Impacts of heatwaves and toxic algal blooms on the physiological performance and future aquaculture of the oysters *Ostrea edulis* and *Magallana* (*Crassostrea*) *gigas*

Ellen Grace Montejo Funesto

A thesis submitted for the degree of

Doctor of Philosophy

School of Life Sciences

University of Essex

January 2023

Declarations

I declare that this thesis, “Impacts of heatwaves and toxic algal blooms on the physiological performance and future aquaculture of the oysters *Ostrea edulis* and *Magallana* (*Crassostrea*) *gigas”,* is my work. The following people made the stated contributions to specific chapters:

- Chapter Three: Dr Thomas Cameron and Dr Alice Lown (University of Essex) conceived the research ideas and designed the methodology for determining the thermal performance of *Ostrea edulis.* Dr Lown developed the methods to measure oxygen consumption rate, while I built upon her work to include appropriate control groups and simultaneously measure oxygen consumption and heart rate.
- Chapter Four: Dr Adam Lewis and Dr Andrew Turner (Centre for Environment, Fisheries and Aquaculture Science) conducted the determination of diarrhetic shellfish toxin content.
- Chapter Five: Dr Alexander Shakspeare (University of Essex) processed the raw voltage data into normalised gaping values from the non-invasive oyster sensor.

Summary

This thesis aims to increase our understanding of the vulnerabilities of bivalves under future climate change (CC) scenarios and identify adaptations aquaculturists, policymakers, and conservation managers should employ for optimal success. After the General Introduction, Chapter Two presents a systematic review of the state of research on the impact of multiple CC stressors on bivalves. Future studies should include less-studied, economically important bivalve species, early life stages, understudied vital responses (e.g., reproduction and behaviour), and relevant CC stressors combinations (e.g., warming and harmful algal blooms). Chapter three suggests that *Ostrea edulis* is vulnerable to the increased frequency of heatwaves. The upper thermal optimum of *O. edulis* is 24°C, while the lethal temperature is above 33°C. Sustained periods between these temperatures reduced body condition and increased mortality. Chapter four investigates the combined effects of a heatwave and the presence of the dinoflagellate *Prorocentrum lima*, a species that produces diarrhetic shellfish toxin-associated algal blooms (DSTB). *P. lima* significantly dampened the typical metabolic response of *Magallana gigas* to warming. Oysters exposed to this toxic alga displayed a subdued reaction to warming, raising concerns about their ability to adapt effectively to sudden temperature shifts. *P. lima* and heatwave-exposed oysters accumulated more toxins than those exposed to *P. lima* alone, suggesting more toxic shellfish as heatwaves become more frequent. Chapter five investigates whether bivalves can be used as biosensors for DSTB. The behaviour of *M. gigas* changed after DSTB exposure, spending less time wide open. Bivalve aquaculturists and restoration practitioners are recommended to choose subtidal and lower intertidal habitats. Monitoring blooms and shellfish meat should be more frequent and efficient as warming may

induce higher toxin accumulation. Valve sensors should be deployed in sites where there is a regular bloom occurrence for the advancement of a system that uses bivalves as algal bloom biosensors.

Acknowledgements

I would like to thank the University of the Philippines for funding my studies through the Faculty, REPS and Administrative Staff Development Program. I will be forever grateful to my supervisors Michael Steinke and Tom Cameron, and my supervisory panel chair Nat Hicks, for the guidance and the pieces of advice they gave me that were integral to completing my studies. Thanks to Alice Lown, who shared her knowledge on oxygen consumption measurements, and was always there to answer my queries online, despite being on maternal leave. I would also like to thank our laboratory technicians, Russ Smart, Mel Anderson, Mata Antoneria, John Green, Tania Cresswell-Maynard, and Farid Benyahia, for their help, especially when I was new in the laboratory and was still finding my way into things. To my CEFAS collaborators Andy Turner and Adam Lewis, thank you so much for being very helpful not only in doing the toxin quantification of algal and shellfish meat samples but also in giving suggestions that made my experimental design and write-up better.

To Neve Edullantes, thanks for helping me when I started my life in Colchester. I love the meals we shared and the errands we did together, which became enjoyable times to discuss valuable research ideas. To my fellow oyster researcher, Alex Shakspeare, thank you for being there from the start when I was fresh from Cebu, and for and bringing me along to COF, which made me see what a bivalve production site is like. Thanks also for the assistance in the NOSy raw data processing, but most of all for being a great friend.

