 2023. The conditions are C – control, L - low salinity variation, M - mid salinity variation and H - high salinity variation. Median, quartiles and full range are shown by the box and whiskers, outliers by shaded diamonds and the mean values by white circles.

3.2.3 Differences Between Runs

In 2020 there was no significant difference found in either time open or closure rate across all treatments between runs (Figure 3.5). The median time open differed by under 12 percentage points and median closure rate by 0.6 closures d^{-1}.

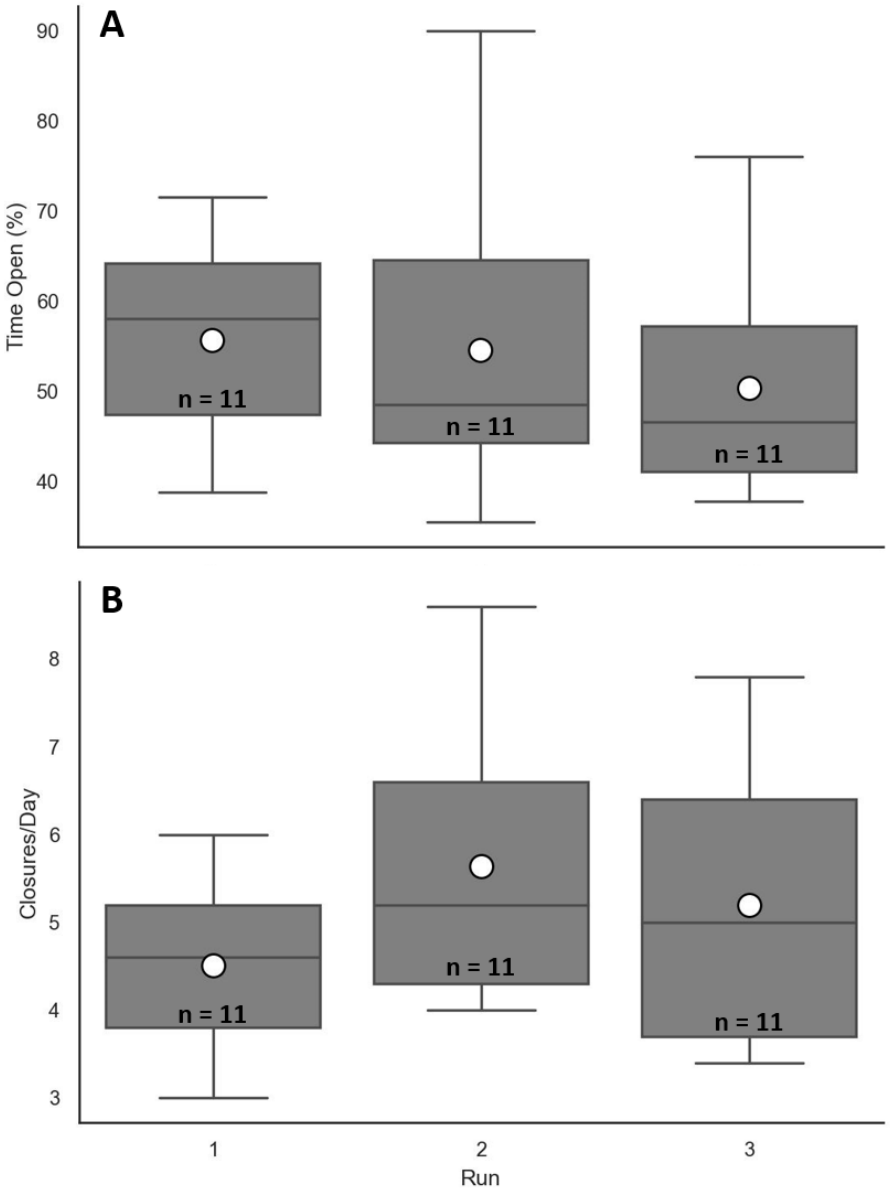

Figure 3.5 Boxplots showing time open (**A**) and closure rate (**B**) in each run in 2020. Median, quartiles and full range are shown by the box and whiskers, outliers by shaded diamonds and the mean values by white circles.

Time open across all treatments differed significantly (Kruskal-Wallis, $H_2 = 9.06$, $p = 0.01$) between runs in 2023 (Figure 3.6A). Oysters in run 1 spent the most time open, there was a difference of between 10 and 36 percentage points in the time spent open between run 1 and runs 2 and 3 respectively. The difference in median closure rate was insignificant between runs, with all rates within 1.8 closures d^{-1} (Figure 3.6B).

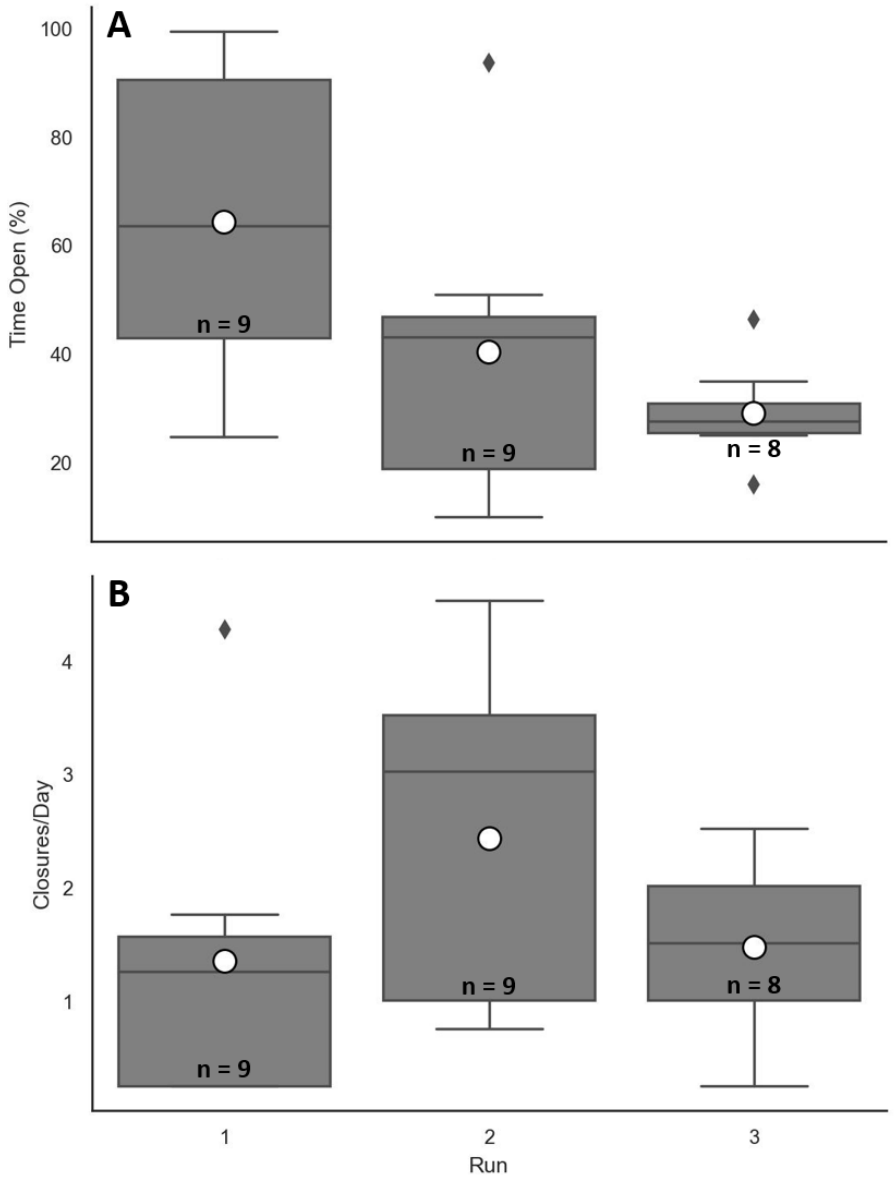

Figure 3.6 Boxplots showing time open (**A**) and closure rate (**B**) in each run in 2023. Median, quartiles and full range are shown by the box and whiskers, outliers by shaded diamonds and the mean values by white circles.

3.2.4 Effect of Salinity Direction

There was no significant difference in either behavioural index between salinity directions in 2020 or 2023. This remained the case when only 2020E and 2023H populations were analysed.

3.2.5 Difference Between Salinity Bins – 2020E and 2023H Only

There was no significant difference in either behavioural index in oysters from the experimental condition between the 4 salinity bins in 2020, although there was a trend of reduced time spent open with increasing salinity, from 60.1% in the lowest salinity bin to 40.1% in the highest. Variability in time spent open also increased with salinity, with an interquartile range of 11 and 23 percentage points in the lowest and highest salinity bins respectively (Figure 3.7A). Median closure rates were higher, at 7.2 closures d^{-1}, in the lowest salinity bin than the rest which ranged from 4.1 to 4.4 closures d^{-1} (Figure 3.7B)

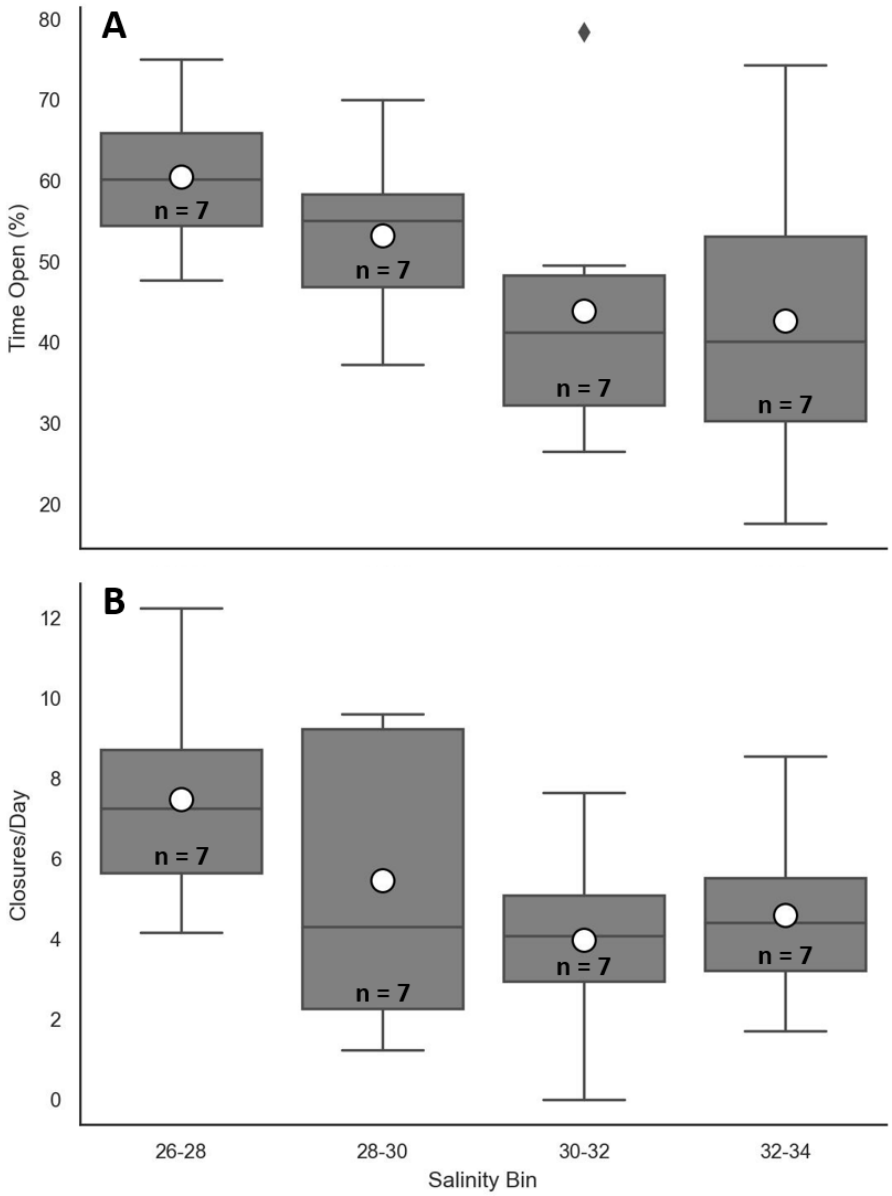

Figure 3.7 Boxplots showing time open (**A**) and closure rate (**B**) for experimental treatment oysters between each salinity bin in 2020. Median, quartiles and full range are shown by the box and whiskers, outliers by shaded diamonds and the mean values by white circles.

There was no significant difference in either behavioural index in oysters from the high salinity variation condition between the 4 salinity bins in 2023. Similarly to 2020, there was a trend of reducing median time spent open with increasing salinity (Figure 3.8A), from 30.5% in the lowest bin to 8.5% in the highest. Variability was also greatest in the highest salinity bin, with an interquartile range of 45 percentage points, this ranged from 9 to 16 percentage points in the other salinity bins. Median closure rates also reduced with salinity, from 7.2 closures d^{-1} in the lowest salinity bin to 4.4 in the highest (Figure 3.8B).

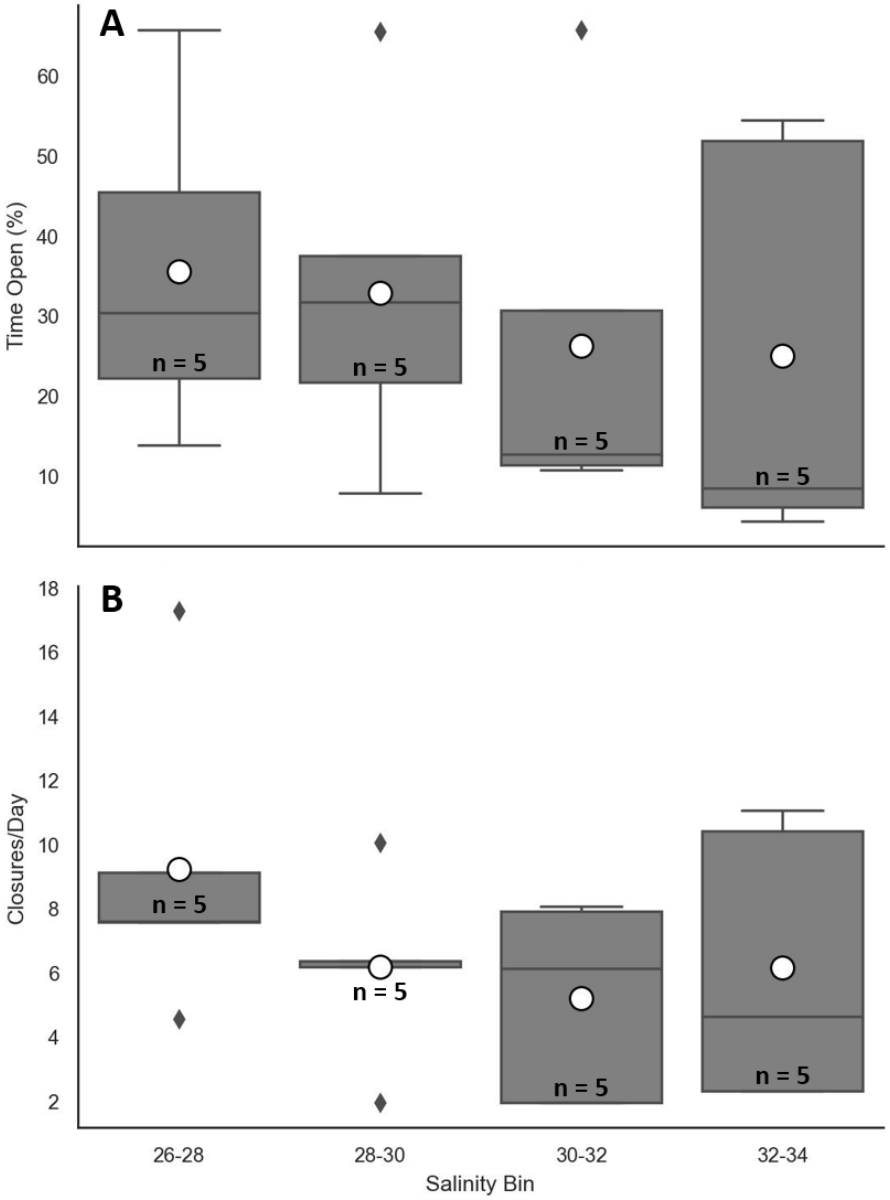

Figure 3.8 Boxplots showing time open (**A**) and closure rate (**B**) for high variation treatment oysters between each salinity bin in 2023. Median, quartiles and full range are shown by the box and whiskers, outliers by shaded diamonds and the mean values by white circles.

4 Discussion

Neither of the two experiments showed a significant impact of variation in salinity on either behavioural index. We are confident that our sensing equipment functioned properly and was sensitive enough to have detected relevant oyster behaviours, having done so in field trials. However, high mortality rates, particularly in 2023, resulted in small and uneven sample sizes.

Replication of salinity cycles in the laboratory also proved challenging. The 'Victorian-Plumbing' system used in 2020 was very effective, but was extremely sensitive to small user errors (Figure 2.1A). Mistakes such as incorrectly positioning an airtight valve by <1 mm could lead to early release of water from vessels, altering the salinity cycle and nullifying 24 hours of experimental data. As a result a number of runs of the experiment were unusable and the decision was taken to redesign the system.

Initially the redesigned system was conceived to use two peristaltic pumps with programmed cycles, the first delivering a constant flow of feed-dosed ~20 salinity water at a high rate. The other a low rate of hyper-saline water at a changing rate to provide salinity variation. In principle this would have been an effective design. However, whilst the engineers at Watson-Marlow assured us it was possible to program an Arduino to control the pumps, we did not succeed in doing so. One of the pumps also failed, and the cost of fixing it was prohibitive.

As a result the system as used in 2023 was designed, initially for use in 2022. This design had two significant drawbacks. Firstly it was not practical to run for 24 hours, as it required the hoses to be swapped over every 6 hours, meaning that oysters were left in a control salinity overnight. Secondly the replicated cycle was linear, rather than sinusoidal, and not a true replication of a typical tidal cycle (Figure 2.2B). Use of this system was also delayed by safety concerns around the use of standard voltage electronic pumps in aquaria at the University of Essex, which were needed for circulation in the header tanks. This meant that

only 3 runs of the system were possible in the available time. Further work is required to integrate the use of high-precision, low-volume and programmable pumps into this experimental methodology, but to date no suitable product has been identified.

It is perhaps not surprising that we did not detect significant impacts of salinity, given that all values examined were within *M. gigas*' optimal range (Shatkin *et al*., 1997). However, some non-significant trends and results (e.g. sections 3.2.2 and 3.2.5) were sufficient to suggest that salinity has some impact on *M. gigas* behaviours, even within their optimal range. Where these trends were seen they were broadly in line with the patterns of reduced opening at higher salinities observed in the field (Chapter 2, Figure 3.3). This indicates that greater sample sizes (either with more oysters or longer term experiments), or a greater salinity variation would clarify the impact of relatively fine-scale salinity variation on *M. gigas* behaviours.

The difference in time spent open between runs in 2023 (Figure 3.6) suggests that there may have been a loss of condition during the longer acclimation period for runs 2 and 3, despite food levels being kept in excess and regular water changes taking place. No such difference was seen in 2020, although the acclimation period was of a similar length and oysters were taken from the river at a similar time of year. The significant difference in results between the two years (Figure 3.2) confirms that the two experiments were sufficiently different to require totally separate analysis, although the broad conclusions from both are the same.

These experiments, which replicated the observed tidal salinity variation from the site of the field trials, show that the observed behaviours in *M. gigas* in the Pyefleet are likely as a result of factors other than salinity. Some authors have suggested that gaping in bivalves has an endogenous circadian rhythmicity, driven by internal cues. For example, Bertolini *et al.* (2021) monitored the gape of wild Mediterranean mussels (*Mytilus galloprovincialis*) in Venice Lagoon for 6 months, demonstrating periodic rhythms on both 12 and 24 hour cycles,

with a seasonally differing dominance between the two. In this case, the authors concluded that the rhythm seen was endogenous. Retailleau *et al.* (2023) showed that Great scallops (*Pecten maximus*) have significantly greater gaping overnight, although they suggest that these patterns are driven by light intensity rather than internal rhythms.

Studies with *M. gigas* however suggest that their circadian rhythms are highly plastic, and apparently entrained by external cycles, primarily the twice-daily tidal cycle, photoperiod on a 24h cycle and lunar cycles of 27-29 days, (Mat *et al*., 2014, 2012; Tran *et al*., 2011). This suggests that behavioural rhythmicity in *M. gigas* is primarily exogenous, driven by external cues. More recently Tran *et al.* (2020b) examined gaping activity and the activity of circadian clock and clock-associated genes in *M. gigas*, the organisms they examined exhibited clear endogenous cycles, in both gene expression and behaviours. Behavioural rhythms in marine organisms are highly complex, in this case the authors conclude that whilst endogenous cycles exist in *M. gigas*, external cues are capable of entraining these cycles. They suggest photoperiod and current direction as candidates for this, but there are many other possibilities.

The relationship between gape and feeding behaviour is indirect but well established in bivalves (Ballesta-Artero *et al*., 2017; Riisgård and Larsen, 2015; Williams and Pilditch, 1997). Blue mussel (*Mytilus edulis*) gape increases and decreases relatively quickly (<1 h) in response to increasing and decreasing availability of food (Riisgård *et al*., 2006). Higgins (1980a, 1980b) demonstrates that juvenile Easter oysters (*Crassostrea) virginica* are significantly more open in the presence of food, reacting within 30 min to the introduction of algal cells, clearance rates were greatest immediately after valve opening and, even in the presence of constant food volumes, decreased thereafter, indicating that satiation may take place.

Suspended particulate matter (SPM) concentration in estuaries, a proxy for bivalve food availability, varies considerably over a tidal cycle (Lindsay *et al*., 1996). Whilst the patterns of

variation in the Colne are not well characterised, in the Tay Estuary the highest concentrations are found near mudflats, such as those found on the banks of the Colne and Pyefleet (McManus, 2005). Fegley *et al.* (1992) analysed seston quantity and quality near the benthic boundary layer over the tidal cycle in Great Sound, New Jersey. They showed that intra-cycle variability was nearly as large as intra-annual variability. Huang *et al.* (2003) showed similar patterns in the seston in saltmarshes around Delaware Bay, USA.

Without further in-depth study of local patterns of SPM concentrations over the tidal cycle it is not possible to draw firm conclusions, but there is the possibility that the observed patterns in the NOSy data collected in the field are as a result of variation in SPM/seston levels, as these are closely correlated to food availability for the filter feeding *M. gigas*.

Temperature must also be considered as a possible influence on *M. gigas* gaping behaviour, although during the period highlighted in Chapter 2, Figure 3.3 the temperature remained between 18 and 22 °C, comfortably within the optimal range for *M. gigas* (Bayne, 2017e). Further work in the laboratory, as well as an in-depth analysis of temperature and gaping patterns in the field, would be required to address whether tidally linked temperature variations are significant in determining gaping patterns in oysters around the Essex coast.

Predator presence can also affect gaping behaviour in bivalves, even without direct disturbance (Carroll and Clements, 2019; Clements *et al*., 2020; Dzierżyńska-Białończyk *et al*., 2019). Predator presence induces rapid closures in *M. edulis*, at the cost of reduced feeding and respiratory opportunity (Robson *et al*., 2010). This cost can be significant, Hard-shell clam populations have significantly higher mortality when indirectly exposed to Blue crabs (*Callinectes sapidus*) for extended durations (Smee and Weissburg, 2006). C*. virginica* on a natural bed in North Carolina, USA, close in the presence of *C. sapidus*, remaining so for ~5 min after the crabs have departed.

Shore crabs (*Carcinas maenas*) prey on oysters of all size classes and are the predator species most commonly observed in the presence of *M. gigas* at the NOSy study site (Miron *et al*., 2005). *C. maenas*, particularly males larger than 35 mm carapace width, migrate with the tide, moving inshore with the flood and departing with the ebb. If *C. maenas* in the Colne follow this pattern, then there is likely to be an increased predation pressure at high water, concurrent with the high closure rates observed in the field.

The changing chemical balance of estuarine water over a tidal cycle may also be a factor, the patterns of which are extremely variable between estuaries (Middelburg and Herman, 2007). Bivalve gape is known to be responsive to chemical cues, e.g. heavy metal concentrations (Kelleghan *et al*., 2023). It is therefore possible that tidally synchronous water chemistry patterns may play a role, but patterns in the Colne are insufficiently characterised to suggest a direct relationship.

5 Conclusion

We were unable to reproduce the behavioural patterns of *M. gigas* observed during NOSy field trials via salinity manipulation. Whilst the observed trends suggest that salinity plays a role in these patterns there were no significant impacts of salinity on the behaviour of experimental oysters and we conclude that other factors also influence the gaping behaviour of *M. gigas* in the Pyefleet.

Replicating salinity cycles, whilst keeping all other conditions constant, is challenging in a laboratory. It is surprising that we were unable to find an established laboratory methodology for manipulating salinity in a controlled environment, but the protocols established during this investigation were relatively effective. Further optimisation of the methodologies we developed for these experiments has clear potential in studying the impact of controlled salinity variation on aquatic organisms, something which is underrepresented in the literature. Greater understanding of the factors that influence gaping behaviours could also have practical benefits in enhancing the efficiency of depuration protocols, as well as enable

producers to make more informed decisions about optimal stock locations in estuarine environments.

Future work to build on these experiments would require two avenues of investigation. Firstly in the laboratory, where methodological refinement would increase the efficiency of experimentation and expansion of the salinity range could clarify the relationship between tidal-scale salinity variation and *M. gigas* gaping behaviour. Secondly, further field deployments of the NOSy unit, accompanied by comprehensive water monitoring and sampling, would allow a wider range of potential influencing factors to be monitored and analysed.

Chapter 4

Trial of a Land-Based Oyster Nursery System for Commercial Use

Abstract

The majority of oysters grown by Colchester Oyster Fishery are originally purchased from hatcheries, representing a considerable annual cost. The current methods of growing these purchased oysters (seed) to a size at which they are sufficiently likely to survive in the company's main growing areas are labour intensive and expose the oysters to a high degree of risk. In this study an alternative, land-based, upwelling system was trialled over the course of three summers (2020-2022).

In 2020 the systems were stocked at two densities (n = 3), initially 90 and 110 % of hatchery recommended levels. The system was only operational for 34 days due to technical failure, but within that time significant differences in growth rates were seen between the two densities. In 2021 stocking levels were dropped by 90 % due to low seed availability and the system was stocked with oysters that were 80 % smaller than in 2020. Two different densities, at the same ratios as in 2020, were trialled, with no impact (n = 3) on averaged growth. Growth uniformity was notably different between the two densities; double the number (23%) of oysters in the high density treatment remained below 0.2 g throughout the 177 day trial. In 2023 stocking densities were uniform and a layered container system was introduced in one treatment (n = 3). In the layered treatment overall growth rates and growth uniformity were significantly improved in relation to the non-layered treatment. We suggest that lowering the localised density reduced competitive asymmetry, preventing larger individuals dominating and allowing all the oysters in the population sufficient access to resources. The trials demonstrated the feasibility of land-based on-growing systems, and the significant impact of simple improvements in stocking protocols.

1 Introduction

1.1 Seed Oyster Growing Systems

Bivalve aquaculture facilities rely on two main sources of seed (juveniles) to be grown to market size; natural settlement and from hatcheries, over 90 % of the oysters currently

harvested for sale by Colchester Oyster Fishery were originally purchased from hatcheries (P Harding, Colchester Oyster Fishery, personal communication). Naturally settled oysters are a substantially cheaper option for growers, cultching (discussed in Chapter 1, Section 3.2) is the only cost involved. However, interannual variation in naturally settled seed numbers can pose a challenge to culture operations, which must ensure that sufficient numbers of high quality animals are recruited to their stock each year to maintain supply to the market.

Many operations therefore rely on bringing in seed from hatcheries, a more controllable seed source, albeit one requiring capital investment. In order to maximise the return on this investment it is important that on-farm mortality is minimised and growth maximised. Seed purchased by COF are typically too small to be laid out directly on the company's grounds and must be grown on until they reach a minimum 2 g. The current river-based methodologies for ongrowing of seed at COF, and their significant drawbacks, are discussed in detail in Chapter 2, Section 3.4. In short, the river-based method requires the use of relatively large, and therefore costly, seed and relies on regular cleaning visits by large vessels and crew to keep the oysters healthy.

Land-based systems are an alternative method for seed ongrowing with potential to address some of the issues with current COF methodologies. These systems require a capital investment and manufacturing time. They also do not negate the need for regular cleaning of the oysters, as pseudofeces build-up, sediment deposition and biofouling still occur. However, as they are situated on land these operations can be carried out at a fraction of the time and vessel cost of the current river-based system. This in turn means that cleaning events can be far more frequent, and as a result smaller mesh sizes than are currently used in the river-based methods at COF are practicable.

The pump that provides water to such systems acts as a crude mechanical filter, preventing ingress of nearly all predators, any that do get into the stock (as larvae for example) are

simply removed once spotted as the systems are easily accessible. Whittington *et al.* (2015a, 2015b) also demonstrated that the use of standing water to supply upwelling systems can confer a degree of protection from pathogens, to which small oysters are particularly vulnerable (Dégremont, 2013). These factors combine to mean that it is possible to stock the system with far smaller seed (0.15 g versus >1 g in the floating cages) without increasing mortality, a saving of ~50 % in seed price.

Siting equipment on land has the further benefit of reduced exposure to harsh weather conditions. Extreme weather events are forecast to become more frequent, which presents a significant challenge to equipment and vessels in the marine environment (Mölter *et al*., 2016). Land-based systems are not fully shielded from the impact of these events, but have a substantially reduced vulnerability compared to equipment kept in coastal and estuarine waters. Poor weather conditions are also less likely to prevent growers accessing their systems for prolonged periods, making maintenance and husbandry less problematic.

An example design for a land-based unit, as used in these trials, is shown in Figure 1.1. The illustrated system is a semi-recirculating ‘upweller’ design, in which a constant flow of water, and therefore food and oxygen, is passed up through the oyster seed before being returned a reservoir. Upwelling systems are commonly used in hatcheries, but are a novel approach at COF (Allan and Burnell, 2013).

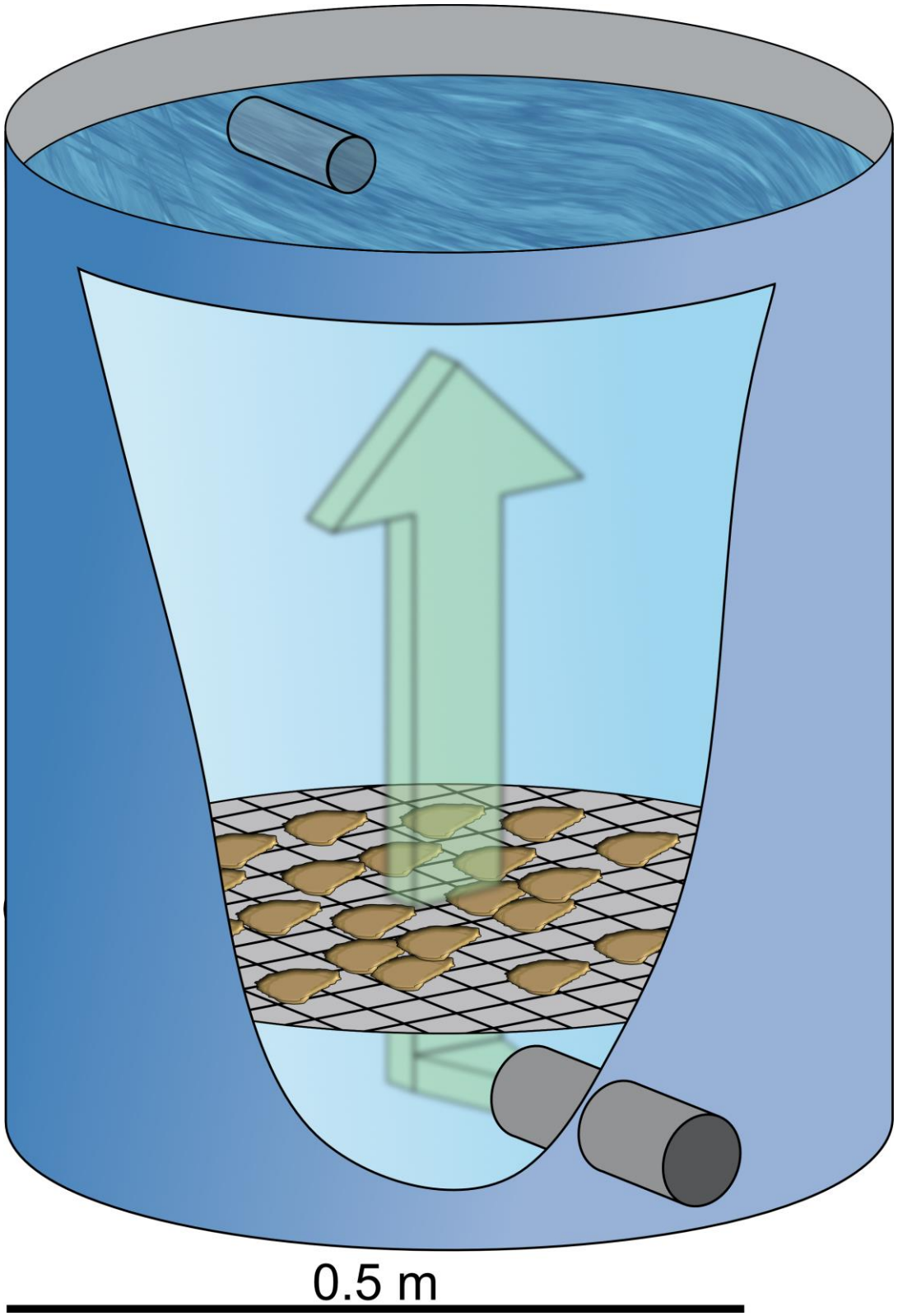

Figure 1.1: Schematic representation of the 200 L land-based upwelling nursery system developed for this study. Water enters at a rate of approximately 1 L s^{-1} through the pipe at the bottom of the unit, flows through the oysters, which sit in a mesh basket, and exits through an overflow drain near the top of the unit.

1.2 The Effect of Stocking Density

1.2.1 Competitive Symmetry

Asymmetric competition, where groups or individuals dominate others within an area or population, causes size variation in monospecific populations and is more pronounced in resource-limited conditions (Begon *et al*., 2012; Keddy, 2001; Lawton and Hassell, 1981). A positive feedback loop, in which dominant individuals outcompete subordinate ones, grow faster and increase in dominance, can arise (Cameron *et al*., 2007; Lekve *et al*., 2002; Schmitt *et al*., 1987; Weiner, 1990).

The impacts of competition in such regimes can be significantly more pronounced on the subordinate individuals within the population, which in extreme cases may fail to grow at all (Ziemba and Collins, 1999). Dominant individuals are not unaffected by competition however, limitation of resources can still impact their growth, even when they are outcompeting other individuals, reducing overall population growth (Keddy, 2001).

In contrast, where resources are less limiting, or dominant animals are removed from the environment, competition is more symmetric and growth is typically much more consistent between individuals (Obeid *et al*., 1967). Resource limitation is in part a function of population density, increasing biomass (through growth or recruitment) increases the degree of limitation in a system with constant supply, such as the upwelling units.

1.2.2 Density and Competition in Bivalves

The impact of competition increases in line with stocking density and has a major impact on growth in bivalve hatcheries (Fan *et al*., 2021), farms (Carlucci *et al*., 2010; Cubillo *et al*., 2012) and wild populations (Vincent *et al*., 1994). Competition in these populations is primarily for food, although it can be for physical space, sufficiently close proximity between bivalves can impede valve opening enough to reduce food intake (Jørgensen *et al*., 1988). In commercial culture operations the working stocking density is a compromise between a

need to grow as many individuals as possible in a cycle, and maintaining a low enough stocking density that competition does not significantly reduce growth or condition (Fan *et al*., 2021).

The degree to which density-influenced competition affects growth rates can be moderated by several factors, Vincent *et al.* (1994) showed that growth of intertidal *Macoma balthica* is significantly more impacted by stocking density in areas with lower immersion time, a proxy for food availability. Competition regimes also change over time as a result of the growth of individuals within the stock increasing the population's overall food requirements; competition in rope-grown Mediterranean mussels does not become significant until they reach over 60 mm length, regardless of stocking density (Cubillo *et al*., 2012). Bivalve hatcheries control for this by stocking relative to total biomass and water flow (K Thompson, Morecambe Bay Oysters, personal communication).

1.3 Aims and Objectives

The aim of this study was to carry out the first phase of a trial to assess the economic feasibility of upwelling land-based ongrowing systems of *M. gigas* seed oysters in comparison to the current methods used at COF. The primary objective was to optimise stocking practices of hatchery-supplied *M. gigas* in a small-scale, but commercially practical, land-based nursery system. Our intention was to make the most efficient possible use of purchased seed by obtaining high growth rates and low mortality whilst using as little energy, water and space as possible.

Growth, survival, growth uniformity and shell shape of oysters are impacted by a range of factors, discussed in detail in Chapter 2, Sections 2.2 and 3.4. Food limitation and hypoxia, both of which are functions of overcrowding in circulating systems, are perhaps the most relevant, so stocking density will be the first factor considered. Results from the 2020 trial

suggested that stocking pattern also has an impact, trials in 2022 therefore tested the impact of reducing localised density by spreading oysters out within a system.

2 Materials and Methods

2.1 System Design

All work for this chapter took place on-site at COF (Chapter 2, Figure 1.4) The system built to trial this method (Figure 1.1 and Figure 2.1) was constructed from six ~200 litre food-safe plastic barrels, with a constant pumped flow (~200 L min^{-1}) of marine water from an on-site reservoir of approximately 2.5 million L. The reservoir also supplies the main fishery building and tanks at COF and is replenished every fortnight, on spring high tides. Oysters were stocked in mesh baskets, allowing for easy changes in mesh size, and therefore optimal flow through the stock at all sizes.

The system consisted of six virtually identical barrels with a 60 cm diameter and ~170 L working capacity (Figure 2.1). Water entered via an inlet pipe near the bottom of the barrel. A 90° elbow directed the flow downwards into the bottom of the unit in order to reduce the impact of relatively high pressure water hitting the oysters, as well as induce a degree of turbulence with the aim of reducing sediment settlement on the base of the units. The water then drained by overflow at the top for return to the reservoir.

The units were fed via a manifold from a single submersible electronic pump (Nova 600, DAB Pumps, Mestrino Padova, Italy) submerged in the reservoir, which supplied ~200 L min^{-1}, the residence time of water in each unit was between 3 and 6 minutes. In this system flow rates were constant throughout each trial. Due to the single manifold construction, flow was not identical between the barrels, the central units received slightly more water than the ones on the outside. The magnitude of this difference varied between pumps and over time as fouling built up. Experimental treatments were placed in alternate

units (i.e. treatment A in units 1, 3 and 5 and treatment B in units 2, 4 and 6) to control for the impact of flow variation.

Figure 2.1: The trial upweller system in operation at COF. Shown are: The six individual units (**A**), equipment and spares storage (**B**), the inlet and drain hoses (**C**), the River Colne (**D**) and the marine reservoir that supplies the units (**E**). Not shown is the pump, which is submerged to the right of the image

There was no non-return valve fitted to the inlet hose, this meant that in the event of pump or power failure the units would drain, preventing stagnation of the relatively small volume of water in the barrels. In 2021 a 4G enabled solar powered security camera (Go-Plus, ReoLink Hong Kong) was mounted near the drain hose and checked remotely twice-daily, on top of on-site staff carrying out sporadic checks when convenient. This meant that in the event of pump or power failure the oysters in the system could be transferred to the reservoir within a few hours until the failure could be rectified.

The system was partly redesigned in 2022, with the addition of layers within the units to allow the stock to be spread (Figure 2.2).

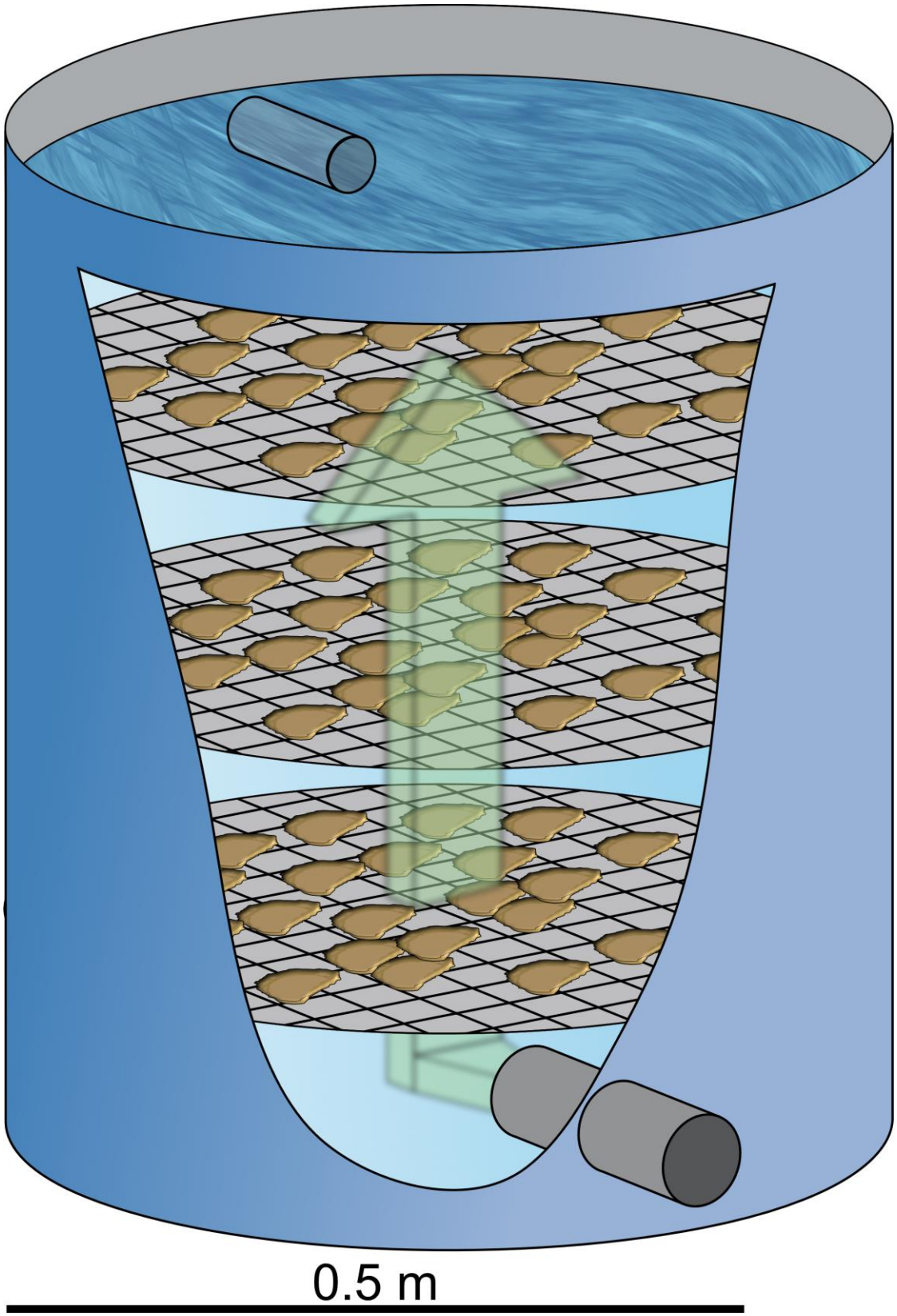

Figure 2.2 Schematic representation of the 200 L land-based upwelling nursery system developed for this study showing the additional layers added to the upweller units for the 2022 trial. Water enters at a rate of approximately 1 L s^{-1} through the pipe at the bottom of

the unit, flows through the oysters, which are distributed between the three layers, and exits through an overflow drain near the top of the unit.

2.2 Check-In Procedure

The size and quantity of the oysters used to stock the units were determined by COF, who contributed part of their seed order to the project. In 2021 a specific request was made to the hatchery for small seed, which was used in the upwelling units. A similar request was made in 2022, but none were available.

On arrival of the seed oysters, 1000 oysters were haphazardously selected and weighed to establish an initial average oyster weight. The stocking program for each year is summarised in Table 2.1.

Table 2.1: Stocking summary for each trial of the land-based upwelling units, summarising oyster weights, experimental treatments and stocking biomass and number per unit.

Parameter	**2020**		**2021**		**2022**	
Estimated individual weight (g)	0.28		0.06		1.12	
Stocking density/pattern	High	Low	High	Low	Single	Layered
Initial unit total biomass (kg)	4.4	3.6	0.41	0.34	1 × 2.44	3 × 0.82
Initial unit stocking number	16 000	13 000	6 400	5 200	2 200	3 × 700

2.2.1 Experimental Set-Up - 2020

The aim of the 2020 trial was to assess the impact of stocking density on growth and survival, as such the units were split into high and low density treatments.

Oysters for 2020 were delivered from Morecambe Bay Oysters, UK as part of the COF primary seed order on the 29th of July. COF allowed us access to as many as necessary to stock the units. Initial stocking of the units was at 90 % and 110 % of the hatchery-recommended level of 10 L min^{-1} kg^{-1} oyster; 3.6 kg and 4.4 kg respectively. Experimental treatments were placed in alternate units to distribute them between potential flow rate variations.

A further 3 samples of 3.6 kg were placed in mesh bags on a floating raft in the river (Chapter 2, Figures 2.6 and 2.7) for comparison to the land-based units. However, after two

weeks, likely as a result of two very hot days with midday slack waters, all oysters on the floating raft died.

2.2.2 Experimental Set-Up - 2021

The aim of the 2021 trial was also to assess the impact of stocking density on growth and survival using the same pattern of treatment but with much smaller oysters than in 2020.

Oysters were delivered on the 28th of April 2021 from Morecambe Bay Oysters, UK. A separate order was made specifically for this trial in order to test smaller oysters in the system, a limited quantity of these were delivered, resulting in a stocking density 90 % lower than the previous year. Stocking was carried out at 90 % and 110 % of an even division between the units (336 and 410 g in the high and low stocking density treatments respectively) and distributed in the same way as in 2020.

2.2.3 Experimental Set-Up - 2022

The aim of the 2022 trial was to assess the impact of stocking pattern on growth and survival. The stocking density was uniform in all 6 units, but in three there was a layered treatment, in which the oysters were spread out over three layers. A single layer was used in the other three units.

Oysters for 2022 were shipped from the France Naissain hatchery, France, arriving at COF on the 16th of March 2022 (day -28). The seed were in poor condition, with >10 % dead on arrival. Accurately assessing the mortality status of a small oyster is very difficult as they can be dead but remain fully closed for some time. It was therefore decided to portion the oysters evenly between the upweller units and allow them to settle for 4 weeks.

On the 13th of April (day 0) a second check in was carried, out. Each oyster was inspected and a further ~10 % were discarded, leaving ~13 100 individuals. The units were then stocked with six even divisions of the total weight of oysters. Three of the units were stocked

as a single layer of ~2 200 oysters whilst three were further split into three even vertical layers of ~700 oysters within the same upwelling unit. The position of oysters within the layered treatments remained consistent throughout the trial.

Nine oysters per unit (3 per layer where applicable) where glued to a plastic strip with UV curing fibreglass resin (Solarez Ding Repair, Vista, CA, USA) for ongoing size (height and length) monitoring. Unfortunately these oysters experienced high (>50 %) mortality, so the data were not usable.

2.3 Ongoing Data Collection and Maintenance Procedures

At each data collection event oysters were removed from the units and cleaned of settled sediment, pseudofeces and fouling organisms. They were then allowed to drain and weighed as a full sample. During this period the units were cleaned of any built up sediment.

Once the water flow through the units was restored, a triplicate water sample was taken and a known volume filtered through a GF/F filter (Whatman UK; poresize 0.45 µm). The filtrate (~45 ml) was retained and frozen from each of the three samples for later nutrient analysis, which was carried out using a Seal AA3 colorimetric autoanalyser. See Table 2.2 below.

Table 2.2 Details of the nutrient analysis methods used to analyse water samples taken during the trials of the land-based upwelling units (SEAL, 2020)

Analyte	Seal Method	Detection Limit	Coefficient of Variation
Ammonia	G-327-05 Rev. 6	0.003 µmol/L	0.3 %
Nitrate and Nitrite	G-172-97 Rev. 13	0.015 µmol/L	0.21 %
Silicate	G-177-96 Rev. 10	0.030 µmol/L	0.5 %

A temperature logger (HOBO Pendant MX, Onset, Bourne, USA) was submerged in one of the units throughout each deployment. The logger recorded temperature every 15 minutes.

2.3.1 2020

Data collection in 2020 took place roughly weekly, depending on availability of personnel. All procedures followed those described above.

2.3.2 2021

As in 2020, data collection took place on roughly a weekly basis. Cleaning and weighing procedures were as in 2020. In addition a haphazardously selected 10 oysters were measured for height. After two months, the samples were graded and separated (see section 2.4) and both grades returned to the units.

From the first grading onwards, the two grades were treated separately at each data collection event. Further to this 10 oysters per unit, 5 from each size grade, were tagged for ongoing individual size tracking. These were measured for height and length at each data-collection event. However, the tagging method was ineffective, and too many tags came unstuck from the oyster, rendering the data unusable.

2.3.3 2022

Data collections were reduced to fortnightly intervals in 2022. The units were drained and samples allowed to drip dry before weighing. Each layer was treated as a separate sample when weighing. Individual tracking was again attempted, but high mortality meant that these data were unusable.

Cleaning and water sampling took place on the same occasions, water sample analysis was carried out as per previous years.

2.4 Grading

2.4.1 2021

Grading is the process of separating the stock by size, removing larger individuals to prevent them dominating the population. The method used for this trial was to sieve the entire population of each upweller through a 15mm square mesh, which retained oysters at ~2 g, the size at which they are deemed sufficiently likely to survive from a commercial point of view. Oysters which passed through the mesh were placed back in the unit they came from, those which were retained were removed from the system, other than the first grading event when they were placed back in the unit in a separate mesh container for 24 days.

The first grading event took place on the 2nd of July (day 66), the large oysters were then removed on the 26th of July (day 90). Further grading events took place on the 18th of August (day 112) and the 13th of September (day 139), when the large oysters were removed from the units. Removed oysters were kept in a marine water tank and then taken to a marked area of the intertidal for ongrowing on the 22nd of September (day 148).

The oysters graded out of each unit were drained and weighed as a full sample. One hundred haphazardously selected individuals were weighed, and an estimated average individual weight established for each group. This was used to estimate the number of individuals removed and remaining in each unit.

Oysters were graded following the same procedure in the final processing on the 22nd of October (day 178). Additionally the small grade oysters were passed through another, smaller, mesh at ~0.7 g grade, quantifying small and medium size classes.

2.4.2 2022

The grading procedure used was as per 2021. The first grading took place on the 25th of May (day 42). Each grade was weighed and the large oysters were removed from the

system, the small ones were returned to their original location. The same procedure was used in the final processing on the 6th of July (day 84). A final mortality count was carried out by individually inspecting all oysters left in the small grade by inserting and gently twisting a fingernail between the valves at the umbo. Live oysters remained shut under this pressure while dead ones opened.

2.5 Post-Upweller Monitoring

The marked area of the intertidal oysters where oysters were laid out on to in 2021 was initially free of other oysters of the same size class. Oysters were placed on the area at a known density. A visit was planned in spring 2022 to assess growth and survival of the oysters. However, severe storms in early 2022 washed a large proportion of COF's stock away from its original position, so it was not possible to carry out the planned monitoring.

2.6 Oyster Shape Tracking

On 27th of May (day 44) 20 haphazardously selected oysters from the layered units and 20 oysters from the same batch of seed that had been in a cage in the Pyefleet were measured (height, length and depth) using callipers. Average height to depth and length to depth ratios were calculated for each sample group to compare shape.