Thank you very much to Mama, Papa, and the rest of the family for cheering me on. I hope I made you proud. To Riz, who has supported me from start to finish, who despite having a completely different background, has read through my chapters, and gave beneficial instructions for improvement, patiently listened to my oral presentation practices, and given me comfort in the times I was down, thank you very much. You have made this PhD journey sweeter and easier.

Lastly, I am very grateful to the One who planted this dream in my heart and made it possible. SDG.

Contents

List of Tables

List of Figures

List of Abbreviations and Acronyms

ABT	Arrhenius breakpoint temperature
AFDW	Ash-free dry weight
CC-OA	Climate change and ocean acidification
CEFAS	Centre for Environment, Fisheries and Aquaculture Science
CFH	Cell free haemolymph
CI	Condition index
DSP	Diarrhetic Shellfish Poisoning
DST	Diarrhetic Shellfish Toxin
DSTB	Diarrhetic Shellfish Toxin-associated algal bloom
DTX	Dinophysis toxins
DW	Dry Weight
GDP	Gross Domestic Product
GLM	Generalised Linear Model
GLMM	Generalised linear mixed model
HAB	Harmful algal blooms
HE	Hall Effect
HPC	Haemolymph protein concentration
HR	Heart rate
IUCN	International Union for Conservation of Nature
LC-MS/MS	Liquid chromatography coupled to tandem mass spectrometry
LT	Lipophilic toxins
MO_2	Oxygen consumption rate
NORA	TheNative Oyster Restoration Alliance
NOSy	Non-invasive oyster sensor
NR	Neutral red
OA	Okadaic acid
OR	Odds ratio
PSP	Paralytic shellfish poisoning
PST	Paralytic shellfish toxin
RO	Reverse Osmosis
THC	Total haemocyte count

CHAPTER ONE

Introduction

"*All aquaculture is not created equal,*
and should not be treated as such."

Shumway et. al., 2012

1.1. Motivation

1.1.1. Bivalve aquaculture may solve the food security crisis

The world's population recently crossed the significant milestone of eight billion, symbolizing our achievements in healthcare and life expectancy. However, this demographic triumph is accompanied by growing concerns that extend beyond mere numbers. Issues such as poverty, hunger, malnutrition, and the looming spectre of climate change have taken centre stage. A noteworthy aspect of this population surge is its concentration in the world's poorest nations. Ironically, these countries contribute far less to global greenhouse gas emissions than their developed counterparts. Yet, they are poised to bear the brunt of climate change's devastating impacts (Chen, 2022). These repercussions encompass a heightened frequency of extreme weather events, diminished agricultural output, and the depletion of vital water resources. The cascading effect includes global food inflation and localized shortages, particularly in impoverished African and Asian regions, where soaring food prices push vulnerable populations to the brink (Misra, 2014; Wheeler & von Braun, 2013). Starkly illustrating the gravity of the situation, nearly two billion people worldwide grapple with food insecurity, with the highest prevalence clustered in the beleaguered regions of sub-Saharan Africa and South Asia (Wheeler & von Braun, 2013) (Fig 1.1).