2.7 Costing

The forecast cost per oyster was modelled for a number of grow-out scenarios in order to assess the economic viability of using a land-based nursery system at COF. The model accounted for the cost of labour, fuel, vessel usage, energy consumption and seed oysters at various sizes.

Ongoing (labour, fuel and vessel usage) costs were estimated for the current river cage system based on values from COF and assumed that the cages would be visited for a full day (1 vessel with 2 crew) once a week in order to maintain high quality husbandry. Ongoing

costs for the upwelling systems were estimated based on energy usage and an hour per day of labour.

Energy consumption calculations were based on (historically high) October 2022 energy costs and data supplied by pump manufacturers on the energy required to pump a given volume of water. The required volume was calculated by using hatchery recommended flow rates, initial stocking weights and estimated growth rates to estimate the required flow rate, and therefore energy cost, for each day.

Ongoing costs for both systems were calculated on a per day basis, and the number of days required was then estimated based on the weight of oysters at stocking and an averaged growth rate from all runs of the upwellers (2% d^{-1}). This was likely to have been conservative given the optimisation of stocking techniques that took place over the trials.

Seed costs for the model were Morecambe Bay Oyster's anticipated 2023 prices for oysters at various weights at the time of model creation, October 2022. Seed was the only capital cost accounted for in the model, the estimated cost of setting up an upwelling system of the required size is £55 000. The final output of the model was the price per oyster at 3 g, the size at which COF preferentially lay oysters out onto their layings, based on stocking 300 000 oysters. Four scenarios were modelled:

A Stocking river cages with 0.5 g oysters (the current practice)

B Stocking upwellers with 0.1 g oysters using mains power

C Stocking upwellers with 0.5 g oysters using mains power

D Stocking upwellers with 0.1 g oysters using 90% on-site generated renewable power

2.8 Data Processing and Plotting

Data processing, plotting and analysis were carried out using Microsoft Excel and Python (v 3.9) the packages *NumPy* (Harris *et al*., 2020) and *pandas* (McKinney, 2010) were also used.

Mortality was calculated as the difference between initial estimated sample sizes and estimated numbers at the final processing. Daily temperature averages, maxima and minima were calculated for each deployment.

Growth rates were calculated using the change in full drained sample weights. These were used to estimate a daily percentage change for the time period since the prior measurement. Grading events were observed to result in a quantity of shell fragments being knocked off the oysters, which would have resulted in an unavoidable underestimate of growth. Any negative mass gains were corrected to 0.

Growth rates were averaged per treatment and compared between stocking densities, stocking patterns and layers where appropriate using repeated measures ANOVA, or Friedman's test where assumptions of parametric tests were not met. Data were checked for normality using *SciPy* Shapiro-Wilk testing (Virtanen *et al*., 2020) and sphericity with *Pingouin* Mauchly testing (Vallat, 2018).

In 2021, the year with the most grading events, treatment averages for the percentage removed at grading and average weight of graded out oyster at each grading were also calculated, and tested using the same procedures used for growth rates.

Estimated growth rates, population sizes and temperature traces were plotted using *Matplotlib* (Caswell *et al*., 2022) and *Seaborn* (Waskom, 2021).

3 Results

3.1 2020

The 2020 start date was delayed by COVID-19 and the pump supplying the units failed after 34 days, on the 29/08/20. The pump was replaced by COF staff with a less powerful unit and this was not communicated immediately. Sourcing a replacement pump was complicated by University of Essex pandemic shutdown resulting in total mortality by 07/09/20.

Growth rate data from the 2020 trial are presented in Figure 3.1. Growth rates (n = 5) in the low-density units averaged 2.1 ± 0.6 % d^{-1}, significantly higher than the 1.7 ± 0.7 % d^{-1} in the high-density units (R-M ANOVA, (F(1,2) = 19.88, p = 0.047).

Treatment averaged growth rates ranged from 1.4 ± 0.2 to 2.9 ± 0.4 % d^{-1} in the low-density units, and from 0.7 ± 0.2 to 2.4 ± 0.2 % d^{-1} in the high-density unit.

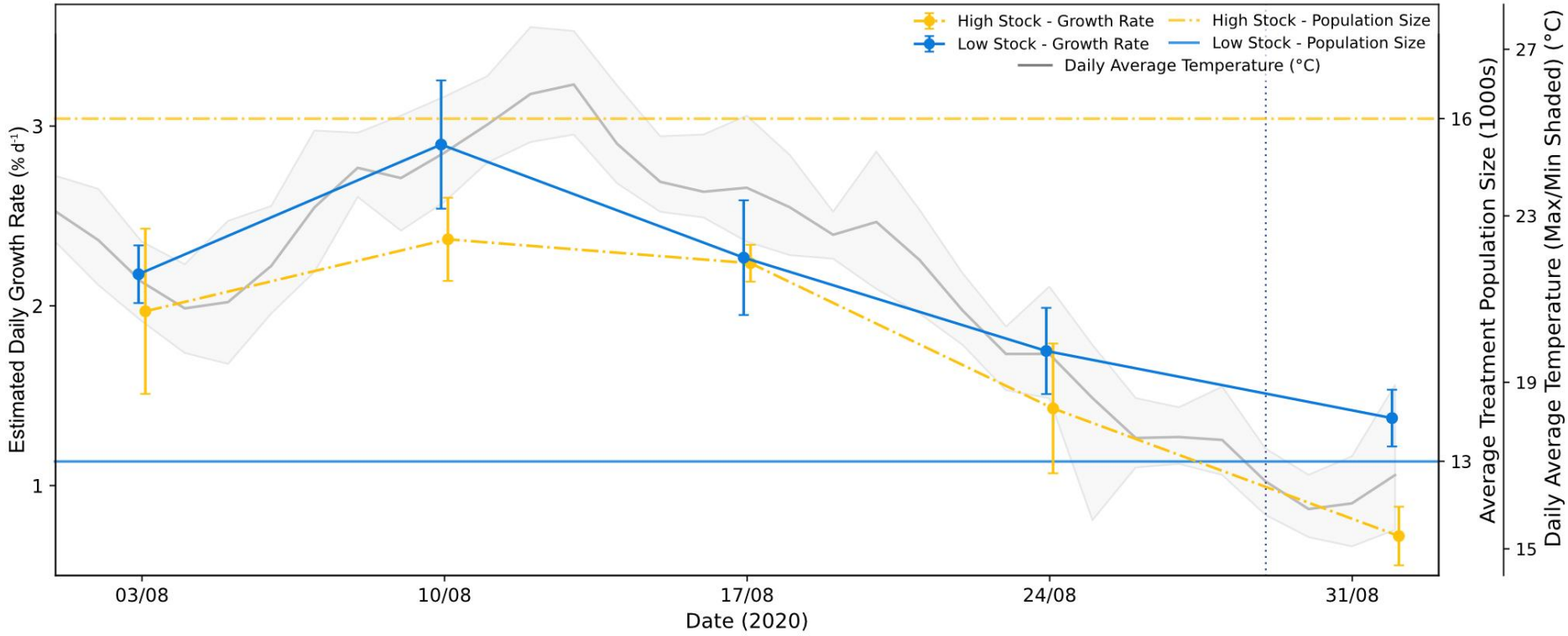

Figure 3.1: Treatment averaged estimated daily growth rates (mean ± SD), average treatment/unit population size and temperature readings from a logger in one of the units for the 2020 trial. Pump failure is indicated by the dotted vertical line. Plots are offs*et al*ong the time axis for clarity.

3.2 2021

Despite issues with power supply early in the run monitoring by security camera ensured that the oysters were protected from any adverse effects of pump failure by temporary storage in the reservoir. Mortality throughout the 177 day run was negligible (<10%).

Growth rate data from 2021 are summarised in Figure 3.2. Splitting and grading events are shown by a decrease in the population levels. Treatment average growth rates (n = 24) were 2.1 ± 1.0 and 1.8 ± 1.0 % d^{-1} in the low and high-density treatments respectively. There was no significant difference between the stocking treatments for any of the three tested parameters: growth rate (Friedman's test, $X^2(1) = 0.33$, $p = 0.56$), percentage graded out (R-M ANOVA, ($F(1,2) = 5.87$, $p = 0.14$) and average weight of graded-out oyster (R-M ANOVA, ($F(1,2) = 0.79$, $p = 0.47$).

Treatment averaged growth rates between the 30/06/22 and 03/07/22 had a notably high variance. This was driven by a single unit with particularly high (>6 % d^{-1}) growth in each treatment. The two units were adjacent, so these differences may have been caused by an issue with flow through the manifold, possibly blocking other units and providing more water to the units with high growth. The difference in growth rates between the treatments remains insignificant when these data are excluded from the analysis (Friedman's test, $X^2(1) = 3$, $p = 0.08$).

Treatment average growth rates varied from 0 to 4.9 ± 0.6 % d^{-1} in the high-density units, and from 0 to 5.1 ± 0.5 % d^{-1} in the low-density units.

At the final processing, 13% of oysters from the low-stock treatments were classified as small (<0.2 g), compared to 26% in the high-density treatments, a random sample of 20 of these oysters were checked for mortality, and all were alive. In total 68% of oysters in the low-density treatment reached a high-grade size (>2 g), compared to 50% in the high-density treatment.

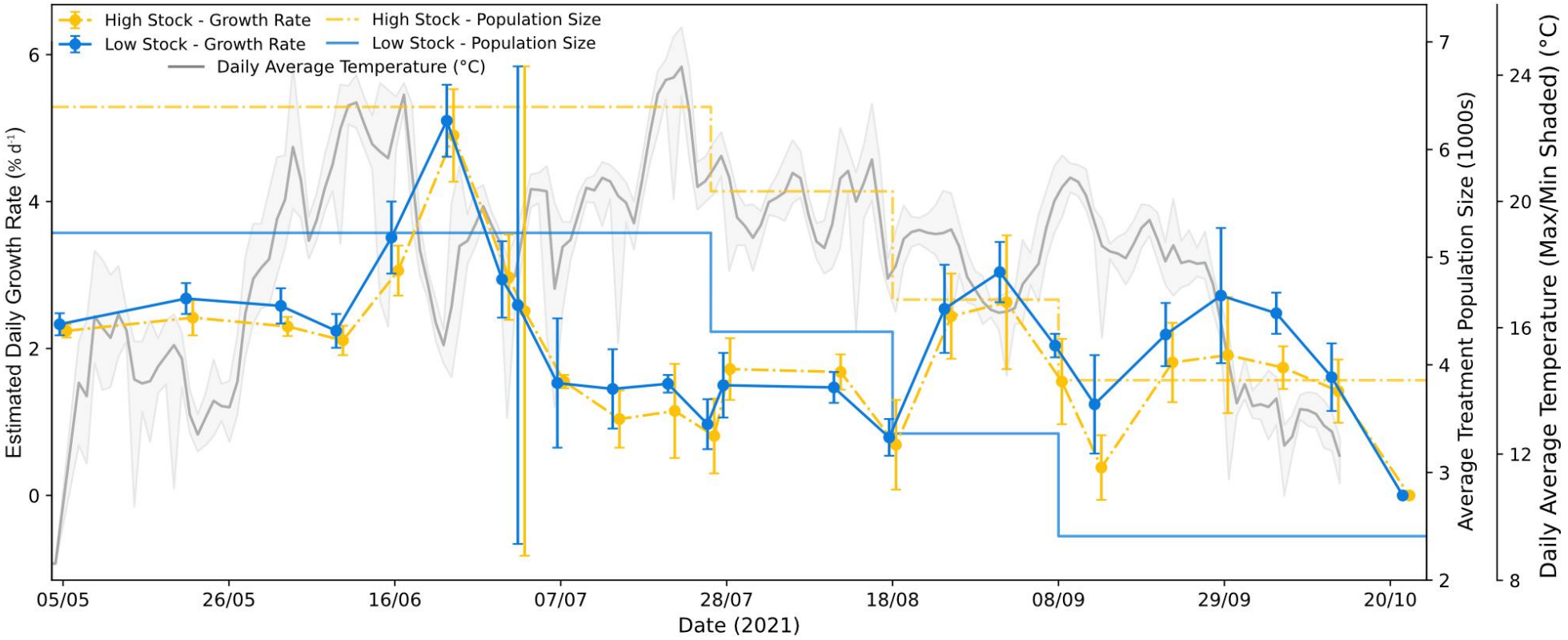

Figure 3.2: Treatment averaged estimated daily growth rates (mean ± SD), average treatment/unit population size and temperature readings from a logger in one of the units for the 2021 trial. Plots are offs*et al*ong the time axis for clarity. Note that the first grading event took place on the 26/06/21 but large oysters were not removed until 27/07/21, these were counted in the unit population until that point, but excluded from growth-rate calculations

3.3 2022

Mortality was higher in 2022 than the two previous trials, at 36 and 37 % in the layered and single-stocking pattern treatments respectively.

3.3.1 Layered vs Single Stocking

Growth rate data compared between stocking pattern treatment from 2022 are summarised in Figure 3.3. Growth rates in the layered units averaged 2.8 ± 1.6 % d^{-1}, significantly higher than the 1.1 ± 0.4 d^{-1} in the single units (Friedman's test, $\chi^2(1) = 3.0$, $p = 0.01$). Treatment average growth rates varied from 1.0 ± 0.3 to 5.5 ± 0.3 % d^{-1} in the layered units, and from 0.6 ± 0.5 to 1.8 ± 1.1 % d^{-1} in the single units.

The differences in grading between the two treatments are summarised in Table 3.1. More and heavier oysters were removed from the layered stocking treatment in both grading events. The average weight of oysters remaining after the first grading was very similar between the treatments, 0.96 ± 0.1 g in the layered and 0.94 ± >0.1 g in the single stocking.

Table 3.1. Comparison between the grading results (% graded out and average weight of graded-out oysters) at grading and final processing events between treatments in 2022.

Treatment	Event	% graded out (treatment average)	Average weight of graded out oysters (g)
Layered	Grading 1	47 ± 2.0	4.4 ± 0.4
Single	Grading 1	36 ± 8.4	2.8 ± 0.3
Layered	Final processing	98 ± 0.9	6.4 ± 0.7
Single	Final processing	45 ± 15	4.5 ± 1.0

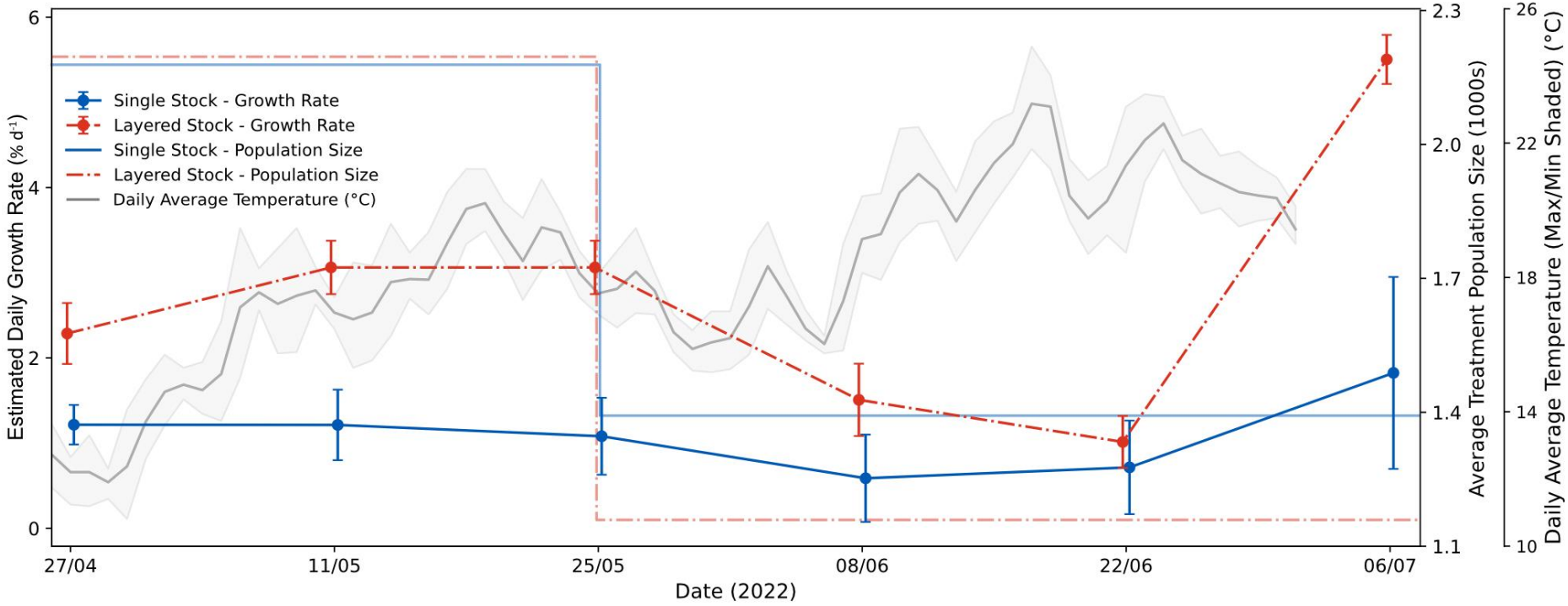

Figure 3.3: Treatment averaged estimated daily growth rates (mean ± SD), average treatment/unit population size and temperature readings from a logger in one of the units for the 2022 trial. Plots are offs*et al*ong the time axis for clarity.

3.3.2 Layer Comparisons

Average growth rate data from the three layers in the layered stocking pattern units are summarised in Figure 3.4. There was no significant difference in growth rates between the layers (R-M ANOVA, $F(2,4) = 0.74$, $p = 0.53$).

Table 3.2: Percentage graded out and average weight of large-grade oysters from the first grading and final processing in 2022.

Layer	**Population Graded Out (%) (±SD)**		**Average weight (g) (±SD)**	
	Grading 1	**Final**	**Grading 1**	**Final**
Top	45 ± 3.3	30 ± 6.8	4.8 ± 0.3	6.0 ± 1.1
Middle	41 ± 2.1	34 ± 7.9	4.5 ± 0.8	5.7 ± 0.5
Bottom	55 ± 2.4	28 ± 5.7	4.0 ± 0.3	7.5 ± 1.6

Table 3.2 summarises the differences in proportion and size of graded-out oysters in 2022. At the first grading more oysters were graded from the bottom layer, but they had a smaller average weight, the inverse of this happened at the final processing.

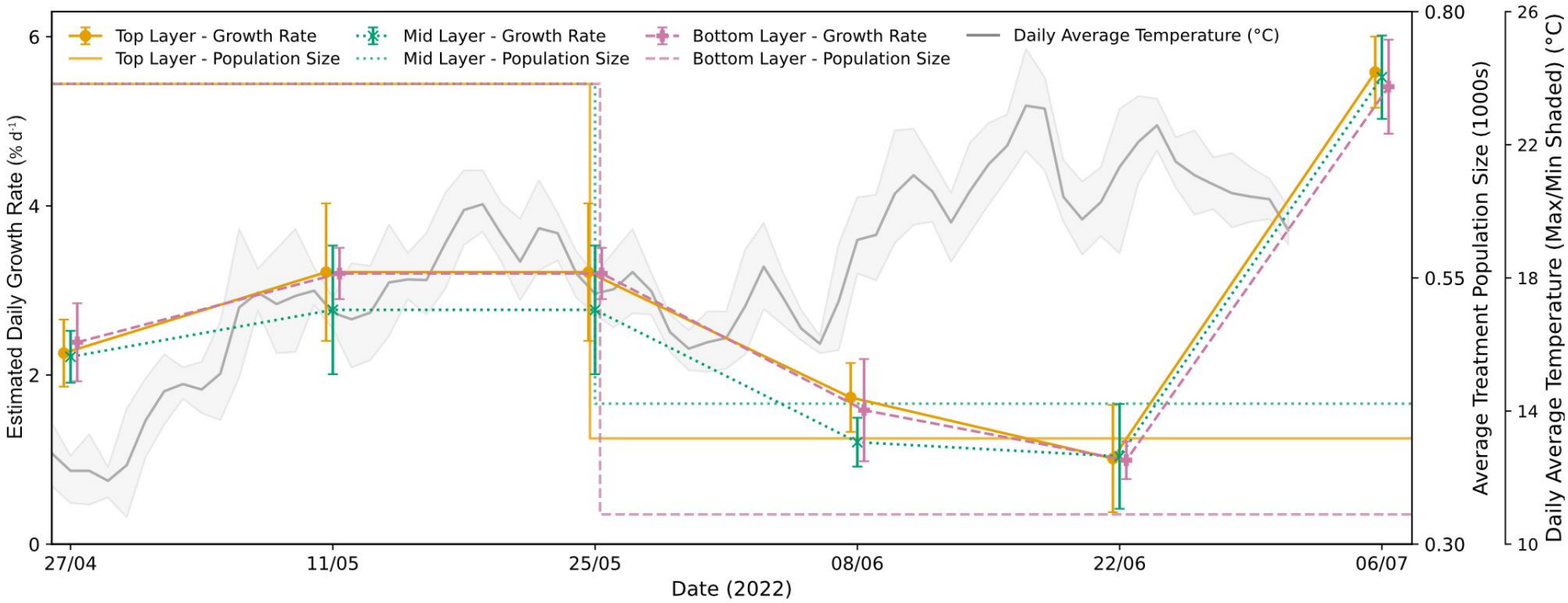

Figure 3.4: Layer averaged estimated daily growth rates (mean ± SD), average layer/unit population size and temperature readings from a logger in one of the units for the 2022 trial. Plots are offs*et al*ong the time axis for clarity.

3.4 Shape Tracking

There was no significant difference in the height-to-depth ratio between oysters from the river and the upweller units. The length-to-depth ratio in oysters from the river was 2.36 ± 0.32, significantly higher than the ratio of 2.08 ± 0.27 found in oysters from the upwelling units (Kruskal-Wallis, $H(1) = 9.2$, $p < 0.01$).

3.5 Nutrient and Temperature

Nutrient sample data are presented in Appendix 4. Nutrient levels remained within the standards set by The Water Framework Directive (2017) for estuarine waters. Temperature remained within the range of *M. gigas* (Bayne, 2017d). Results from the analyses were variable, but no correlation was found between nutrient levels or temperature and oyster growth rates over the duration of all three trials.

3.6 Costing

Table 3.3: Forecast costs per oyster at 3 g, the size at which they are considered suitably likely to survive when introduced to the intertidal layings. Costs under COF's current procedures, and three realistic scenarios for upwelling units are provided for comparison.

	Growing System	Seed cost (p)	Stocking Size (g)	Power Source	Cost at 3 g (p)
A	River Cage	3.2	1.0	N/A	**4.2**
B	Upweller	1.3	0.1	Mains	**5.2**
C	Upweller	1.9	0.5	Mains	**5.0**
D	Upweller	1.3	0.1	Renewable (90%)	**2.6**

4 Discussion

4.1 Project Summary

The upwelling units were in operation for a total of 43 weeks over the course of the three trials. 135 000 oysters from two hatcheries passed through the system, growing at an average of 2 % d^{-1}. Husbandry visits took place nearly 120 times, with sampling and measuring on 35 of these visits, during each of which the oysters from all 6 units were drained and weighed, and up to 120 oysters individually measured and each unit cleaned. There was no evidence of any disease occurrence in the units, likely as a result of high quality conditions, but also possibly due to use of a reservoir as a water source which may have resulted in settlement of particulate disease vectors, resulting in reduced exposure to pathogens (Whittington *et al*., 2015b)

These trials were designed to be small-scale but mimic commercial practices, and grading was therefore a key part of the process. Well implemented grading processes are a crucial element in many aquaculture systems, preventing slow-growing individuals being outcompeted by faster-growing individuals, and can significantly improve the final outcome of a growing cycle (Ahvenharju *et al*., 2005; Gunnes, 1976; Mgaya and Mercer, 1995). However, it must be noted that on a fully commercial scale, the grading practices would have been noticeably different. Commercial shellfish grading machines are gentler and faster than the sieving approach used in these trials. Our methods required that the oysters were out of the water for up to half an hour, with a relatively strong movement needed to grade them effectively. This in turn meant that grading resulted in a loss of weight, particularly the fragile newly grown shell was lost, resulting in an underestimate of growth. There may also have been significant stress associated with this process, which could have had a negative impact on the oysters.

4.2 Annual Summaries

Lessons were learned from each year's trial, enabling us to improve our methodologies and system design throughout the trials. Summaries of the data, conclusions and modifications each year are provided in the following subsections.

4.2.1 2020

Due to the short duration of the 2020 trial only limited conclusions can be drawn. When the system was fully functioning the growth and survival rates were sufficient to prove the concept of the upwelling units. The estimated average oyster size at stocking in 2020 was larger (0.28 g vs 0.06 g in 2021) than the units were designed for as COF donated a portion of their normal seed order rather than purchasing specifically for the trial.

The final weight prior to the pump failure showed a stocking density 76 % and 53 % above the hatchery-recommended level in the high and low-stock units respectively, with no apparent impact on mortality. Growth rates calculated from the final visit were by far the lowest seen during this trial, particularly in the high-stock units. This suggests that stocking density was beginning to have an impact on growth rate.

4.2.2 2021

After 2020 two main changes were made to the protocols. Back-up pumps were purchased in advance of stocking the system in 2021, and a remote monitoring system was installed. We were therefore able to respond to pump failure or power loss rapidly, preventing any impact on the oysters in the units.

Work in 2021 proved that small seed could be used in the system with very low (< 10 %) mortality in both treatments. With an estimated average weight of 0.06 g, the oysters used for this trial were unsuitable for river cages. The size of mesh required to avoid loss would have required daily cleaning to prevent clogging and it was likely that mortality from predation would have been high.

The lack of a significant difference in treatment averaged growth in 2021 is likely due to the low overall stocking density (340-410 g per unit), which initially averaged 10 % of the levels recommended by hatcheries (~4 kg biomass per unit). This is lower than we would have

liked, but COF's supplier was unable to provide a larger amount of seed oysters at the requested size. Due to the grading procedures, overall stocking weights remained below hatchery-recommended density for nearly the full duration of the trial, only rising above it on three occasions.

There was also no significant difference between treatments in the number or weight of oysters removed at each grading. However, in the low-density treatment 36 % more oysters reached a size at which they were graded out, and 50 % fewer remained under 0.2 g. This suggests that the growth of some individuals within the stock was negatively affected in the high density treatment. This may also have occurred to a lesser degree in the low-density treatment. However it is not possible to discount that the individuals that failed to grow substantially may have done so as a result of other reasons, possibly genetic or resulting from poor conditions experienced prior to being stocked in the upwelling units.

We suggest that the differences between treatments may have been a function of localised density, the oysters in 2021 were stocked as a single group, meaning that each oyster was in close proximity to all others in the unit, the number of which was a reflection of the stocking density. Competition for freshly pumped, and therefore food-rich and well oxygenated water was potentially a cause of these differences in outcome between the treatments.

4.2.3 2022

The trials in 2022 were designed to assess whether localised density had an impact on seed growth rate, growth uniformity and survival. This was accomplished by splitting the stock into 3 layers, using the modified units (Figure 2.2).

Work in 2022 was heavily impacted by the low quality of the delivered seed, an estimated 10 % of which was removed on arrival due to mortality. After allowing the seed to recover for a month in the upwelling units a further 10 % of oysters were dead and were removed prior

to the start of the trial. Mortality assessment is challenging in small oysters, which do not necessarily open when dead, and our assessment was likely to have been an underestimate. Combined with the poor quality of the seed this accounts, at least in part, for the higher (36.5 % post check-in) mortality in comparison to 2021.

The impact of stocking pattern was clear and highly significant, oysters in the layered treatment grew 2.5 times quicker and were 1.5 times larger when graded out. All but 24 of the 6 300 oysters from the layered treatment that were alive at the end of the trials had reached a large grade size, compared to 980 of 6 600 in the single stocked units. Mortality was <1 % different between the two treatments, suggesting that the negative impact of the single stocking was insufficient to increase mortality.

There was no impact of position within the layers, suggesting that the oysters in the lower layers, closer to the water inlet, did not remove sufficient resources from the water to adversely impact those above them. This was likely to have been due to the relatively low stocking in relation to the overall flow. It may also reflect the turbulent flow induced by the system design, which would have resulted in some water flowing round the oyster containers (which did not fully cover the horizontal cross-section of the units) and in the sides rather than consecutively through the layers.

4.3 The Impact of Stocking Density

4.3.1 The Impact of Density and Competition in Upwelling Units

Overall density in the units was rarely limiting in our trials, possibly with the exception of late 2020 when growth rates were substantially lower in the high density treatment Inter-individual competition, however, was an important factor, with some individuals reaching sizes nearly an order of magnitude greater than others. The intra-population differential in growth rates, most notably in the high-stock units in 2021, suggests that competition between individuals in the units was heavily asymmetric (Fréchette *et al*., 2005; Weiner, 1990). Unfortunately attempts to track the relationship between size and growth at an individual level failed due to tag loss and high mortality of tagged individuals, but between grading events the increased growth rate of the larger oysters is likely to have increased the competitive asymmetry in the population, underlining the importance of effective grading. More frequent grading may have helped reduce the asymmetry in competition between individuals, but would not have been without drawbacks, as discussed in section 4.1.

In the layered treatment in 2022 no individuals failed to reach a large grade size, whereas in the single treatment 15% did not grow sufficiently. The contrast to 2021 may in part be due to the units having been initially stocked with much larger (1.1 g rather than 0.06 g in 2021) oysters. However, we suggest that the difference in the layered units was primarily a function of lower localised densities reducing resource limitation. Competition theory predicts precisely the outcome of these trials, more even growth as a result of reduced asymmetry (Begon *et al*., 2012). The higher overall growth rate seen in the layered units suggests that the increased resource availability also reduced overall competition for resources, indicating that the layered design increased the overall carrying capacity of each unit.

4.3.2 Competition and Grading

Grading serves to artificially reduce size differences within a population by removing the largest individuals. This reduces the impact of competitive asymmetry, preventing slower growing individuals from being dominated by allowing them to either make more efficient use of available resources or have greater access to existing resources and therefore, in theory, grow faster. It is common practice across the aquaculture industry as a result (Fan *et al*., 2021; González *et al*., 2011; Gunnes, 1976).

However, grading is a time-consuming and costly process that causes a degree of stress regardless of how it is carried out, and ideally would not be necessary. In reality however it is unavoidable, growth in *M. gigas*, as in most organisms, is strongly affected by genetics with inter-individual variability inevitably arising as a result (Gutierrez *et al*., 2018; Meyer and Manahan, 2010). We have demonstrated that optimisation of stocking protocols in upwelling systems can strongly influence the degree of asymmetry in competition and growth, in turn reducing the frequency of grading events but not removing their necessity.

4.4 Product Quality

The shape-tracking data, while limited in scope, suggest that oysters grown in the upwelling units may go on to have a deeper shape, and therefore are a more desirable product than those grown in river cages. Reliable production of deeper oysters would certainly be of interest to some markets, potentially allowing producers to charge a higher price (Marshall and Dunham, 2013). Due to the movement of oysters after being laid out in the river and following a storm, it was impossible to investigate this effect further.

4.5 Costing

The forecasted costs of operating the upwelling units show that they have the potential to produce cheaper oysters at a suitable size to be laid out on COF’s grounds than the current

methods used at the fishery, particularly given that the estimates were based on a conservative (2% d^{-1}) growth rate that we expect an optimised system to exceed, and historically high electricity prices. The potential for reduced grading frequency and production of oysters with a more desirable shape may provide further economic advantages.

Capital cost for the installation of suitably sized upweller units remains a stumbling block, but we believe that the trials described in this chapter provide a strong evidence base for revision of practices at the fishery in principle, particularly if renewable electricity generation capacity is incorporated into the system.

5 Conclusion

Over the course of three summers we have demonstrated the potential benefits of land-based oyster ongrowing systems. Reducing localised density by layering the stock in 2022 reduced asymmetry in competition between the oysters, substantially increasing evenness of growth within the population. Overall resource limitation was also reduced, resulting in increased population growth rates and possibly the unit carrying capacity. These effects are all of major benefit in commercial shellfish aquaculture. Addition of layers was a low-cost intervention, needing only a small time and material investment to construct and minimum additional maintenance other than a small increase in cleaning requirements.

Future work should investigate several factors. Using smaller and, hence, cheaper seed oysters in the layered system should be explored further as it represents additional potential cost savings. Similarly a trial using triploid oysters could enhance the utility of the systems due to their inherently faster growth, although they would represent an increased up-front cost (Guernsey Sea Farms, 2023; Wadsworth *et al*., 2019). Further reduction in localised density may realise additional gains. Tracking of the relationship between size and growth at an individual level was unsuccessfully attempted in both 2021 and 2022. Accurate data of

this nature would add substantially to our understanding of the competitive dynamics within the units.

Chapter 5

Gaping Behaviour of Blue Mussels (*Mytilus edulis*) in Relation to Freshwater Runoff Risks

This chapter is a version of a manuscript published in Aquaculture Reports (https://www.sciencedirect.com/science/article/pii/S2352513423002582) that has been edited to remove information that repeats content published elsewhere in this thesis.

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 to 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

The concepts and potential uses of gape sensing are discussed in detail in Chapter 2, Section 4.3. This chapter focuses on the use of the NOSy system in a different context to those discussed so far, the monitoring of a Blue mussel (*Mytilus edulis*) population in Dundrum Bay, an area with substantial water contamination issues (H Moore, Agri-Food and Biosciences Institute Northern Ireland, personal communication). Dundrum Bay is located on the south-east coast of Northern Ireland (Figure 1.1). Historically productive mussel beds in the southern end of the bay are currently deemed unsuitable for harvest due to the identification of local contamination and continual food safety testing failures (H Moore, Agri-Food and Biosciences Institute Northern Ireland, personal communication).

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.

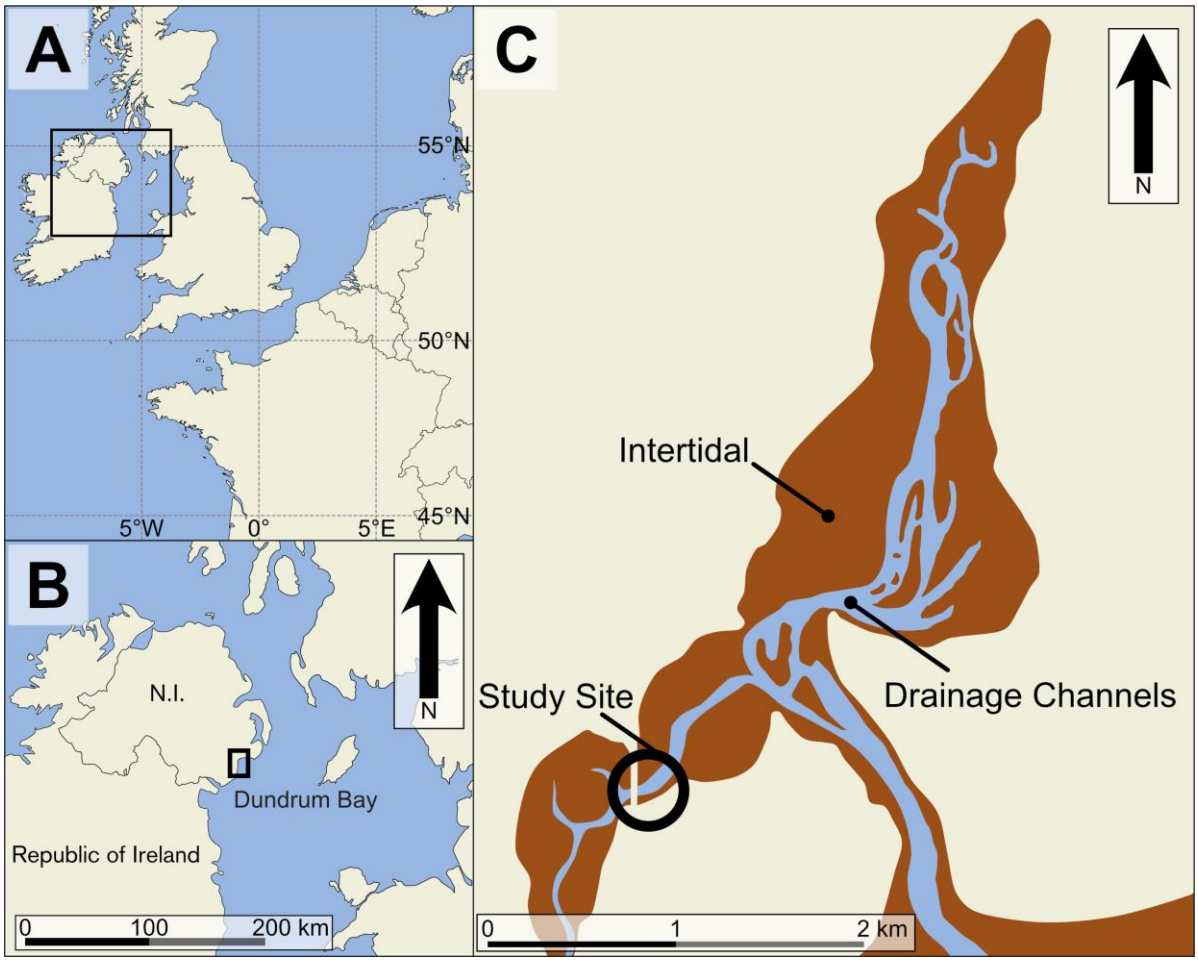

Figure 1.1: Study site location. **A** and **B** locate the study site on a continental and country scale. **C** provides study site scale detail. The drainage channels that remain full at low tide are shown in blue with intertidal areas indicated in brown. The study location is highlighted by a black circle, which the floating pontoon was at the centre of (54.251N, -5.849W).

2 Materials and Methods

2.1 Study Area

Dundrum Bay, Northern Ireland (Figure 1.1) covers an area of approximately 4.5 km^2 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 in the drainage channels is virtually fresh, whilst it reaches nearly full-marine salinity at high tide. As in many estuaries, the restriction of water to relatively narrow drainage channels at low tide suggests that contamination entering the bay would be relatively concentrated at low tides.

2.2 *Sensing Equipment*

The NOSy system, as described in Chapter 2, was used to monitor *M. edulis* gaping behaviours throughout this study.

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 19-25 September 2020; Xylem Analytics UK, Letchworth, UK) which were deployed on the seafloor in close proximity to the pontoon, logging depth and salinity every 15 min (Seabird 19 CTDi) or 20 min (YSI 6080) throughout the deployment. Depth data from the CTD instrument used on 19-25 September 2020 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 minutes apart) to calculate an estimated salinity equivalent to each minute-averaged gape data point.

2.3 Study Animals and Experimental Conditions

Mussels for the study were retrieved from the study site, a mussel bed in the south west of the bay (Figure 1.1C), on 15 September. 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 following the procedure as laid out in Chapter 2, Section 2.3, using a UV-curing fibre-reinforced polyester resin (Solarez Ding Repair, Vista, CA, USA) (Figure 2.1). 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 (Figure 2.1). 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. Deployment of sensor unit and mussels started on 16 September and ended 9 days later on 25 September 2020, with gape logged at 3.7 Hz throughout. Battery replacements were carried out regularly to ensure sufficient power supply.

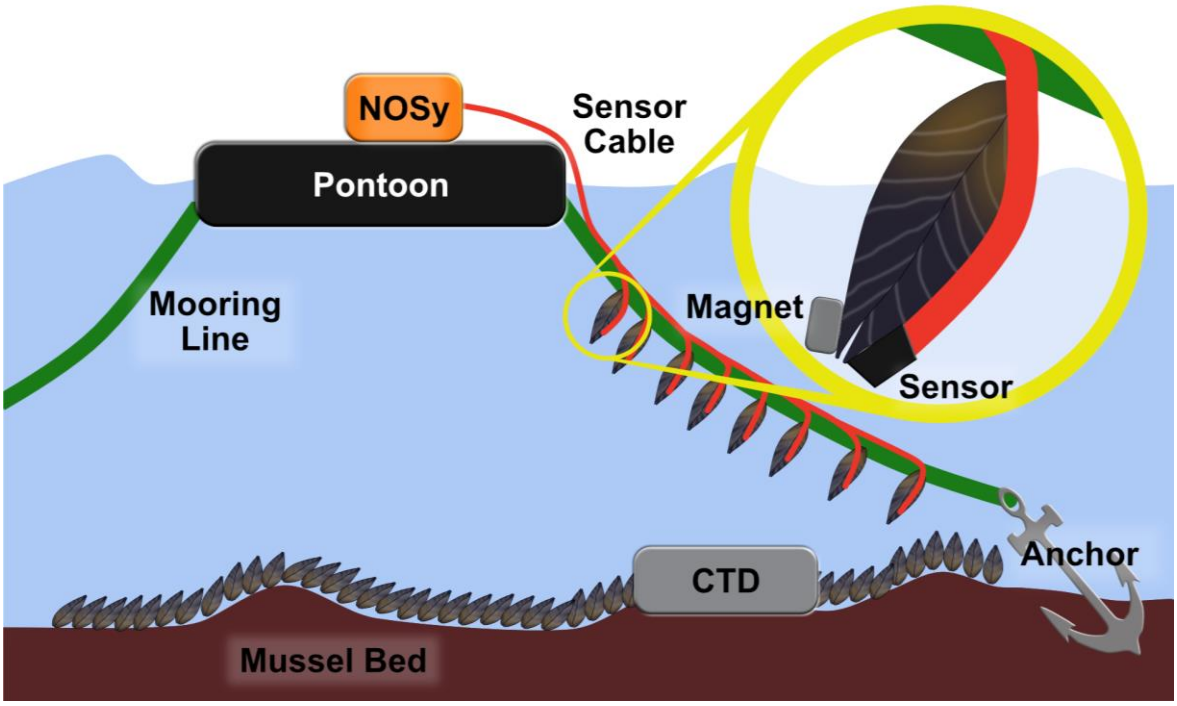

Figure 2.1: Schematic of the NOSy installation for the deployment in Dundrum Bay, September 2020. Shown are the principle set-up of the NOSy sensor with mussels suspended from a mooring line, and the location of autonomous CTD instruments to measure conductivity, temperature and depth. The inset illustrates the relative positioning of a neodymium magnet and the Hall-Effect sensor on the study animals.

2.4 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 sec) 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 were initially processed as per the protocols laid out in Chapter 2, Section 2.4. Data were then averaged to the minute and normalised using the *sklearn* Python package (Pedregosa *et al*., 2011). 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 (not directly verified during NOSy retrieval) and were removed. Population averaged gape per minute was then calculated (n = 12-15).

Inflection point analysis (Muggeo, 2003) was carried out using the *piecewise-regression* (Pilgrim, 2021) Python package to explore the relationship between depth (as a proxy for all tidally influenced factors) and gaping behaviour. Significant inflection points, where found, indicated depths at which the relationship between depth and gape alter. Data were plotted using the *matplotlib* (Caswell *et al*., 2022) and *Seaborn* (Waskom, 2021) Python packages.

3 Results and Discussion

This study demonstrated the successful field deployment of the NOSy system with *M. edulis* with complete data recovered from 15 mussels for 83% of the deployment period. A figure showing processed gape data for all individuals is provided in Appendix 5.

There was a difference in raw-data quality between mussels, as inter-individual morphological variation affected the distance between sensor and magnet (Appendix 6). 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 (24.1 ± 10.1). Mean temperature was 14.3 ± 1.3 °C, with a range of 9.7 to 16.9 °C. These environmental data are summarised alongside population-averaged gape in Figure 3.1 and demonstrate that 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 that was synchronous with tidal height was found across the study population, with high gape readings occurring at high tides and vice versa. Frequency distributions of the population's average gape in 0.5 m depth bands are presented in Figure 3.2. There was a clear trend of increasing average gape with depth, most notably between the < 0.5 m and 0.5 to 1 m bands, where the average gape increased from 0.09 to 0.43.

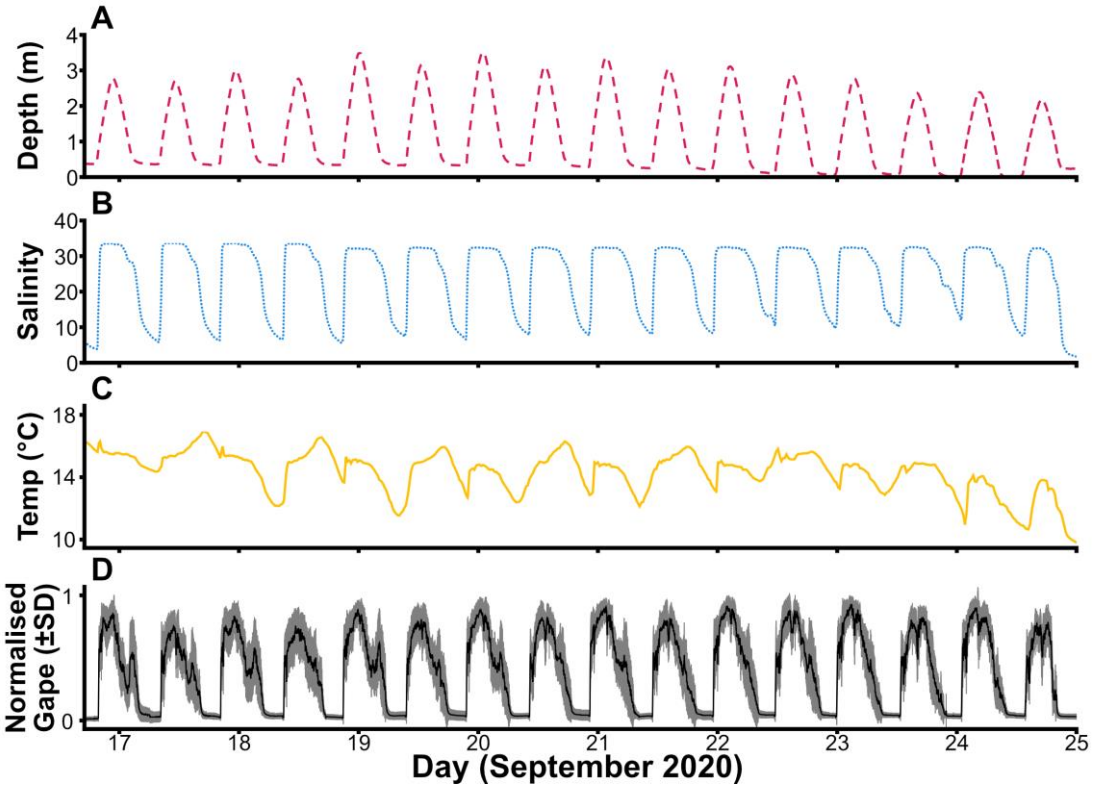

Figure 3.1 CTD and gape data summary from the full NOSy deployment in Dundrum Bay, Northern Ireland. Shown are data for depth (**A**), salinity (**B**) and temperature (**C**), and a summary of the population and minute-averaged *M. edulis* gape (± one standard deviation) (**D**). A normalised gape value of 1 indicates maximum opening and 0 indicates minimum opening.

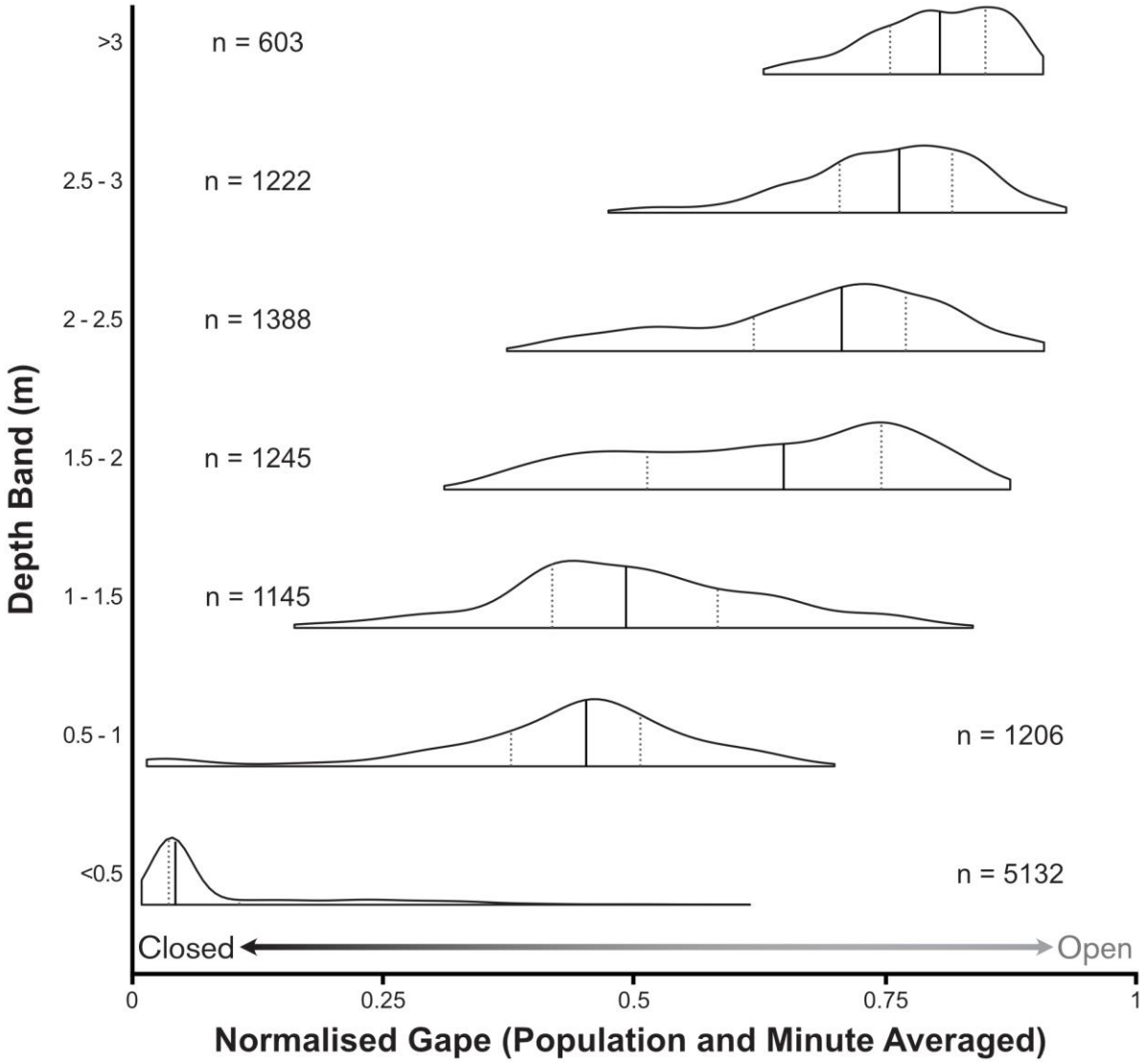

Figure 3.2 Population average gape frequency distributions classified by 0.5 m depth bins. Vertical lines indicate the mean (solid line) and quartiles (dashed lines) for each depth bin. n values indicate population and minute-averaged gape data points, representing the number of minutes spent in each depth band over the deployment.

There was a significant inflection point in the population average gaping behaviour at 0.92 m depth (Davies Test, df = 11937, p = 0.021) (Figure 3.3). Below this depth, gape was strongly positively related to depth, above it there was a positive but shallower relationship between the variables. Analysis of individual mussels showed that 8 of the 15 study animals also had a significant inflection-point in their gaping behaviour with depth, ranging from 0.35 to 0.91 m, averaging 0.76 m (Table 3.1).