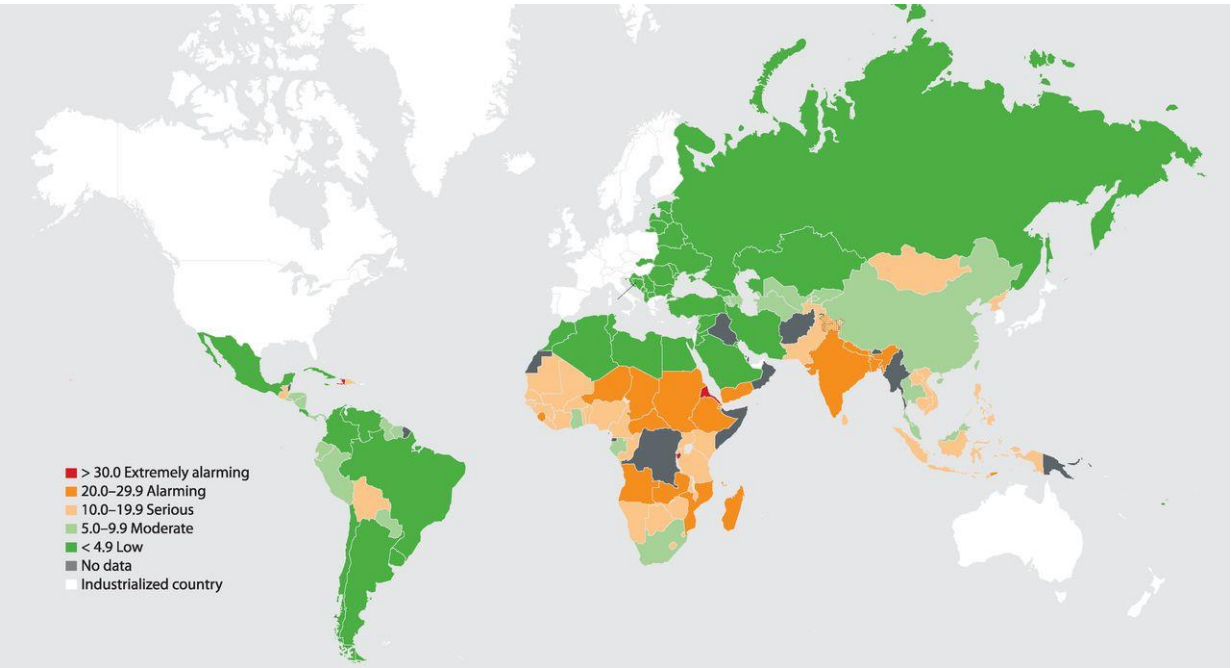


Figure 1.1 Global distribution of hunger calculated by the 2012 Global Hunger Index. Welthungerhilfe, IFPRI and Concern Worldwide Hunger map 2012 calculated a global hunger index for 120 countries from the proportion of malnourished people, the proportion of underweight children under the age of 5, and the mortality of children under the age of 5, weighted equally. Image taken from Grebmer et al., (2012).

The rise of aquaculture over the past two decades fuels optimism among scientists and policy analysts anxious about global food security. Global fish and shellfish cultivation grew at 7.8% annually between 1990 and 2010, a rate that significantly exceeded that of poultry (4.6%), pork (2.2%), dairy (1.4%), beef (1.0%), and grains (1.4%) over the same period (Troell et al., 2014). Aquaculture presently delivers about half of the fish consumed globally (FAO, 2018), and its share is anticipated to rise as wild fisheries reach or exceed their sustainability limits and aquaculture technology and management continue to improve. Aquaculture is arguably the most dynamic sector of the global food system. In addition to rapid growth in volume and value, the sector is characterised by significant investment in many regions of the world (Troell et al., 2014). Seafood, both from the wild and aquaculture, is an

important source of protein for humans. It contains micronutrients and essential fatty acids not found in land-based protein sources (FAO, 2016), making them critical in mitigating malnutrition, especially in low-income countries. Hence, aquaculture can be key to our present and future food supply and provide resilience to the global food system amid environmental changes.

1.1.2. Bivalve aquaculture brings ecosystem services

Unfortunately, the term 'aquaculture' has become overly broad, often wielded by various interest groups to criticize coastal farmers for their perceived environmental impact. It is essential to recognize that aquaculture encompasses a wide range of practices, and not all should be treated alike, as Shumway et al. (2012) aptly pointed out in their paper 'Shellfish Aquaculture — In Praise of Sustainable Economies and Environments.' Bivalve aquaculture, which includes species like oysters, mussels, clams, and cockles, differs significantly from other forms of aquaculture, such as finfish farming. Bivalves have distinct growth requirements that impact coastal environments differently. Unlike many other aquaculture or agricultural practices, bivalves feed by naturally filtering suspended particles from the water column. This unique feeding behaviour distinguishes bivalve aquaculture as a 'green' industry, as no artificially added feeds are introduced to the environment. In contrast, finfish aquaculture, often involving formulated feeds high in nutrients like nitrogen and phosphorus, is viewed as more polluting due to its potential contributions to eutrophication.

In addition to their role in filtering turbid waters as they feed, bivalve aquaculture offers a wealth of ecosystem services that profoundly benefit their surrounding environments. These ecosystem services include a broad spectrum of conditions and processes inherent to natural ecosystems, encompassing both the species within them and the functions they perform (Daily, 1997). Bivalve aquaculture supports and enhances human life by directly or indirectly contributing to the production of vital ecosystem goods, including seafood, poultry grit, jewellery, agricultural additives, and construction materials. Furthermore, these services extend to the fundamental process's ecosystems undertake, such as cleansing, recycling, and renewal. Beyond the tangible benefits, ecosystem services also encompass intangible yet invaluable contributions, such as aesthetic and cultural value.

The Millennium Ecosystem Assessment (MA), a notable United Nations-backed initiative, categorized these ecosystem services into four key groups: provisioning, regulating, cultural, and supporting services. Within the context of bivalve aquaculture, these categories help us comprehensively understand the myriad benefits these remarkable organisms bring to both the environment and human society. In the subsequent sections, we will delve into the specific ecosystem services provided by bivalve aquaculture, elucidating their significance across these categories.

1.1.2.1. Provisioning services

Bivalves play a crucial role in providing essential animal protein consumption for approximately 1.5 billion people, accounting for 15% of the average per capita intake (Tan et al., 2020). In the the recent report of FAO (2022), scallops, clams, oysters, and mussels are the most important bivalve mollusc species traded internationally. Most bivalve molluscs consumed are now farmed in various countries including Europe, North America, China, and Chile. The European Union, the United States of America, China, and the Republic of Korea are the main importers of these molluscs. Demand for bivalves has remained constant over time due to the positive perception of these species as healthy and sustainable food options. In 2020, the global export value of bivalve molluscs reached £3.4 billion, accounting for about 2.8% of the total value of global aquatic exports (FAO, 2022).

In 2015, the global aquaculture production of bivalves intended for human consumption reached a substantial 14.65 million tonnes (as shown in Table 1.1), with an estimated market value of £18.90 billion (Olivier, 2020). Upon consolidating the data from Table 1.1, it becomes evident *that Crassostrea gigas* consistently maintains a significant presence among the top aquaculture species cultivated across diverse regions. When considering data from all countries spanning various regions, its annual production impressively totals 468,375 tonnes, boasting a substantial economic value of £714,551.05. Notably, this ranking falls second only to the aggregated production of *Crassostrea* spp. from China, an astounding 12,389,502 tonnes with a total value of £14,582,684.26.

Following *Crassostrea gigas*, the Japanese scallop *Patinopecten yessoensis* records production figures of 413,028 tonnes, valued at £651,772.91. The Mediterranean Mussel *Mytilus galloprovincialis* follows closely with 332,137 tonnes produced, amounting to £254,834.25. Subsequently, the Chilean mussel *Mytilus chilensis* exhibits noteworthy figures, with 214,531 tonnes produced, valued at £1,408,694.03. Other key species include the Eastern oyster *Crassostrea virginica* (159,175 tonnes, £203,095.57), the New Zealand green-lipped mussel *Perna canaliculus* (78,720 tonnes, £400,985.04), the blue mussel *Mytilus edulis* (36,31155 tonnes, £183.08), and the Giant clams (*Tridacna spp*) (5 tonnes, £12.64).

In the most recent Aquaculture Statistics for the UK by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), the UK aquaculture with 248 shellfish enterprises gave livelihood to 705 staff across the UK in 2012 (Ellis et al., 2015). In the same report, the UK's total shellfish harvest is 27,360 tonnes, with an estimated value of £33.2 million. Table 1.2 shows the shellfish production in the UK in 2012, separated by country, species, and estimated farm-gate price and value of production (Ellis et al., 2015).