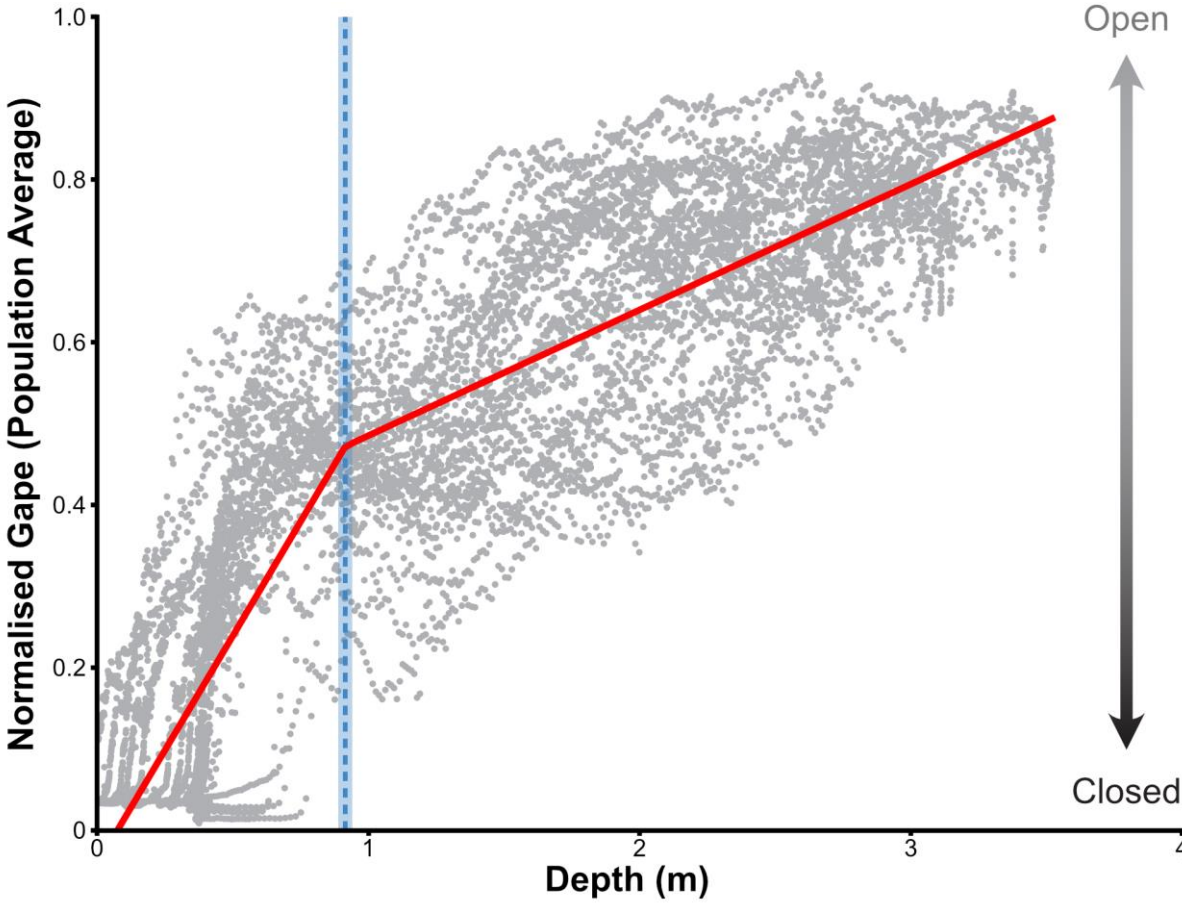

Figure 3.3: Population and minute-averaged gape of *M. edulis* throughout the deployment at all tidal heights. Results of the inflection-point regressions are summarised, solid lines indicate the two separate regressions and the dashed line the calculated inflection-point (± 5% confidence interval in grey shading) of 0.92 m, the depth at which the relationship between depth and population and minute-averaged gape changed (Davies Test, df = 11937, p = 0.021)

Table 3.1: 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)	Davies Test P Value	Number of observations
Average	0.915	**0.021**	11941
M1	0.812	**<0.001**	11941
M2	DNC	DNC	11941
M3	0.133	0.926	11941
M4	1.827	0.826	11941
M5	0.702	**<0.001**	10372
M6	0.794	0.063	11941
M7	0.976	0.106	11941
M8	0.823	**0.002**	11941
M9	1.304	0.195	11941
M10	0.349	**0.026**	11941
M11	0.850	0.019	11941
M12	0.772	**<0.001**	9908
M13	0.909	**<0.001**	11341
M14	1.804	0.446	11941
M15	0.846	**0.001**	11941

Bivalve molluscs are osmoconformers (Krogh, 1939) and *M. edulis* have a salinity tolerance of between 5 and 35, with an ability to tolerate fully fresh water for a short time (Barrett *et al*., 2022; Bayne, 1976; Westerbom *et al*., 2002). Tolerance to low salinity is primarily enabled by the behavioural response of valve closure, which protects the organism from harmful conditions including the ingress of freshwater (Riisgård and Larsen, 2015; Solan and Whiteley, 2016).

Whilst there are several factors, including temperature, food availability, pollutants and harmful algae, which can influence bivalve gaping we suggest that the strong relationship between depth and gaping behaviour is largely driven by the salinity variations (ranging in our experiment from 1.8 to 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 (Figure 3.1). The significant inflection point in population-averaged behaviour at 0.92 m (Figure 3.3)

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 are separated into salinity bands, the gaping-behaviour patterns are clearly different between salinities below and above 25, showing that mussels remain significantly less open at intermediate salinities far above their published tolerance (Figure 3.4). This was supported by the inflection-point analysis (0.9 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.

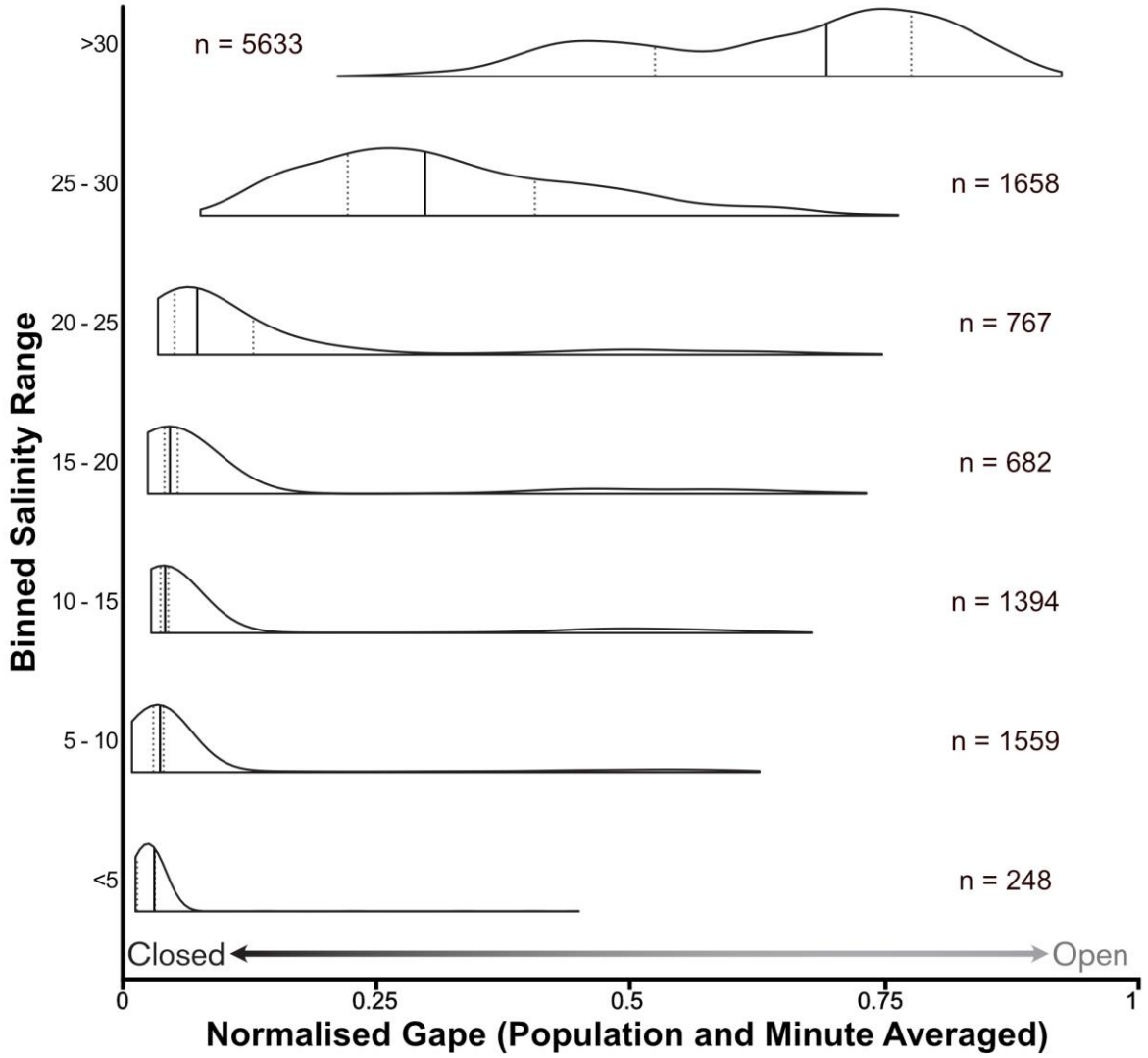

Figure 3.4: Population and minute-average gape frequency distributions classified by salinity. Vertical lines indicate the mean (solid line) and quartiles (dashed lines) for each salinity bin. n values indicate population and minute-averaged gape data points, representing the number of minutes spent in each salinity band over the deployment.

This study demonstrated the suitability of the NOSy system for gape-data collection in the field, providing insight into the behavioural patterns of *M. edulis* over a tidal cycle covering extremes of salinity. Despite this species being tolerant to lower salinities, we have shown that *M. edulis* in Dundrum Bay showed substantially smaller gaping widths at salinities below 25. 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 high levels of background osmotic stress in the animals on the bed, resulting

from the high salinity variations found in the bay, that decrease their ability to cope with salinities below 25.

Regardless, there is a degree of protection inferred by this behaviour pattern against the potentially high contaminant concentrations that can be found in freshwater catchment run-off as estuaries approach low-tide conditions. This does not exclude that local contaminant and water-quality indices are high enough to harm shellfish or their suitability for human consumption at other points in the tidal cycle when the shellfish are feeding, i.e. immediately after the start of flood tide, although further inflection-point analysis showed that opening on the ebb tide occurs at higher salinity (29.6) than closure on the flood when the sea is on average 1.2m deep and significantly diluting the freshwater river.

The quality of the data and our analysis suggest that the NOSy sensor unit would be suitable for monitoring bivalve behaviours for a diverse range of purposes. This approach has potential application in the design and interpretation of shellfish classification monitoring programmes, particularly where waste-water discharges or diffuse inputs are tidally influenced.

Chapter 6

Synthesis

The primary aim of this thesis was to ‘develop technologies and approaches with potential to improve our understanding of bivalve biology, with a primary focus on oyster production methods’. By developing processing and analysis protocols that facilitate the use of data from the Non-Invasive Oyster System (NOSy) for industrial, research and monitoring purposes, as well as successfully trialling land-based nursery systems, we have met this aim. Further to this we have shown that NOSy is capable of detecting spawning events in the Pacific oyster, the original purpose of the system.

Data from field trials of NOSy units provided clear evidence of tidally synchronous gaping cycles, growth traces and behavioural differences between two species, Pacific and Native oysters. We investigated whether salinity was a driver of the observed behavioural patterns by designing novel laboratory methodologies that replicated tidal salinity variations. This is an under-studied aspect of estuarine ecology and bivalve behaviours, possibly due to the challenges of designing effective methodologies.

As well as improving overall productivity of the oysters once growing in the marine environment, fisheries are dependent on sourcing and growing on of young stock and hence we worked closely with our industrial partner, Colchester Oyster Fishery, to develop and trial a land-based system for ongrowing of oysters purchased from a hatchery. This system was conceived to overcome many of the challenges that result from standard procedures at the fishery, particularly the high workload required to maintain the oysters in good condition. The land-based system was deployed for three summers. With iterative improvement in protocols and system design we were able to show that land-based systems have the potential to substantially reduce costs and improve returns on the investment in oysters from hatcheries.

To demonstrate a broader application of NOSy we also deployed the system in Northern Ireland in collaboration with colleagues at the Agri-Food and Biosciences Institute, as part of an investigation into the vulnerability of Blue mussels to waterborne contamination. We showed that, as a result of responses to extreme salinity variation on the site, the mussels

close almost fully over low tide, which we suggest infers a degree of protection from contamination associated with freshwater runoff. Finally, this chapter recaps the findings and their relation to the primary objective.

1 Chapter Recaps

1.1 Chapter 2: Development and Trial of the Non-Invasive Oyster Sensor

Chapter 2 primarily provides an overview of the trial and development of the NOSy system, the principles underlying its construction and how the recorded data can be utilised. The system was originally conceived to automatically alert Pacific oyster growers to when their stock is spawning, monitoring of which is currently an imprecise process that relies on invasive monitoring and inference from environmental data. Use of a reliable automatic detection system would enable precise timing of cultching by growers, a process that maximises the number of oysters retained from natural spawning and can therefore substantially reduce outgoings on hatchery purchased oysters.

The chapter recounts the challenges that arose during the trial and development of the system, including loss of coverage due to shortcomings in the power supply system, unit failure and data corruption. Despite these challenges spawning was successfully detected when induced in the laboratory, proving that the design of the NOSy system was fit for its original purpose, although automatic detection of the behaviour in the field is yet to be accomplished. The data recorded in the field were, once processed, of suitable quality to identify behavioural patterns, trace growth and identify behavioural differences between oyster species. Suggestions are made as to how the system can be improved, namely a reduction in power consumption, the introduction of remote communication capabilities and development of on-board processing capability to recognise departure from normal behaviours.

The chapter concludes that the underlying concept and design of NOSy has been proved capable of high resolution recording of bivalve behaviours. With further improvements the system should be a valuable tool across several sectors. It will enable bivalve growers to monitor stock, supplying data that will aid in making management decisions to increase productivity. Further uses, as part of biosensor networks for large-scale environmental monitoring programs and in behavioural research are also discussed.

1.2 Chapter 3: The Role of Salinity as a Driver of Pacific Oyster Gaping Behaviours

Chapter 3 investigated the importance of salinity variation as a driver of Pacific oyster behaviours. Estuaries are complex environments in which many factors vary in a twice daily cycle, one of the most biologically significant of which is salinity. Observations made during field trials of the NOSy system showed that gaping behaviour of Pacific oyster in an estuarine environment is semi-synchronous to the tidal cycle. We designed an experiment to determine whether the tidal salinity variations were a primary driver of the observed patterns.

Manipulation of salinity is methodologically challenging and the lack of laboratory studies in which tidal salinity variations are replicated suggest that this is an under-studied aspect of estuarine ecology. We designed two systems to replicate tidal patterns of salinity variations as measured in the River Colne, and recorded the gaping behaviour of oysters over multiple replicated tidal cycles. The gaping behaviours observed in the field were not replicated, although there were non-significant trends that suggest the range of salinity variations tested has a small impact. We suggest other factors, such as food availability and predation pressures, as possible drivers of the observed patterns.

The findings from this chapter illustrate that patterns of gaping behaviour are influenced by multiple factors. Increasing understanding of these may allow producers to site their crop in more optimal locations, and to develop more efficient purification protocols. In a wider

research context we suggest that the development of salinity control methodologies will broaden the scope of estuarine and coastal biological research.

1.3 Chapter 4: Trial of a Land-Based Oyster Nursery System for Commercial Use

Chapter 4 details the development and trial of a new system for on-growing of purchased small oysters (seed) at Colchester Oyster Fishery. The current method the fishery uses is to keep the seed in mesh bags in the estuary until they reach a sufficient size to be laid out on the intertidal layings, where they grow to market size. This method has been used extensively by the company for decades, but it is labour intensive and exposes the oysters to the estuarine environment and associated risks. Land-based systems have the potential to reduce both labour costs and environmental exposure, which in turn allows for the purchase of smaller, and therefore cheaper, seed.

We designed and built a system on-site at the fishery, which was trialled over the course of three summers (2020 – 2022). We trialled a range of stocking densities, seed sizes and stocking patterns within the units. In the first trial two stocking density treatments, at a 9:11 ratio, were compared, growth was significantly faster in the lower density treatment. Both trialled densities were above those recommended by hatcheries for the majority of the trial, with no substantial mortality until the pump supplying the system with water failed.

In the second trial we stocked the units with seed that were 80% smaller than the previous year, but at a 90% lower overall density due to a lack of availability of small seed. Two stocking densities were again trialled, with the same ratio as the previous year. No difference was seen in treatment averaged growth rates in this trial. However, double the number of oysters failed to reach a minimum size by the end of the trial. We suggest that this difference was a function of the stocking pattern in the units, in which the oysters were all kept in a single container. The high density treatment therefore had a higher localised, or experienced, density within the container, although the overall density in the unit was

relatively low. This led to greater competitive asymmetry in the higher density treatment, as a result of which dominant animals exhibited disproportionate growth despite regular grading, the removal of the largest animals from the stock.

The stocking pattern in half the units was redesigned for the third trial to layer the oysters within the units. This had the effect of reducing the localised density without affecting the overall stocking density per unit. Only large seed was available for this trial, nearly three times larger than the seed used in 2020, and 19 times larger than in 2021. Initial overall stocking densities were just over half the hatchery recommended levels. Treatment averaged growth rates and growth uniformity were both higher in the layered units. Shell shape, a key aspect of consumer desirability, was also improved in comparison to oysters grown in the estuary. This demonstrates that an understanding of competition regimes within a population can allow for significant gains in the efficiency of production methods without the addition of extra capacity.

Cost forecasts demonstrate that under optimal stocking regimes, and with the use of on-site generated power, land-based methods could be of substantial benefit to the fishery, reducing the cost of oysters at the point of laying out by half. This represents a potential saving of tens of thousands of pounds a year, as well as reducing exposure to the risks incurred when placing small oysters in an estuary.

*1.4 Chapter 5: Gaping Behaviour of Blue Mussels (*Mytilus edulis*) in Relation to Freshwater Runoff Risks*

Chapter 5 focused on the study of Blue mussels. The study site was a mussel bed located in Dundrum Bay, Northern Ireland, that has historically supported regular harvest, but in recent years has become too contaminated to allow economical exploitation. In collaboration with colleagues at the Northern Irish Agri-Food and Biosciences Institute (AFBI) we deployed the

NOSy system for nine days on mussels on the bed to determine if their patterns of behaviour had any impact on their vulnerability to waterborne contamination.

High quality data were recorded throughout the nine-day deployment, proving that NOSy has utility and value in the monitoring of non-oyster species. These data showed that mussels in the bay had clear patterns of closure over the low tide period. The salinity regime in Dundrum Bay has an extreme variation, we recorded salinities from 1.8 to 33.6 over the NOSy deployment and we suggest that the observed behaviours are a response that protects the mussels during low-salinity periods. Contamination carried by freshwater runoff will be at its highest concentration during low tide, when the marine influence on the bay is minimal, and as a result this pattern of behaviour provides a degree of protection from this form of contamination. AFBI hope to carry out further work to determine the source of the contamination that is affecting the mussels.

These data provide an insight into the patterns of mussel behaviour in Dundrum Bay that would not have been possible without the use of gape sensors. We suggest that the use of NOSy to better understand the behaviour of bivalves could be of great benefit in the development of environmental monitoring programmes.

2 Active Synthesis

The work of this PhD project has created knowledge, refined protocols, and developed approaches with application to both the bivalve shellfish aquaculture sector and wider monitoring and research. The work described in chapters two, three and five developed and proved methods to make use of the NOSy system, and indeed any other form of valvometric sensor. We also recorded wild gaping behaviours of *M. gigas* and *M. edulis*. For *M. gigas* we examined the causes of these in a controlled laboratory environment, demonstrating that salinity was not likely to be a primary driver of gaping behaviours in an estuarine environment. In the case of *M. edulis* we were able to determine how vulnerable the population was to contamination resulting from runoff derived pollutants.

In combination with a wider suite of monitoring tools, such as bivalve heartrate monitors (Electric Blue, 2020), electronic logging of temperature and salinity, and water sample analysis for nutrient levels and contaminants, NOSy can contribute substantially to our understanding of how bivalves interact with, and are affected by, their environment. The use of valvometric sensing is increasingly accepted as a powerful tool, and as demonstrated by Ahmed *et al.* (2017, 2016) it is possible to employ algorithmic detection of 'non-normal' behaviours in HFNI valvometric data. As access to machine learning and AI tools become more accessible the power of this approach will only increase, further adding to the utility of the data provided by systems such as NOSy.

By building on the work of this thesis, along with that of other groups (e.g. Borcherding, 2006; Durier *et al*., 2022; Tran *et al*., 2003) it will be possible to leverage an increased understanding of bivalve behaviours to improve production efficiencies in aquaculture. Detection of spawning in *M. gigas* and how it could improve production has been successfully demonstrated and extensively discussed in this thesis. But the use of valvometric tools in bivalve culture is not limited to this; the MusselMonitor (Kramer and Foekema, 2001) provides an example where valvometry can contribute to wider monitoring of cultured shellfish. Adoption of similar tools would allow growers to monitor growth, stress levels and spawning behaviours of their stock, ensuring that husbandry and harvesting operations are targeted as efficiently as possible.

Outside of aquaculture there are a number of applications for HFNI valvometry which are discussed in detail in Chapter 1, Section 4. Perhaps the most impactful of these could be the creation of large scale environmental monitoring networks, employing valvometer equipped bivalves as sentinel organisms to monitor environmental conditions. The work described in Chapter 5 was fundamentally a small-scale version of this concept, in which a sub-set of the local *M. edulis* population was monitored in order to better understand their relationship to their environment. Substantial work is required to ensure that the data generated by such

systems are properly understood, but the potential for bivalves to function as highly sensitive, broad spectrum, sensors as part of a large-scale network is significant.

Chapter 4 focused on a separate aspect of bivalve culture, improving the process of on-growing seed oysters purchased from a hatchery. This work was designed to address the drawbacks of the system currently employed by our industrial partner, Colchester Oyster Fishery, which lacks control and therefore is high in risk. We demonstrated that the use of land-based systems is practical and controlled, can provide high growth rates with low mortality and has the potential to result in substantial cost savings. The results of these trials were sufficiently positive to continue small-scale work for three years, during which iterative improvements in design and methodology resulted in significant improvements in outcome. The next steps in the process, up-scaling of the system and development of on-site hatchery capacity, are currently being discussed and funding applied for.

Hatchery sourced oysters are a key element in many oyster producer's processes, therefore improvements in the initial months of growing the seed oysters have the potential for substantial impact. Further benefits may be realised if renewable power generation is integrated into the design of such systems, both in terms of global impact and reduced operational costs leading to greater profitability.

3 Future Work Direction

3.1 The NOSy System

Chapter 2 makes several suggestions about the work needed to improve the NOSy system, with the aim of developing it into a viable commercial product. Broadly speaking, the improvements needed are: greater reliability; use of the unit's remote communication capability; development of behavioural detection algorithms; and the addition of on-board processing. The sensor design itself has proven simple and effective, with an inherently low power requirement.

The first step in improving reliability is to modify the system so it does not run out of power in the field. The addition of larger batteries with the ability to cope with a large number of charging cycles is a relatively simple first step. Different power generation technologies must be explored, particularly the potential of tidal flow generators when the units are deployed in marine environments. Reduced power consumption should also be a priority, such as the replacement of the Beaglebone board with a computer that is optimised for long-term field deployments.

Use of more specialist computing equipment would have the further benefit of reducing the likelihood of system failures, such as loss of digital access and malfunctioning storage capacity. The Beaglebone unit that is used to manage access and data storage is not designed for non-desk based applications, and is particularly poorly suited for use in physically unstable places. We have been informed by experts that the many of the system issues we have experienced are likely to be as a result of Beaglebone hardware failure and are actively exploring other options.

Remote communication is also vital if NOSy is to become a commercially viable product. At present the remote communication ability of the units is not realised, and users need to physically access the unit to download data. This is time consuming and inconvenient, particularly when units are deployed in remote locations. Remote communications, using the cellular network or lower bandwidth options such as LoRaWAN, would substantially reduce this need, although they are likely to add to power consumption without optimising the protocols.

Automatic detection of spawning was a principle aim of developing NOSy. Colleagues in the original NOSy grant team have expertise in developing signal detection algorithms, and have done so for valvometric spawning detection in the past. Unfortunately, they have not yet been able to tailor these to the NOSy unit, but doing so is a priority for future work with the system.

Valvometric data can, in principle, be used to establish a baseline of normal gape behaviour, deviation from which can be characteristic of a response to environmental perturbations. For example Funesto (2023) demonstrated gaping responses of *M. gigas* to toxic algae, whilst Tran *et al.* (2003) did so for the Asiatic clam in the presence of cadmium. Substantial work is required to develop algorithms that can realise this, but the potential power of a network of sensors deployed to monitor farm stocks or natural populations is significant. The proliferation of AI and machine learning technologies may further add to the utility of gaping data by providing improved pattern recognition and 'big-data' analysis potential.

On-board processing power has the potential to further increase the utility of the NOSy system. Development and use of algorithms to detect behavioural deviation would allow the unit to alter frequency of monitoring, reducing power consumption when behaviours are normal. Remote communication, a power intensive process, could be activated only when certain behaviours are detected.

In summary, whilst the potential of the basic technology behind NOSy has been demonstrated, a lot of work remains to be done to develop the system further, on both hardware and processing. Additional development of end-user training programmes, data-sharing systems and marketing tools, plus identification of further potential uses, would add greater value to the product. NOSy has the potential to be a powerful tool, in the aquaculture sector as originally proposed and in the environmental monitoring and research sectors, but investment will be required to maximise it.

3.2 Experimenting with Salinity

We encountered significant challenges when designing a system for controlled salinity variation experiments. Possibly as a result of the challenges inherent in developing such systems this appears to be an under-studied aspect of estuarine and coastal science. We

suggest that whilst the ‘Victorian Plumbing’ system produced usable results it is not a practical method for extensive use in the laboratory.

Further methodological development should focus on the system as initially redesigned, using two high precision pumps, one to pump a relatively high volume of low saline water, the second to provide a low, variable, flow of hypersaline water to moderate salinity. Despite extensive efforts we were unable to develop flow control protocols with the available pumps, one of which failed during the process. Further development of this methodology, potentially combined with salinity sensors in the experimental chambers to increase the precision of the generated cycle, would increase the ability of researchers to investigate a fundamental aspect of estuarine and coastal biology.