Table 1.1 *Annual aquaculture production by continent in the year 2015, with information on the top three producing countries and the main aquaculture species. The values have been adjusted for inflation to the year 2017 (Table taken from: Olivier, 2020).*

Region	Country	Predominant Species farmed	National total for all Species (Tonnes)	Value (£)
Africa		**Mussels, Oysters**	**8,703**	**6,875.37**
	South Africa	*Mytilus galloprovincialis*	3,987	3,149.73
	Namibia	*Crassostrea gigas*	1,850	1,461.50
	Senegal	*Crassostrea gigas*	1,798	1,462.29
Americas		**Mussels, Oysters, Clams, Cockles, Arkshells, Scallops, Pectens**	**463,419**	**1,817,622.52**
	Chile	*Mytilus chilensis*	214,531	1,408,694.03
	United States of America	*Crassostrea virginica*	159,175	203,095.57
	Canada	*Mytilus edulis*	36,311	55,183.08
Asia		**Mussels, Oysters, Clams, Cockles, Arkshells, Scallops, Pectens**	**13,479,192**	**15,787,256.51**
	China	*Crassostrea spp*	12,389,502	14,582,684.26
	Japan	*Patinopecten yessoensis*	413,028	651,772.91
	Taiwan	*Crassostrea gigas*	323,926	244,802.04
Europe		**Mussels, Oysters, Clams, Cockles, Arkshells, Scallops, Pectens**	**608,957**	**874,035.46**
	Spain	*Mytilus galloprovincialis*	227,805	114,439.40
	France	*Crassostrea gigas*	124,481	405,520.43
	Italy	*Mytilus galloprovincialis*	100,345	137,245.12
Oceania		**Mussels, Oysters, Clams, Cockles, Arkshells, Scallops, Pectens**	**95,054**	**478,497.47**
	New Zealand	*Perna canaliculus*	78,720	400,985.04
	Australia	*Crassostrea gigas*	16,320	61,304.79
	Cook Islands	*Tridacna spp*	5	12.64
			14,649,532	**18,896,162.47**

Table 1.2 *Bivalve shellfish production in the UK in 2012, separated by species (and technique) and country. Also included are estimated farm-gate price and value of production (Table taken from: Ellis et al., 2015).*

Country	Species	Harvest (tonnes)	Estimated Price per Tonne (£)	Imported value (£)
England	*Mytilus spp.*	5,965.70	1000.00	5,965,700.00
	Crassostrea gigas	850.00	4000.00	3,400,000.00
	Ostrea edulis	85.90	7600.00	652,840.00
	Mercenaria mercenaria	8.60	3100.00	26,660.00
	Ruditapes philipinarum	5.00	3100.00	15,500.00
	NATIONAL TOTAL	**6,915.20**	**-**	**10,060,700.00**
	% UK TOTAL	**25%**	**-**	**30%**
Wales	*Mytilus spp.*	89,966.00	1000.00	89,966,000.00
	Crassostrea gigas	3.00	4000.00	12,000.00
	NATIONAL TOTAL	**89,969.00**	**-**	**89,978,000.00**
	% UK TOTAL	**33%**	**-**	**27%**
Scotland	*Mytilus spp.*	6,227.00	1200.00	7,472,400.00
	Carassostrea gigas	216.00	4400.00	950,400.00
	Ostrea edulis	25.00	7600.00	190,000.00
	Pecten maximus	7.00	14300.00	100,100.00
	Aequipecten opercularis	0.40	2500.00	1,000.00
	NATIONAL TOTAL	**6,475.40**	**-**	**8,713,900.00**
	% UK TOTAL	**24%**	**-**	**26%**
Northern Ireland	*Mytilus spp. (off bottom harvest)*	76.60	1200.00	91,920.00
	Mytilus spp. (on bottom harvest)	4,706.00	1000.00	4,706,000.00
	Carassostrea gigas	137.30	4000.00	549,200.00
	NATIONAL TOTAL	**4,919.90**	**-**	**5,347,120.00**
	% UK TOTAL	**18%**	**-**	**16%**
United Kingdom Total	*Mytilus spp.*	26,021.30	-	27,292,012.00
	Carassostrea gigas	1,206.30	-	4,911,600.00
	Ostrea edulis	110.90	-	843,106.00
	Mercenaria mercenaria	8.60	-	26,576.00
	Ruditapes philipinarum	5.00	-	15,500.00
	Pecten maximus	7.00		100,100.00
	Aequipecten opercularis	0.40	-	1000
	TOTAL	**27,359.50**	**-**	**33,189,894.00**

Aside from food and employment provision, bivalve shells serve a dual purpose in poultry farming, aiding in digestion as poultry grit and promoting calcium formation for eggshells. Oyster shells are commonly sold for use as poultry grit,

and their value can range between £250 and £1,900 per metric tonne (Morris et al., 2018). Bivalve shell is also a rich source of macro- and micro-nutrients essential for agriculture. In fact, the nitrogen, phosphate, and potash ratio found in shellfish-based compost matches the nutrient requirements of many crops (Olivier et al., 2020). Crushed oyster shells also make an excellent soil conditioner, promoting the growth of soil and rhizospheric microorganisms. Adding just 0.3 t ha^{-1} can double the number of bacteria, actinomycetes, and nitrogen-fixing bacteria. Oyster-shell has also been shown to significantly increase soil pH and nutrient levels when applied at rates of up to 16 t ha^{-1} (Gouliang et al., 2003).

Beyond farming applications, oyster shells have been used in construction for centuries. Their lightweight and tightly packed nature make them a popular choice for sea defences and burnt oyster shells have been used as lime for building construction. Research has recently explored the potential for crushed shells to replace sand, aggregate, and cement in concrete, with promising results. In fact, shell and lime can replace up to 10% of standard aggregate without impacting strength (Olivier, 2020).

Pearls are another highly prized product obtained from bivalves, long valued for their beauty, and used in jewellery. In 2009, global pearl production reached approximately 40 tons. Mother of pearl or nacre, which lines the inner shell of some molluscs, has a rich history of use in decorative items like pearl buttons and inlaid furniture (Olivier, 2020).

1.1.2.2. Regulating services

Bivalves are known for their ability to filter water by removing bacteria, phytoplankton, and sediments suspended in the water. This process can lead to a significant improvement in water quality. For example, a study showed that a decrease in the population of *Dreissena spp*. (zebra mussels) in the central basin of Lake Erie, caused by hypoxia, was associated with a rise in total phosphorus concentrations and a shift from clear to more turbid water. However, the change in phosphorus concentrations and water turbidity was not observed in the eastern basin of the lake, where the zebra mussel population remained relatively high and constant. It is worth noting that while bivalves like zebra mussels can have positive impacts on water quality, the fact that zebra mussels are an invasive species and can outcompete native species make them a significant concern for conservationists and researchers (Karatayev et al., 2018).

Another regulating service is the nutrient cycling abilities of bivalves. A high amount of particulate matter and dissolved nutrients are recycled during bivalve feeding. Bivalves cycle large quantities of organic material and transform some of it into tissue that can be discharged as gametes or are decomposed when they die. The faeces and pseudofaeces from these animals can be used by coprophages or are directly decomposed by bacteria or fungi. Their ammonium and urea excretion and biomineralisation (during shell formation) of biodeposits greatly affect the microbial activities like nitrification and nutrient production from the sediment, returning

essential inorganic nutrients and supporting the growth of phytoplankton communities (Gazeau et al., 2013).

Bivalves are usually cultured in environments with high abundance of plant material. They help in breaking down this material. Within sediment, for example, bivalves play an important role through bioturbation and bioirrigation. Bioturbation pertains to the changing of aquatic sediments by organisms living in the deposit, like burrowing animals, rooting plants, and microbes. Activities of many animals also cause bioirrigation, which improves the transport of solutes between the sediment and the water overlaying it, including the filling of burrows (Norkko & Shumway, 2011; Vaughn & Hakenkamp, 2001). Bivalve's bioturbation and bioirrigation greatly increase microbial activities by mixing and flushing the sediment in their habitat when they feed and move. This leads to a deeper oxygen penetration in the sediment and enhancement of microbial metabolism, which affects the functioning of the whole system, since the sediment microbial community performs important roles in decomposition of deposited organic material and mineralisation of nutrients (Norkko & Shumway, 2011; Vaughn & Hakenkamp, 2001). Modification of the sediment creates changes in the sediment sorting, grain size dispersal, porosity, and the vertical arrangement of both solids and solutes. These changes modify the rates and processes of reactions in the sediment and between the overlying water (Norkko & Shumway, 2011).