3.3 Land-Based Growing Systems

Chapter 4 details the successful trial of small-scale, land-based, oyster-growing systems. The results from these trials were encouraging, and merit further work. The primary aim of this should be the scaling up of systems to a full commercial level. The evidence from trials conducted so far is that large-scale use of this kind of system could provide a substantially improved return on a fishery’s investment in seed oysters. During further trials investigation into use of small seed oysters, optimisation of stocking patterns and triploid oysters should take place. In addition, the development of a methodology to reliably track the growth of individual oysters would illuminate the effects of competitive asymmetry and size-dependent growth rates.

Of interest as an extension to the work described in this thesis, is the development of a protocol that combines the automatic spawning detection and the use of land-based growing systems. Coupelles, lime-coated plastic structures designed to attract naturally occurring spat, are commonly used by French oyster growers (Koike and Seki, 2020). In theory, by using a system that informs them when spawning has occurred, a grower could achieve

extremely high rates of settlement on coupelles, using them as a source of seed that reduces the need to purchase them from hatcheries.

Unlike the cultch that that is used by COF and other growers, coupelles can be easily removed from the water, after which the newly settle oysters are detached. The oysters could then be grown-on in a land-based system. Proven to provide an environment which encourages rapid growth and low mortality, a land-based system would ensure a high return from the spat collection effort, with potentially substantial savings to the grower.

3.4 Environmental Monitoring

Further work is required in Dundrum Bay to accurately attribute the source of contamination that is currently rendering the mussels in the area un-harvestable. This project clearly demonstrated the potential of valvometry to provide insights into bivalve behaviours and their relation to water quality. The use of bivalves as sentinel organisms is a powerful tool for bivalve growers to monitor their stocks and as a wider tool for environmental monitoring, particularly given the current state of British coastal waters. Development of bio-sensor networks utilising remote sensing technology should be considered by researchers and policy makers as a potentially invaluable addition to current water quality monitoring protocols.

4 Conclusion

The bivalve aquaculture sector produces a healthy, valuable and sustainable crop. Over £28 million (at first sale) income was generated for UK coastal communities in 2018 (Syvret *et al*., 2021). The ecosystem service provision associated with oyster reefs has been valued at £17 376 $ha^{-1}yr^{-1}$ (Syvret *et al*., 2021). The adoption of modern technologies and approaches will improve production efficiencies in the sector, in turn enhancing its positive contributions. Non-invasive gape sensing technology has underpinned most of the research behind this thesis and has great potential to assist bivalve producers in future, through increased

understanding of the factors that affect farmed species and by providing them with accurate and real time data from their stock. The technology also has substantial utility as a research tool, and as part of environmental monitoring programmes.

In this thesis we successfully demonstrated the use of the NOSy system, a gape sensor designed and built at the University of Essex. Using the system we detected spawning in Pacific oysters, developed our understanding of the impact of environmental factors on oyster behaviour and gained important insights into how in situ behaviours of mussels in a challenging environment can affect their vulnerability to contaminants. The challenges of developing these systems has been discussed in detail, and suggestions made as to the priorities for future improvements.

We have also demonstrated the efficacy of a novel, relatively simple, method of on-growing oysters purchased from hatcheries. If scaled up this system has the potential to produce high quality oysters at a substantially lower cost than the current methods in use by our industrial partner, Colchester Oyster Fishery. The success of this system highlights that even relatively low-tech solutions have the potential to improve efficiencies in the bivalve aquaculture sector.

We believe that the work described in this thesis has the potential for substantial impact. The technologies and approaches we have described, if adopted by growers, could improve efficiency and reduce risk in multiple aspects of the bivalve aquaculture industry. Further, the sensor technology we have described has a broad range of applications outside the aquaculture sector, with potential to contribute substantially to research and environmental monitoring.

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Appendices

1 Statistical Analysis Summary of all Results (Chapter 3)

Appendix 1 Table 1.1. Summary of the Kruskal-Wallis testing results from both salinity experiments

All (non-excluded) oysters

Year	Dependent Variable	Independent Variable	Deg. Freedom	H Value	p
20+23	Time Open	Year	1	6.12	0.01
20+23	Closure Rate	Year	1	5.97	0.01
20	Time Open	Experimental Condition	3	7.48	0.06
20	Closure Rate	Experimental Condition	3	2.63	0.45
23	Time Open	Experimental Condition	3	6.90	0.08
23	Closure Rate	Experimental Condition	3	3.28	0.35
20	Time Open	Run	2	1.55	0.46
20	Closure Rate	Run	2	2.64	0.27
23	Time Open	Run	2	9.06	0.01
23	Closure Rate	Run	2	2.80	0.25
20	Time Open	Salinity Direction	1	0.20	0.66
20	Closure Rate	Salinity Direction	1	1.47	0.23
23	Time Open	Salinity Direction	1	0.13	0.72
23	Closure Rate	Salinity Direction	1	0.54	0.46

2020 experimental, and 2023 high salinity variation only

Year	Dependent Variable	Independent Variable	Deg. Freedom	H Value	p
20_E	Time Open	Salinity Direction	1	20.32	0.15
20_E	Closure Rate	Salinity Direction	1	0.33	0.57
23_H	Time Open	Salinity Direction	1	0.24	0.62
23_H	Closure Rate	Salinity Direction	1	0.82	0.37
20_E	Time Open	Binned Salinity	3	6.18	0.10
20_E	Closure Rate	Binned Salinity	3	5.28	0.15
23_H	Time Open	Binned Salinity	3	1.67	0.64
23_H	Closure Rate	Binned Salinity	3	2.04	0.56

2 Oysters Excluded From Experimental Analysis (Chapter 2)

The following oysters were excluded from the analysis due to sensor failure or mortality:

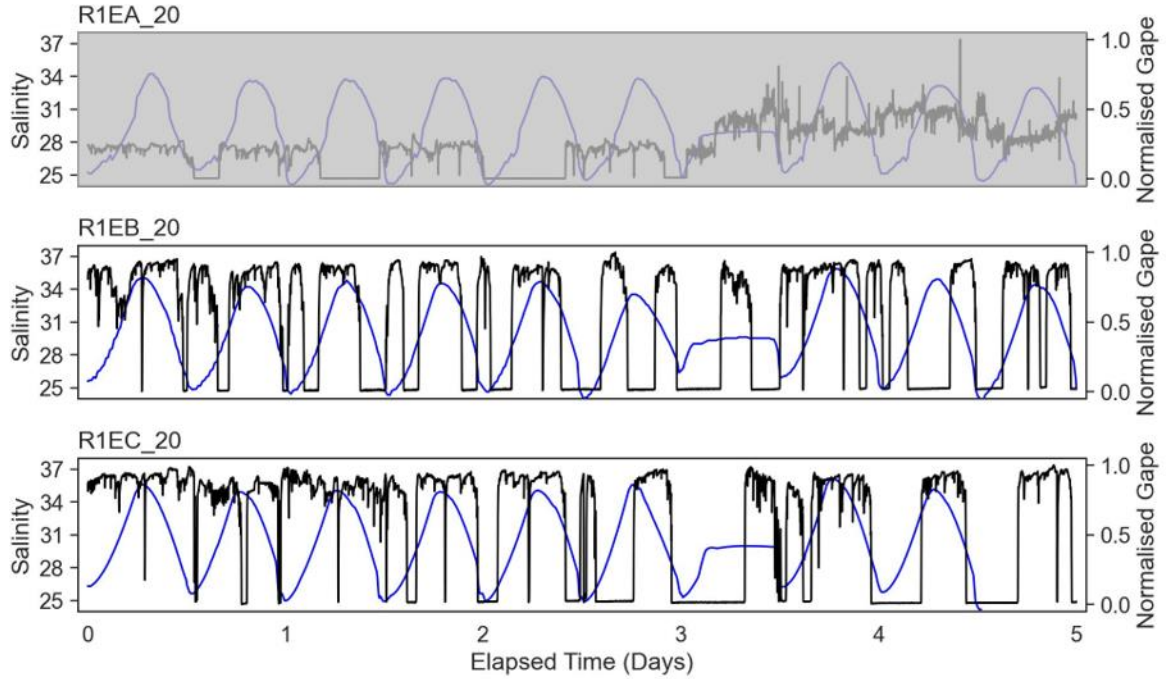
- R1EA_20
- R2LCA_20
- R3EA_20
- R1MA_23
- R1MC_23
- R1HC_23
- R2LC_23
- R2HC_23
- R2MB_23
- R3LB_23
- R3MB_23
- R3HB_23
- R3HC_23

3 Gape and Salinity Plots for all Oysters (Chapter 2)

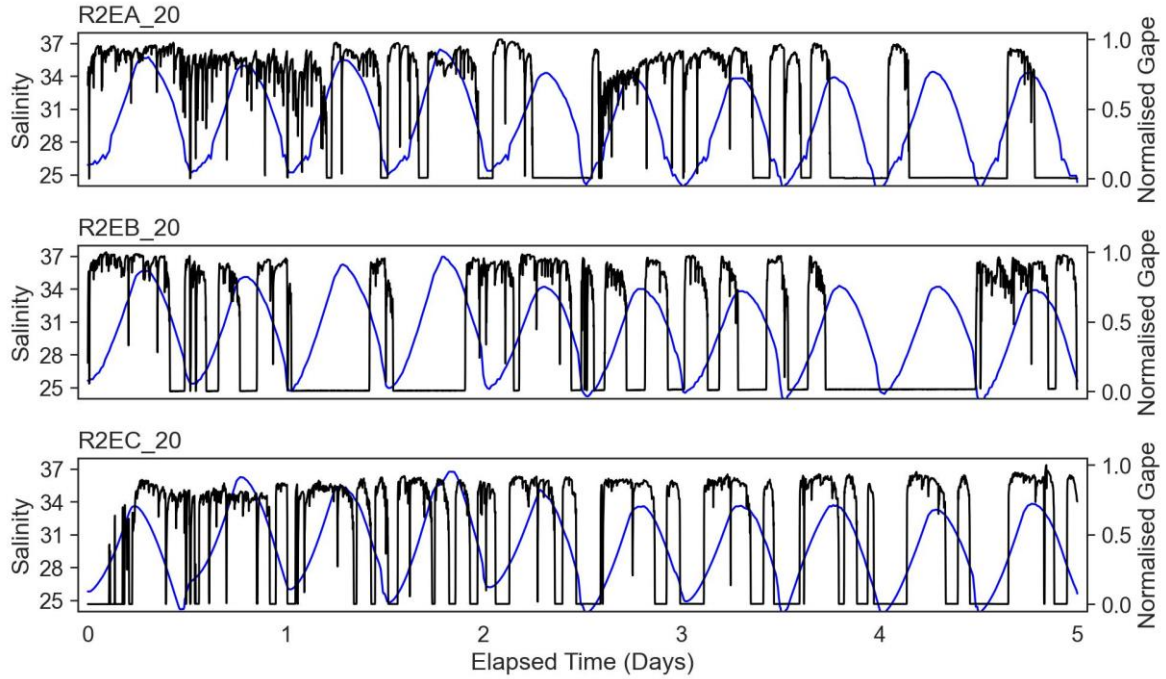
3.1 Data Oysters from the 2020 Experiment

Excluded oysters are greyed out

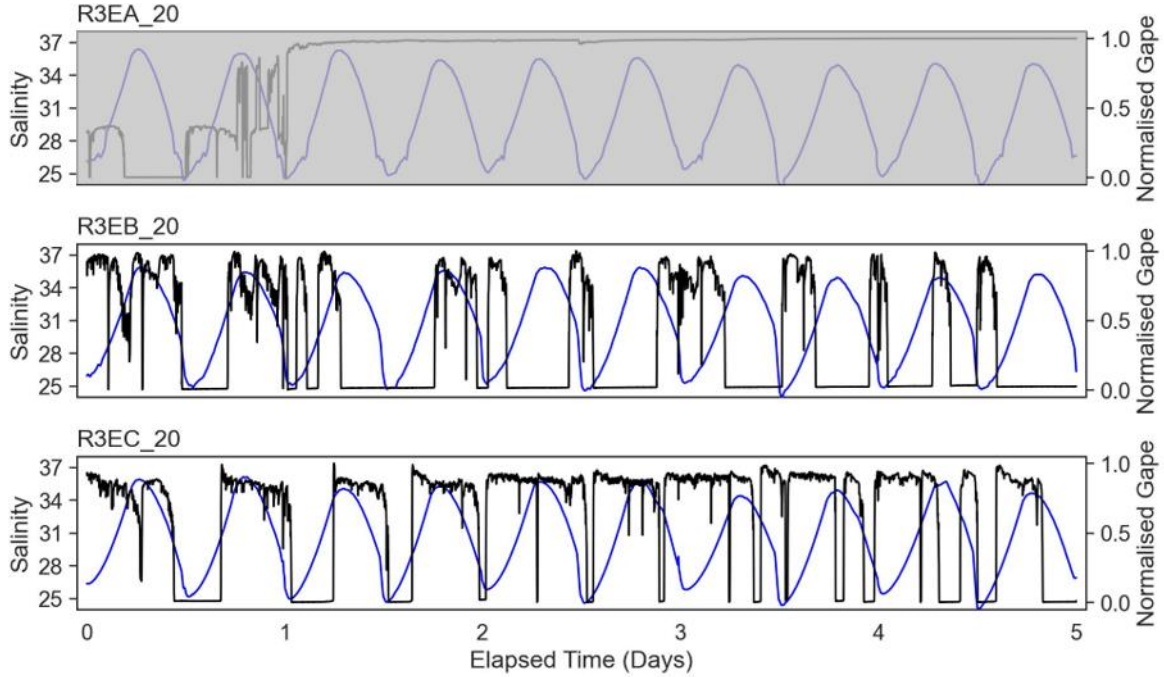
3.1.1 Experimental Oysters



Appendix 3.1 Figure 1: Gape and salinity for the 2020 run 1 experimental oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

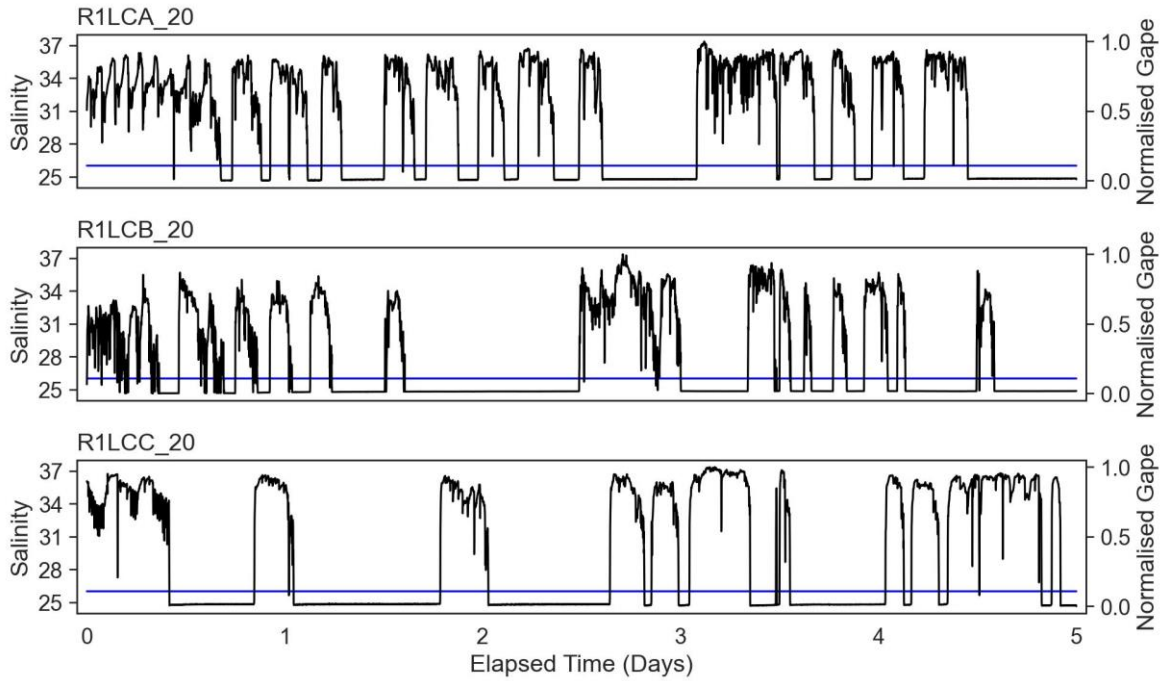


Appendix 3.1 Figure 2: Gape and salinity for the 2020 run 2 experimental oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

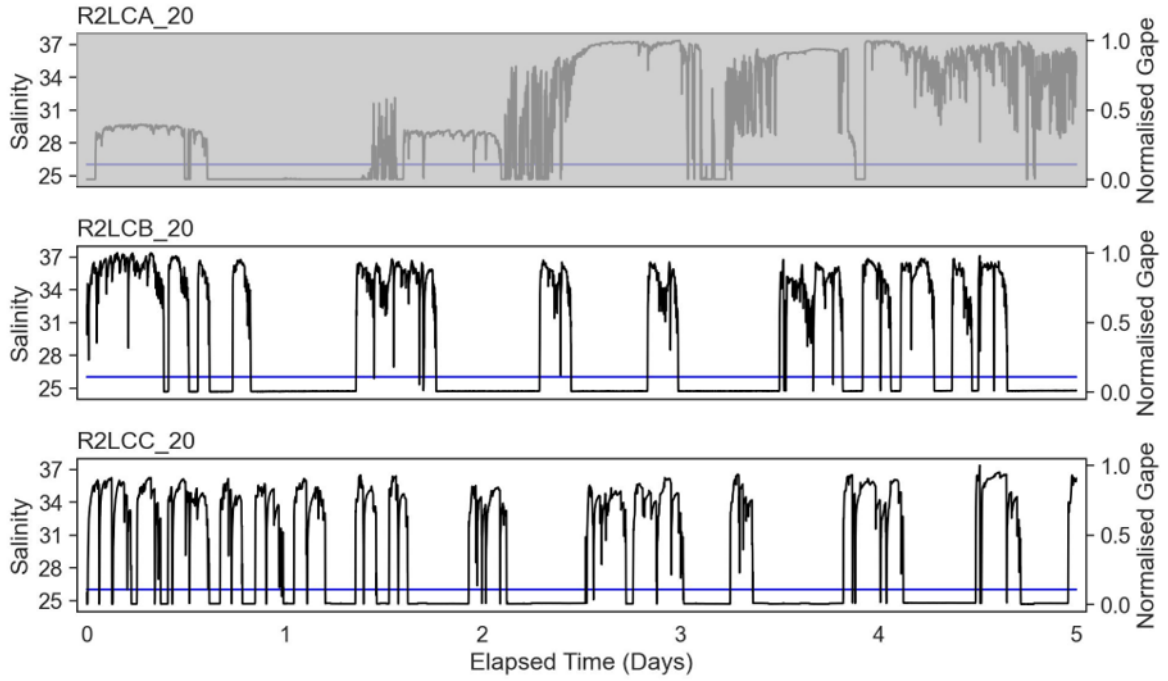


Appendix 3.1 Figure 3: Gape and salinity for the 2020 run 3 experimental oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

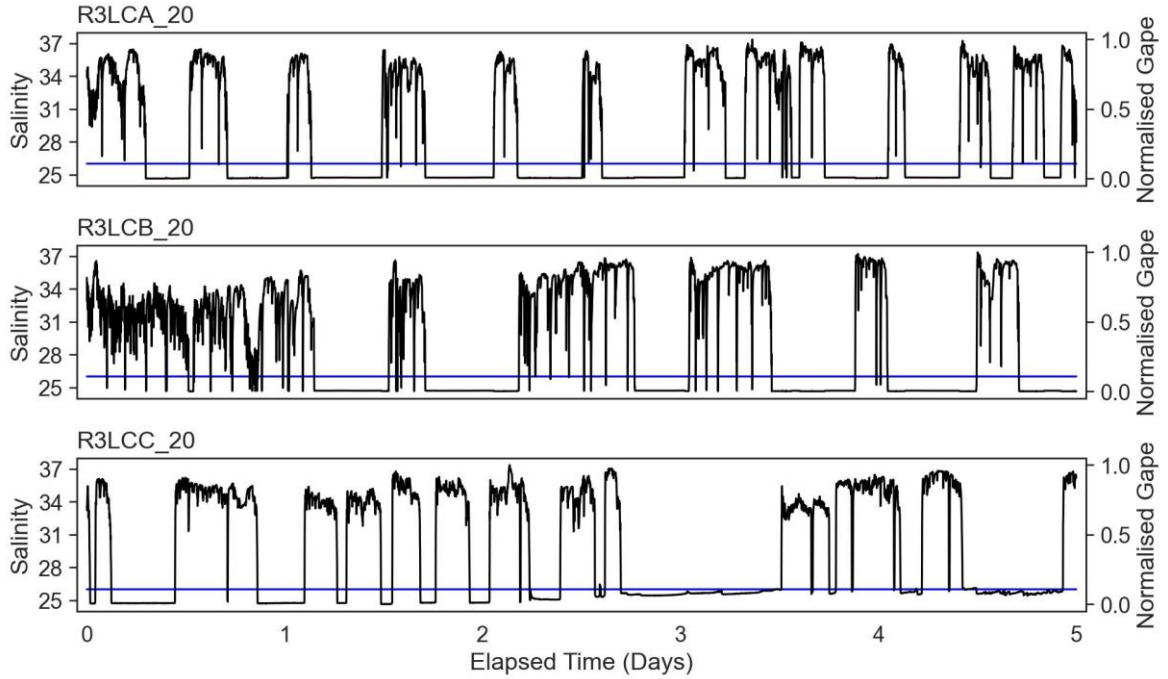
3.1.2 Low Salinity Control



Appendix 3.1 Figure 4: Gape and salinity for the 2020 run 1 low salinity control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

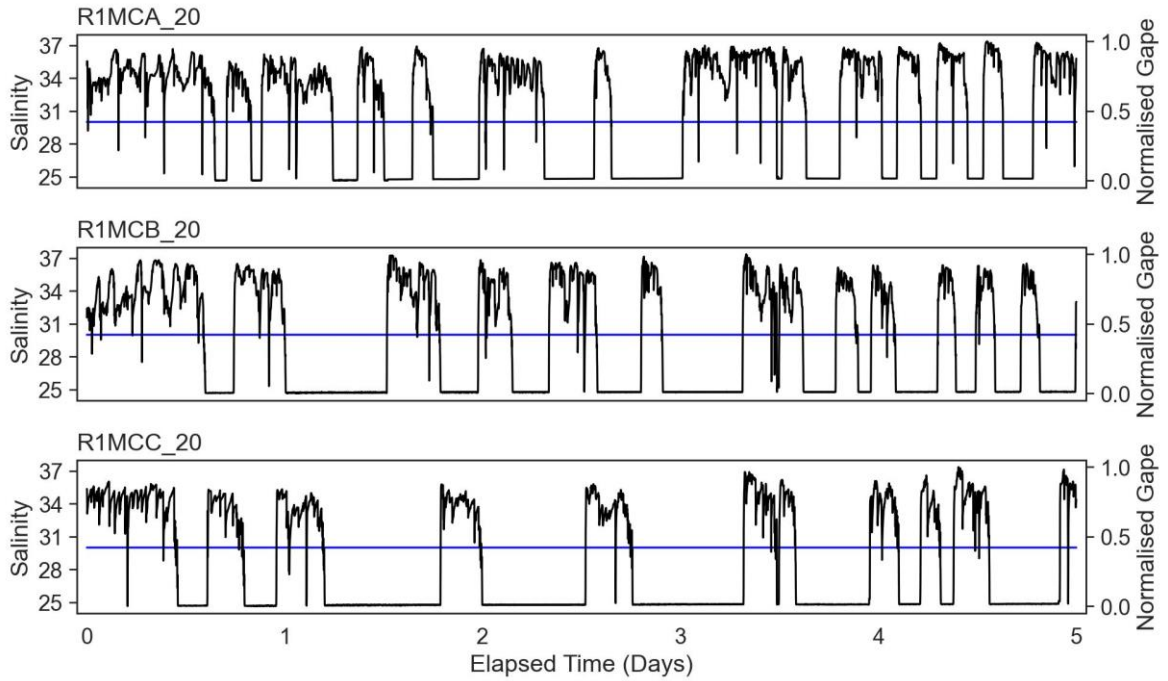


Appendix 3.1 Figure 5: Gape and salinity for the 2020 run 2 low salinity control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

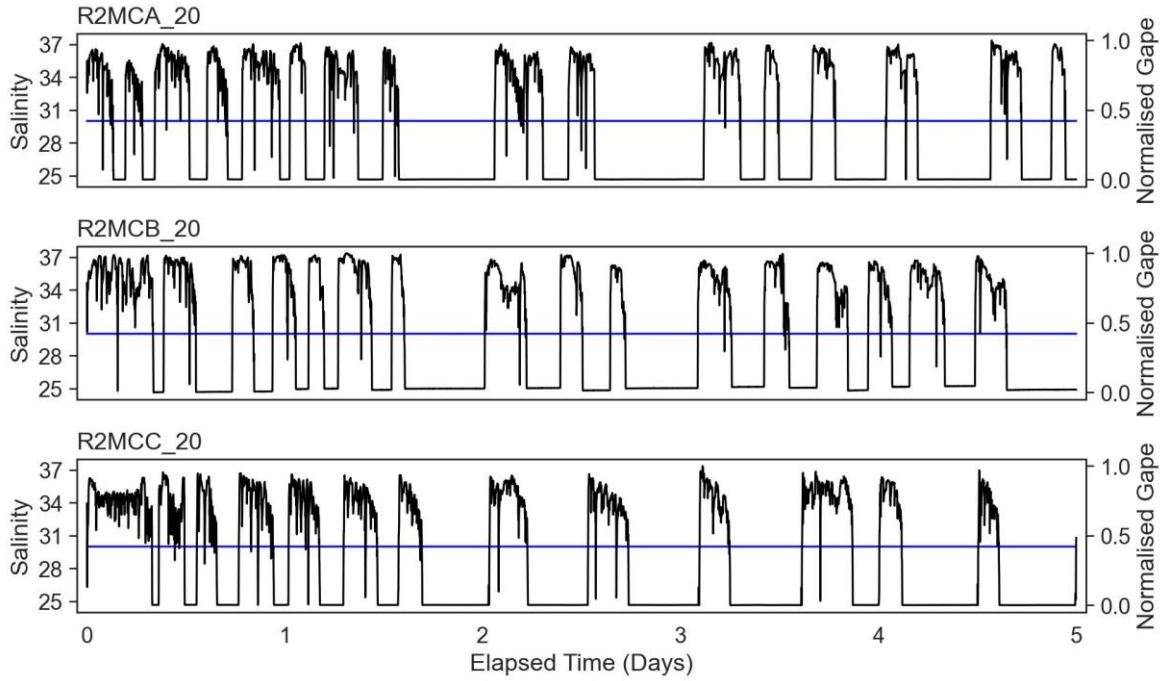


Appendix 3.1 Figure 6: Gape and salinity for the 2020 run 3 low salinity control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

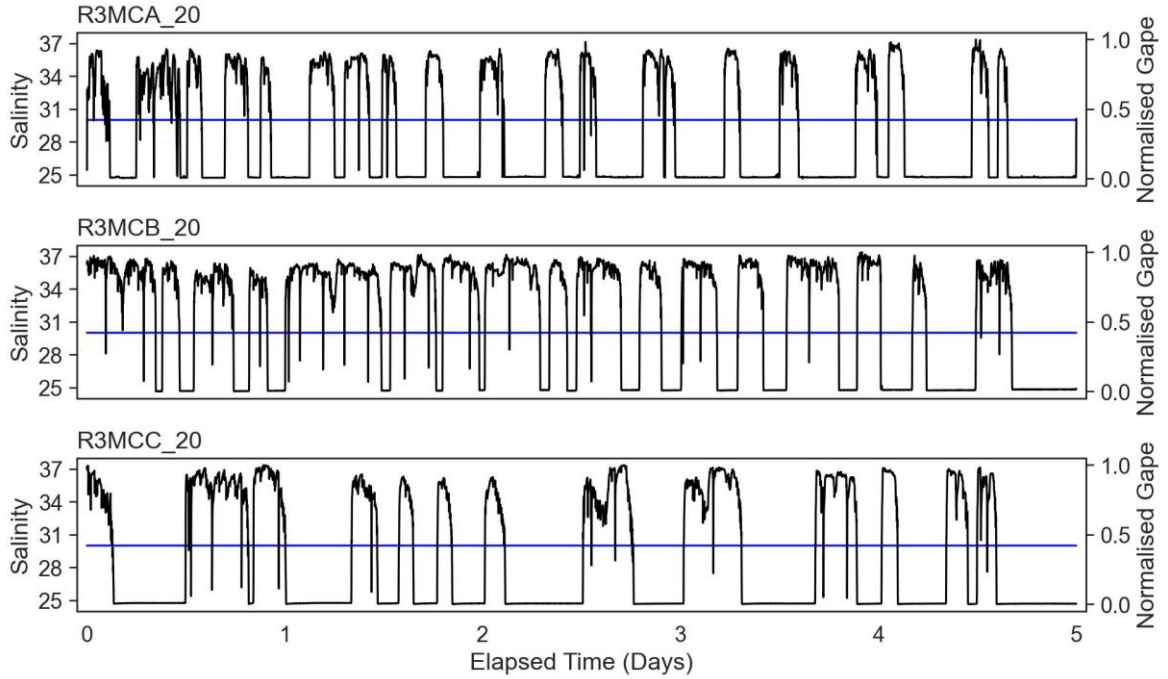
3.1.3 Medium Salinity Control Oysters



Appendix 3.1 Figure 7: Gape and salinity for the 2020 run 1 medium salinity control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

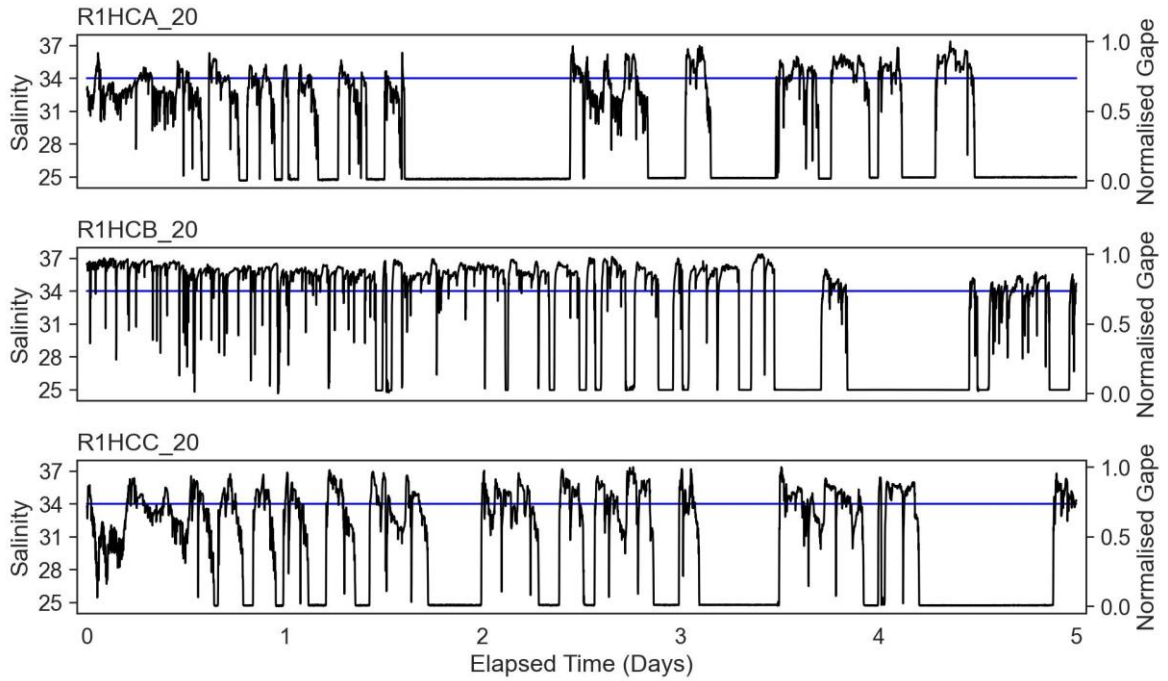


Appendix 3.1 Figure 8: Gape and salinity for the 2020 run 2 medium salinity control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

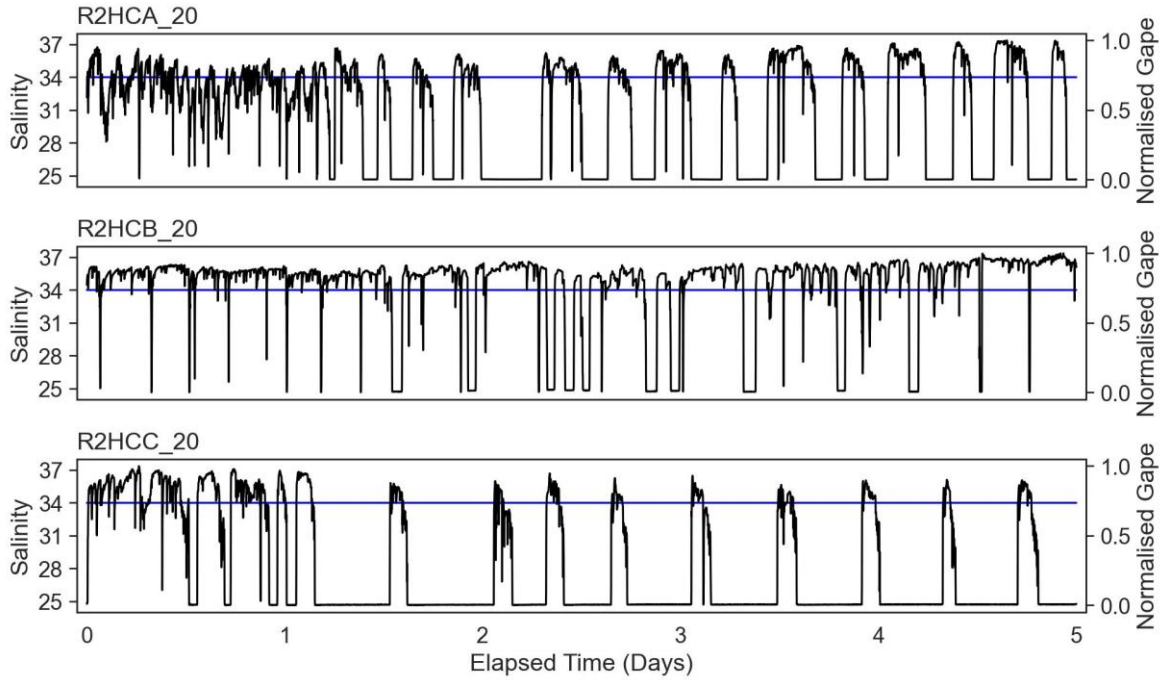


Appendix 3.1 Figure 9: Gape and salinity for the 2020 run 3 medium salinity control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

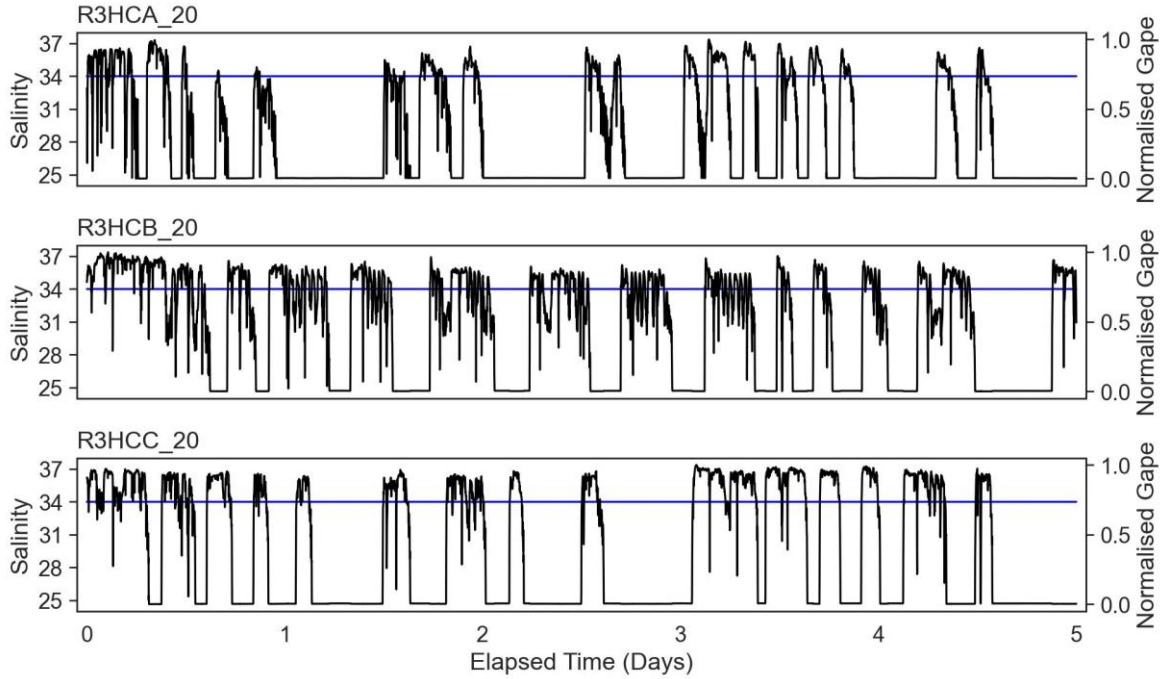
3.1.4 High Salinity Control Oysters



Appendix 3.1 Figure 10: 2020 run 1 high salinity control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.



Appendix 3.1 Figure 11: Gape and salinity for the 2020 run 2 high salinity control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

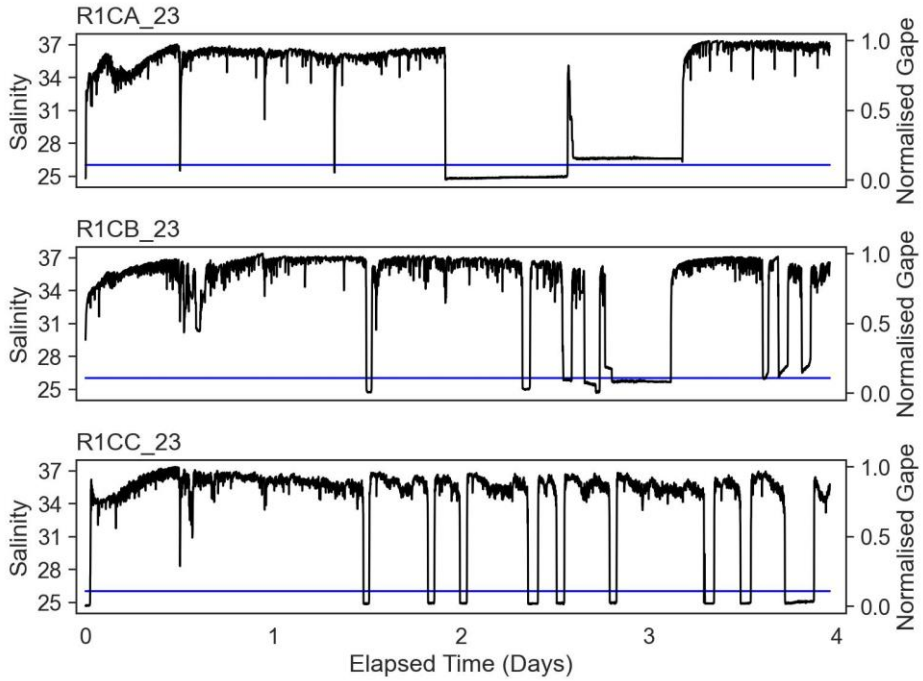


Appendix 3.1 Figure 12: Gape and salinity for the 2020 run 3 high salinity control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

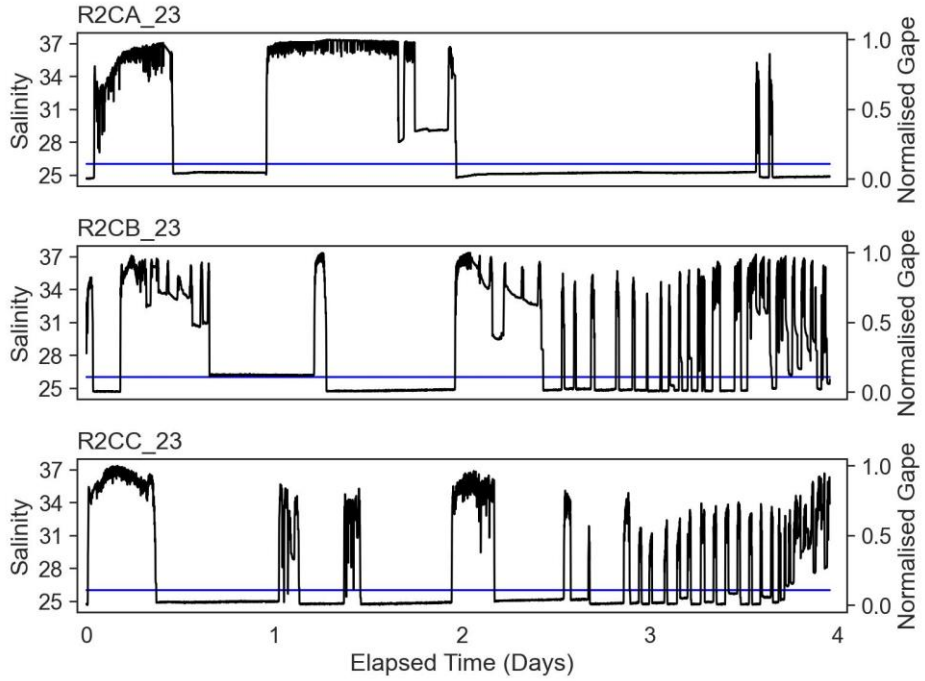
3.2 Salinity (blue) and Normalised Gape (black) and for all Oysters – 2023

Excluded oysters are greyed out

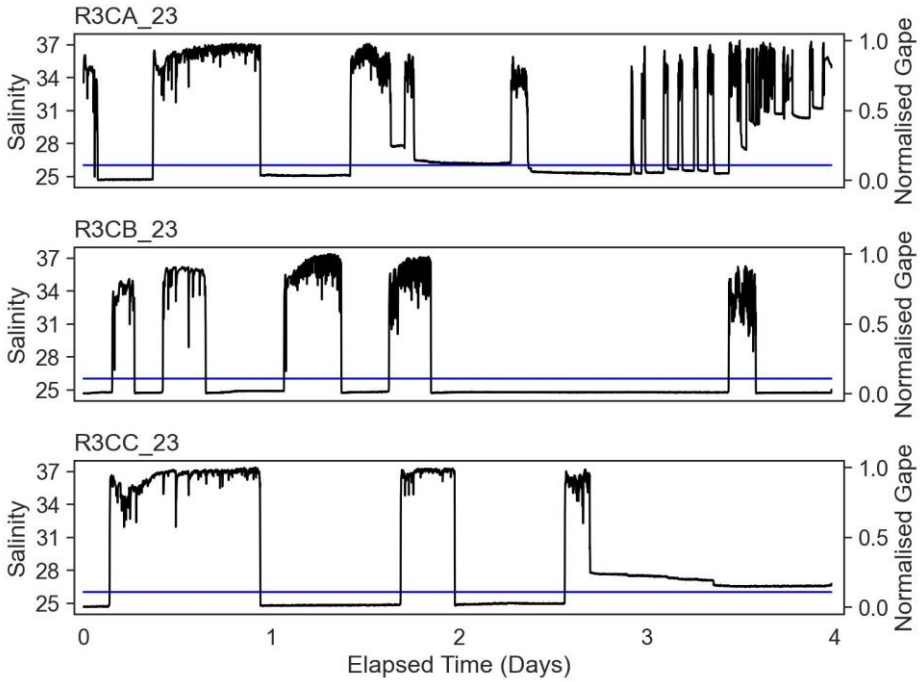
3.2.1 Control Oysters



Appendix 3.2 Figure 1: Gape and salinity for the 2023 run 1 control oysters The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

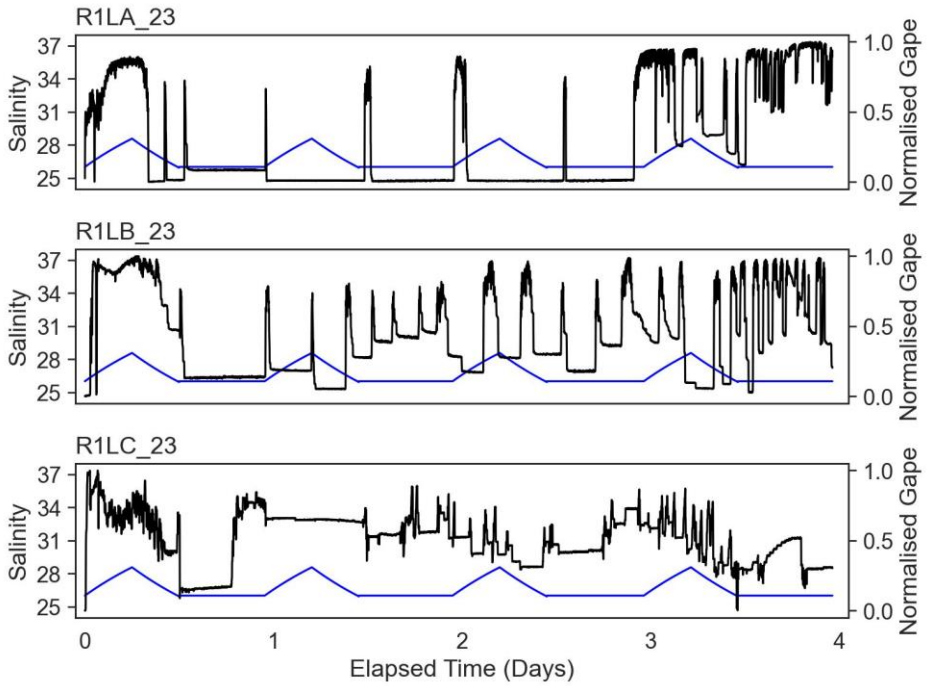


Appendix 3.2 Figure 2: Gape and salinity for the 2023 run 2 control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

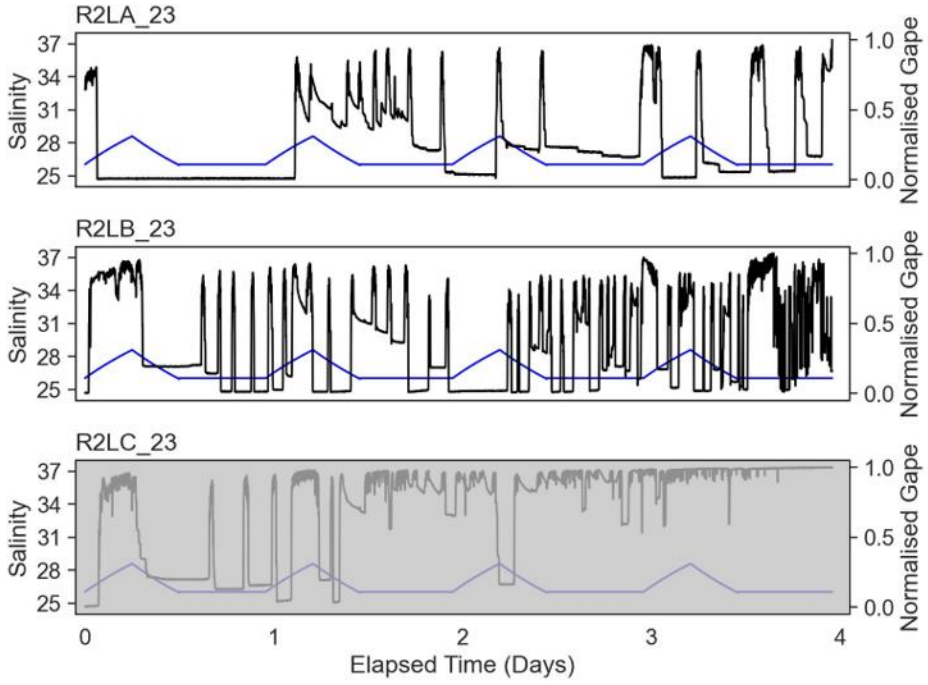


Appendix 3.2 Figure 3: Gape and salinity for the 2023 run 3 control oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

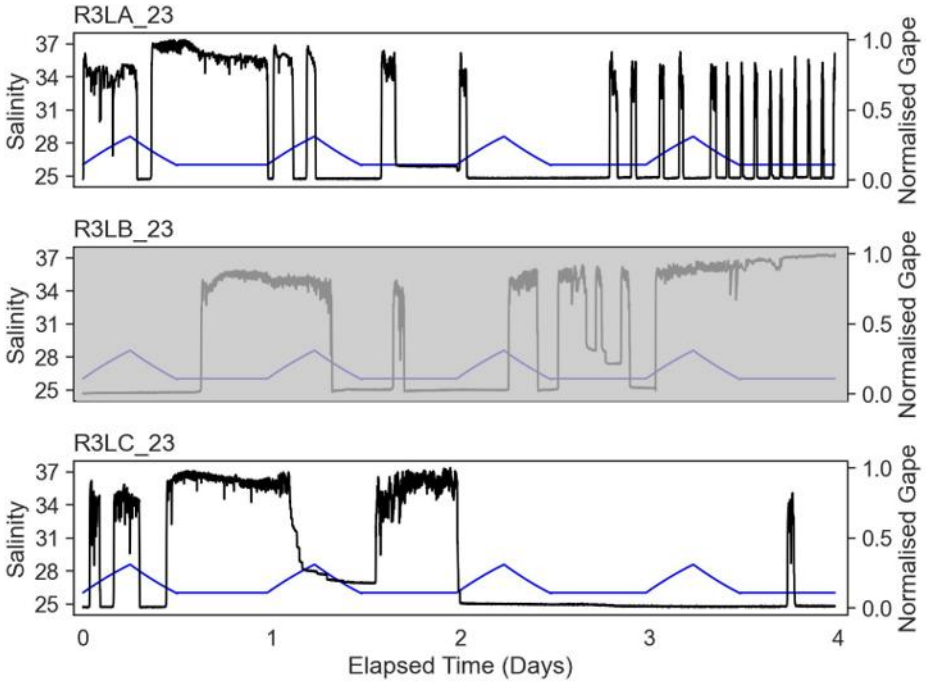
3.2.2 Low Salinity Variation Oysters



Appendix 3.2 Figure 4: Gape and salinity for the 2023 run 1 low salinity variation oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

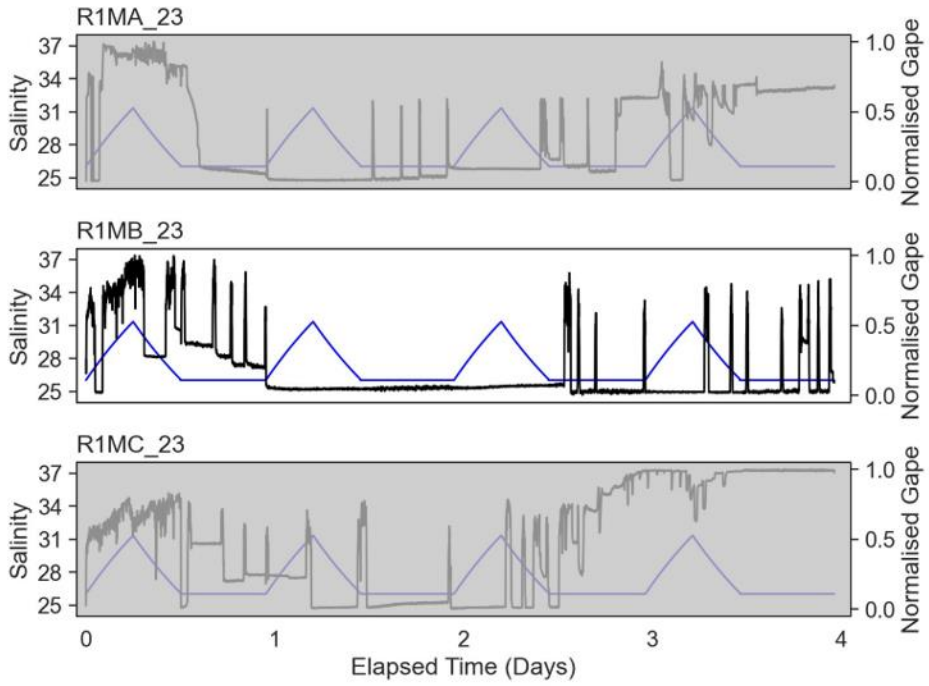


Appendix 3.2 Figure 5: Gape and salinity for the 2023 run 2 low salinity variation oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

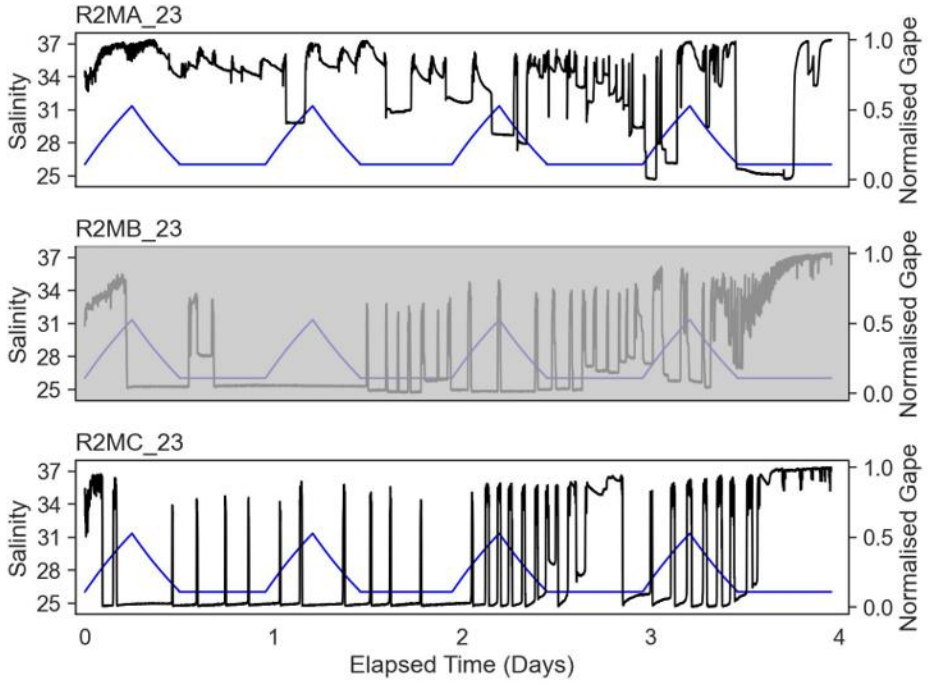


Appendix 3.2 Figure 6: Gape and salinity for the 2023 run 3 low salinity variation oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

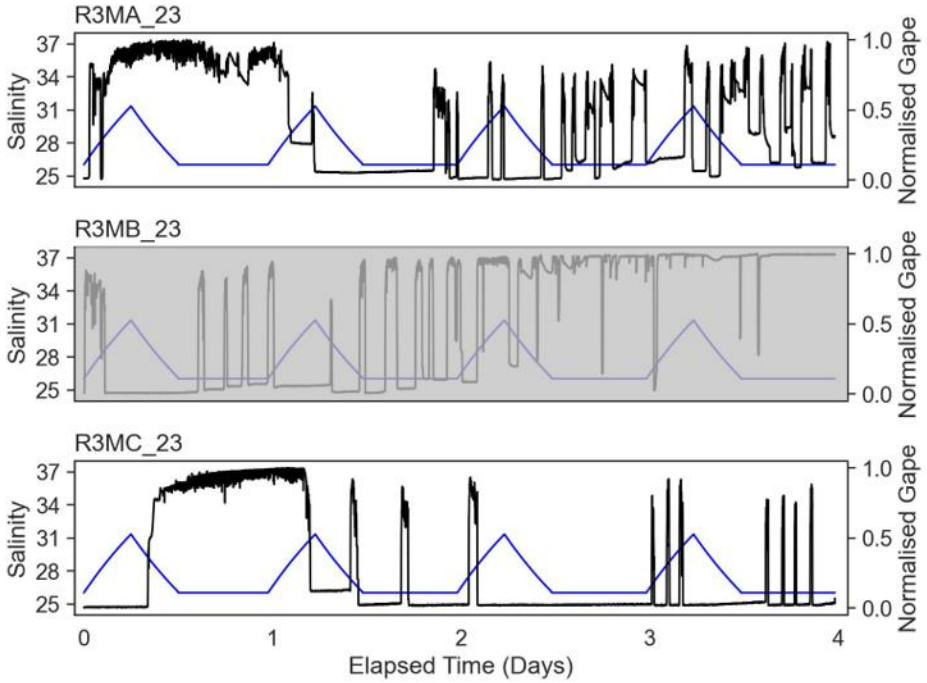
3.2.3 Medium Salinity Variation Oysters



Appendix 3.2 Figure 7: Gape and salinity for the 2023 run 1 medium salinity variation oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

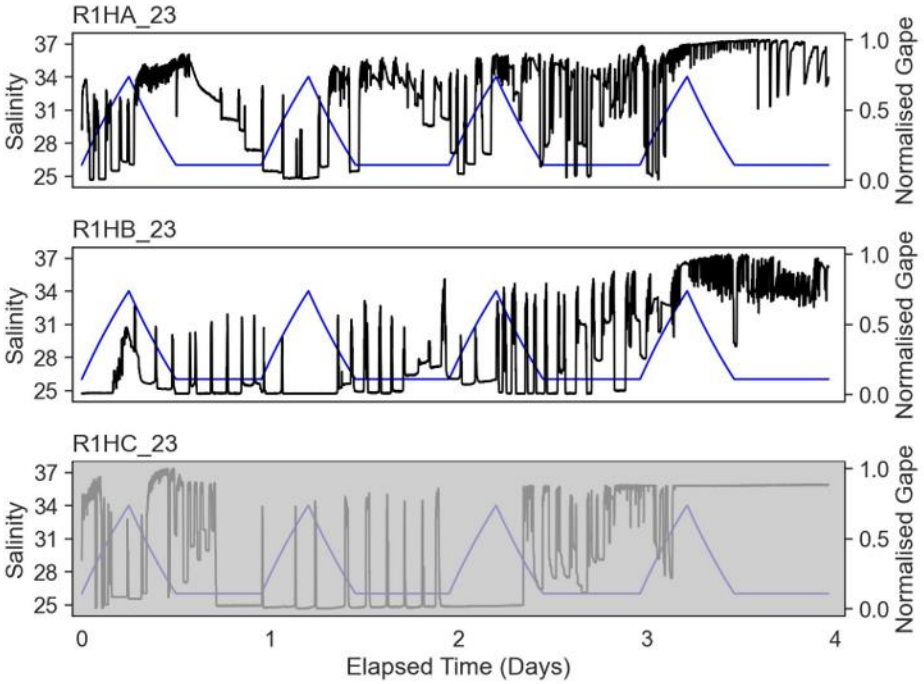


Appendix 3.2 Figure 8: Gape and salinity for the 2023 run 2 medium salinity variation oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

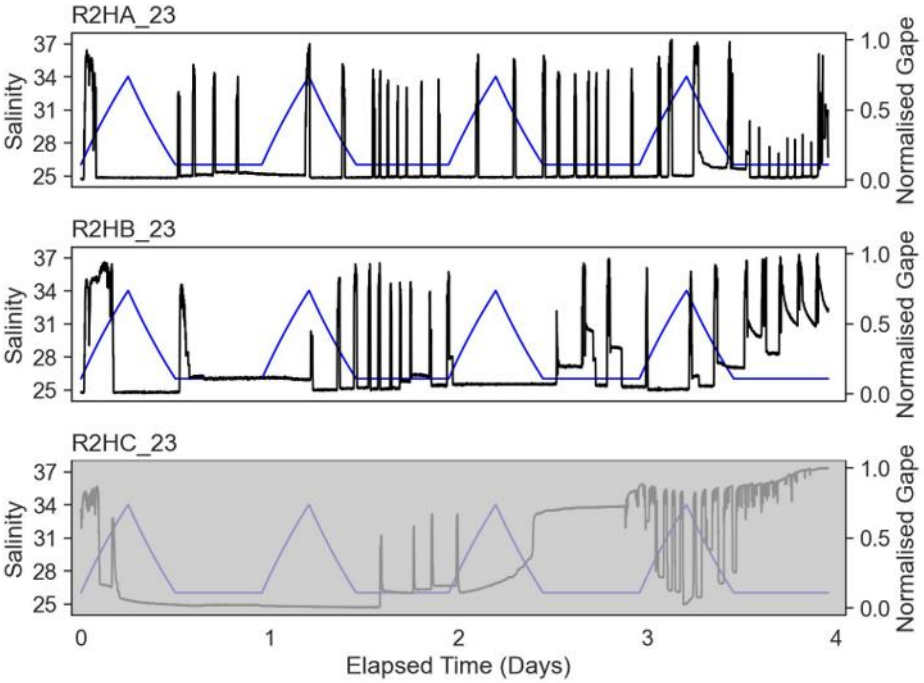


Appendix 3.2 Figure 9: Gape and salinity for the 2023 run 3 medium salinity variation oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

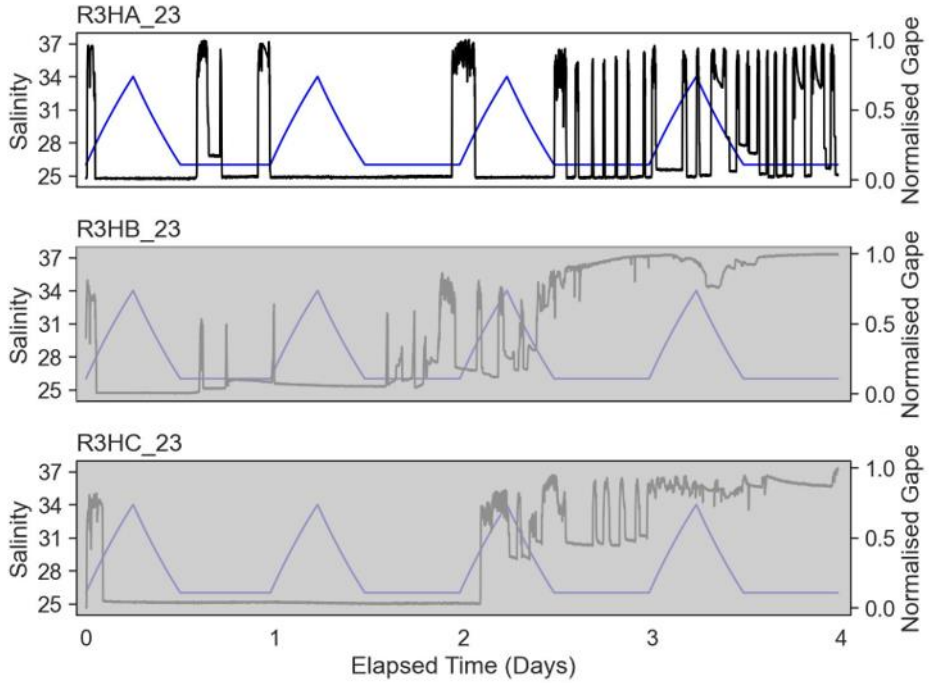
3.2.4 High Salinity Variation Oysters



Appendix 3.2 Figure 10: Gape and salinity for the 2023 run 1 high salinity variation oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

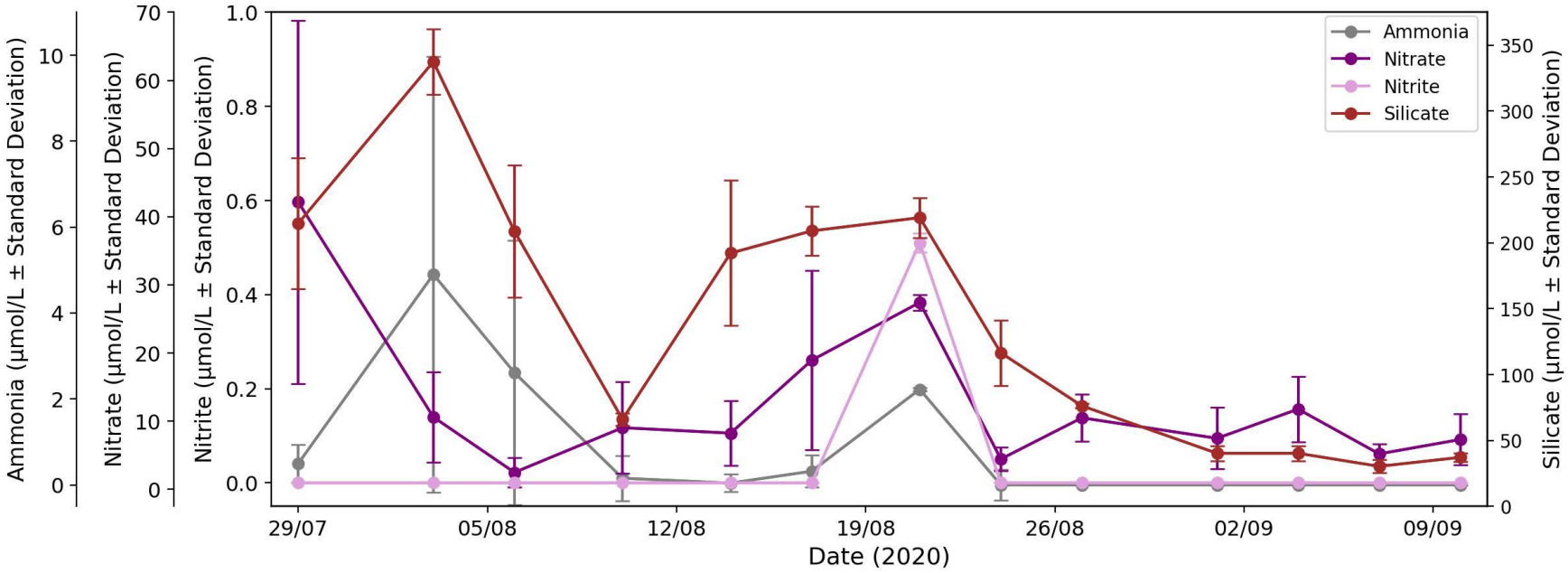


Appendix 3.2 Figure 11: Gape and salinity for the 2023 run 2 high salinity variation oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

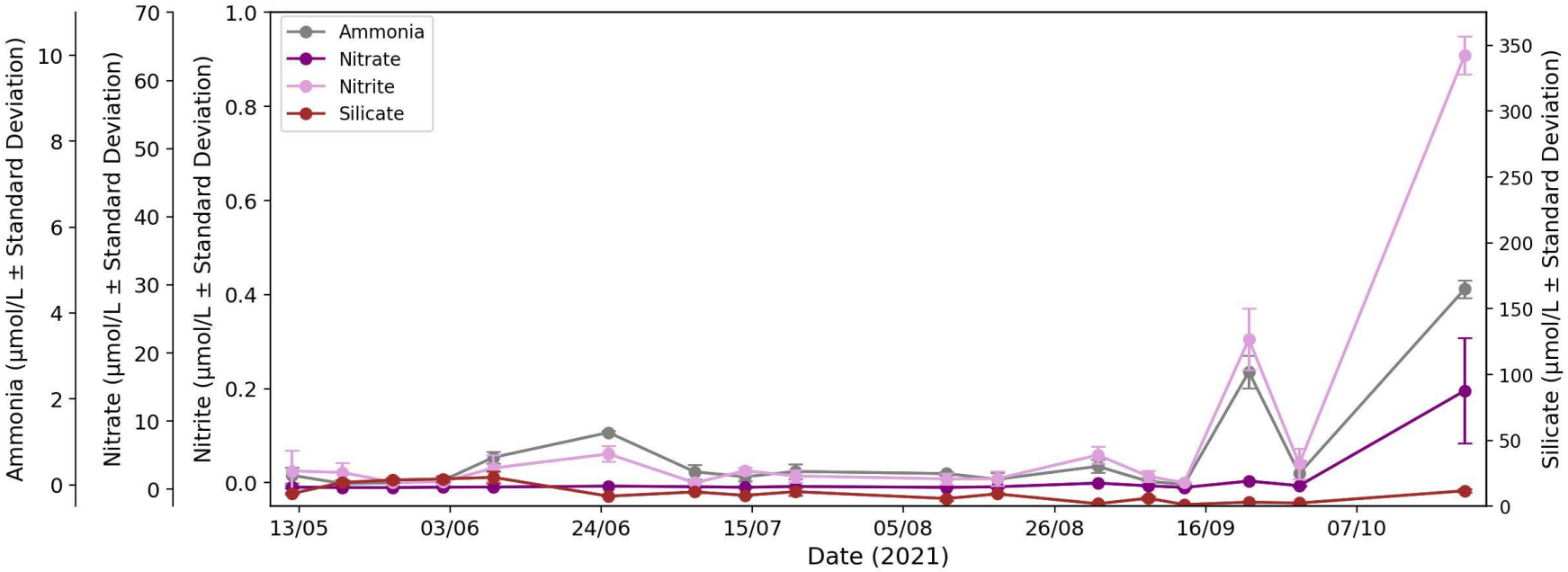


Appendix 3.2 Figure 12: Gape and salinity for the 2023 run 3 high salinity variation oysters. The black line shows recorded gape and the blue line salinity over the full duration of each experimental run.

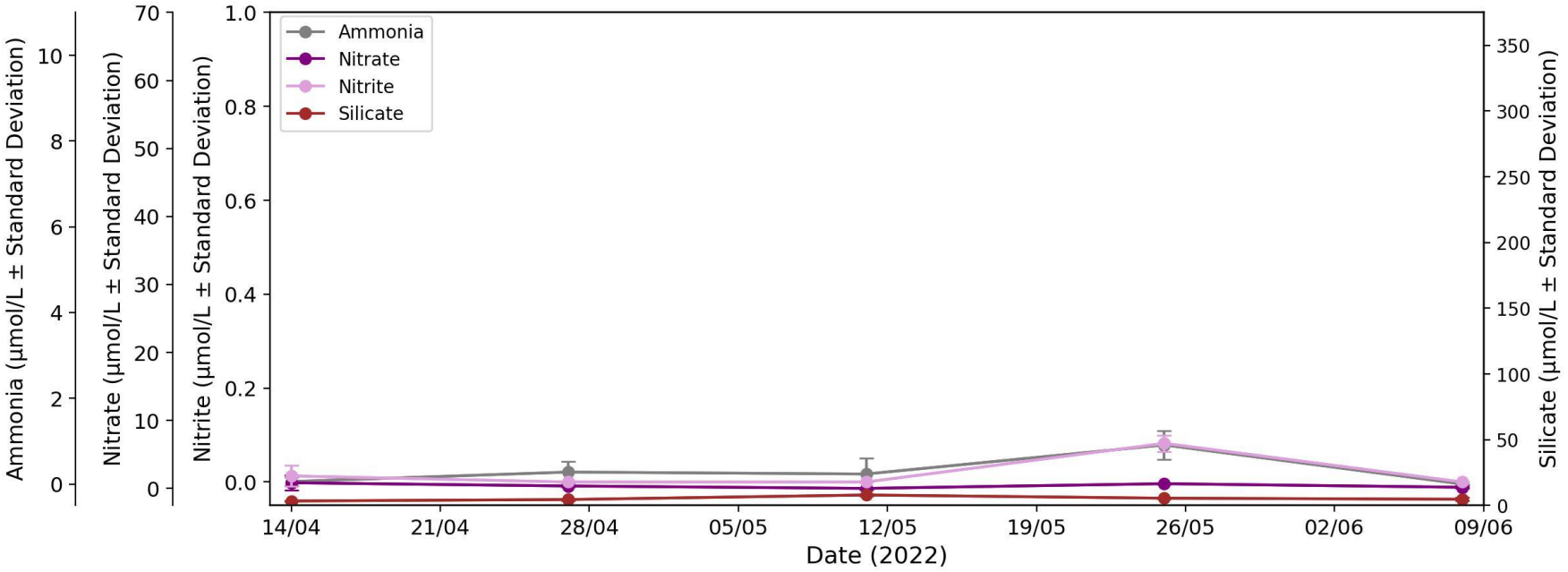
4 Nutrient Analysis Results From The Land-Based On-Growing System (Chapter 4)



Appendix 4 Figure 1: Ammonia, nitrate, nitrite and silicate concentrations (average ± SD, n = 3) from the land-based on-growing units measured during the 2020 trials

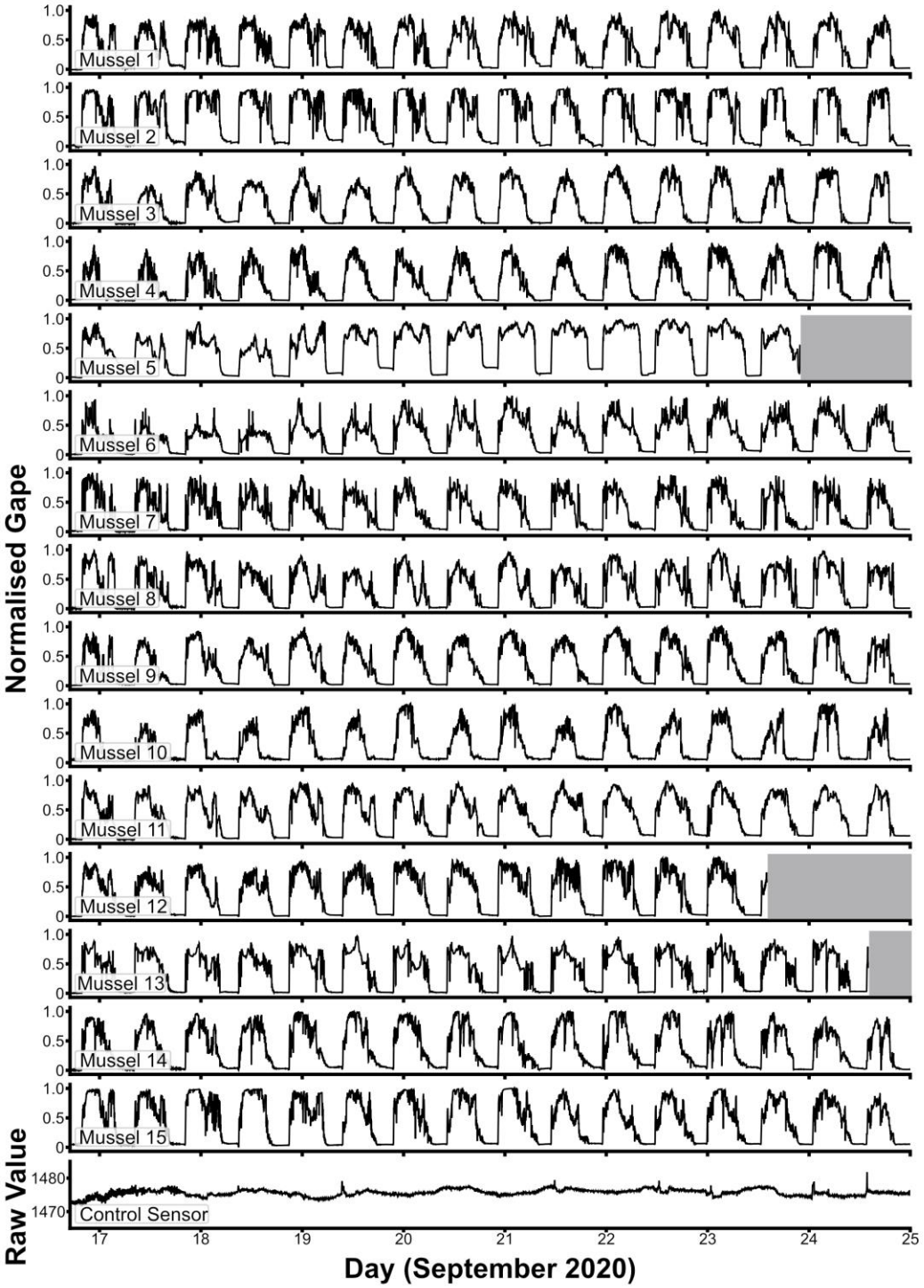


Appendix 4 Figure 2: Ammonia, nitrate, nitrite and silicate concentrations (average ± SD, n = 3) from the land-based on growing units measured during the 2021 trials



Appendix 4 Figure 3: Ammonia, nitrate, nitrite and silicate concentrations (average ± SD, n = 3) from the land-based on growing units measured during the 2022 trials

5 All Gape Records of Mussels Attached to NOSy (Chapter 5)



Appendix 5 Figure 1: Individual normalised gape and raw control values from all mussels monitored during the Dundrum Bay NOSy deployment, September 2020. Greyed-out areas indicate where low quality data were excluded from the analysis.

6 Descriptive Statistical Summary of Raw Data (Chapter 5)

Appendix 6 Table 1: Descriptive statistics of the raw data recorded during the 2020 deployment of NOSy in Dundrum Bay, Northern Ireland to monitor Blue mussels.

Sensor	Minimum	Maximum	Range	Range (%)
M1	870	1385	515	37
M2	1121	1403	282	20
M3	1121	1411	290	21
M4	705	1383	678	49
M5	642	1341	699	52
M6	586	1279	693	54
M7	803	1394	591	42
M8	1139	1403	264	19
M9	500	1352	852	63
M10	1255	1428	173	12
M11	969	1435	466	32
M12	694	1408	714	51
M13	670	1328	658	50
M14	1232	1440	208	14
M15	1015	1388	373	27
Control Sensor	1457	1492	35	2

Summary (Excluding control sensor data)

	Minimum	Maximum	Range	Range (%)
Mean	888	1385	497	36
Standard Deviation	250	44	218	17
Minimum	500	1279	173	12
Maximum	1255	1440	852	63