1.1.2.3. Supporting services

Thick beds of attached bivalves in aquaculture sites, and in the natural environment, such as those of mussels and oysters provide habitat for a wide range of other animals and algae. Larger benthic bivalves with an exposed shell provide potential habitat (Darrigran & Damborenea, 2011). The creation of mussel beds by introducing *Limnoptera fortunei* (golden mussel), for example, leads to the increase in macroinvertebrate richness, favouring oligochaetes, nematodes, and hirudineans (Darrigran & Damborenea, 2011; Sylvester et al., 2007). Benthic substrates made from empty shells of native (duck mussel and swollen river mussel) and invasive bivalve species (Asian clam and Chinese pond mussel) in the Danube River in Hungary are populated by taxa otherwise not found or present in low density in the natural substrates and produce diverse microhabitats (Bódis et al., 2014).

The structures used in aquaculture (racks, cages, nets, ropes, trays, and lines), and shellfish aquaculture in particular, also act like reefs and provide habitat and shelter for a wide variety of other organisms, often serving as rearing areas for fish and other shellfish, like young lobsters (Shumway et al., 2012). They provide protection from predators for juvenile fish and crustaceans, a larger surface area for fouling organisms (phytobenthos and zoobenthos), and an increased food supply for other organisms. Bivalve culture can also reduce the negative effects of benthic disturbance that would occur if the area had been used for harvesting wild stocks instead, since the increased density on shellfish farms means less environmental impact and disturbance for the same yield compared to wild collection (Shumway et al., 2012).

The presence of shellfish beds affects water flow on both small and large scales. On a small scale, the shape of the mussel shells and the water jets from their exhalant siphons create biomixing and increase bed roughness, affecting water flow at the millimetre to centimetre level. On a larger scale of tens of meters, the topographic variation of the mussel bed, such as alternating patches of mussel and bare sediment, impacts water flow. The mixing of water resulting from the shellfish beds is crucial for nutrient cycling, alteration of turbidity, sediment accretion, and moderating wave energy, among other supporting or intermediate services (Olivier et al, 2020).

1.1.2.4. Cultural services

Bivalve beds offer a range of cultural services that are important for human well-being. Recreational fisheries, for instance, are a popular pastime for many people who enjoy fishing for bivalves such as clams, oysters, and mussels. Historical artisanal fisheries for the public, which involve traditional methods of harvesting and processing bivalves, are also an important cultural service that helps to preserve cultural heritage and promote local identity (Olivier et al., 2020).

Education and tourism are another important cultural service provided by bivalve beds. Bivalve beds offer unique opportunities for people to learn about the ecology of the marine environment and the importance of sustainable seafood production. Many areas with bivalve beds also have educational programs, guided tours, and interpretive centres that offer visitors an opportunity to learn

more about bivalves and the ecosystem services they provide (Olivier et al., 2020).

Seafood festivals are another cultural service associated with bivalve beds. These festivals celebrate the culinary diversity of bivalves and provide an opportunity for local communities to showcase their unique food traditions. They also help to promote sustainable seafood practices and raise awareness about the importance of conserving bivalve populations and their habitats (Olivier et al., 2020).

Additionally, bivalves have symbolic and spiritual value for many cultures around the world. Bivalves have been used in traditional ceremonies and rituals for thousands of years and are often associated with themes such as fertility, purification, and renewal. For some communities, bivalve beds are sacred places that are important for their spiritual and cultural identity (Olivier et al., 2020).

1.1.3. Bivalve aquaculturists and policymakers should consider unprecedented environmental change

Bivalves are among the best candidates for environmentally sustainable aquaculture, that may help solve food insecurity. Shellfish farming not only offers humanity a low-impact source of sustainable protein, it also creates jobs and social and economic development, while bringing tangible benefits to the marine environment (Shumway et al., 2012).

The success of an aquaculture venture hinges upon a comprehensive grasp of both the biological and technical facets of the cultivated species, as well as a keen understanding of the environmental prerequisites for cultivation. Moreover, it necessitates the presence of a well-structured policy framework that harmonizes with sustainable aquaculture practices and access to a robust market that facilitates the proliferation of the species (Azra et al., 2021). In this context, sustainability denotes the capacity to perpetuate aquaculture activities without depleting natural resources or inflicting harm upon the environment.

A sound policy framework encompasses a suite of regulations, guidelines, and incentives meticulously crafted to endorse responsible and sustainable aquaculture methods (Hishamunda, et al, 2012). These policies frequently address critical domains, such as safeguarding the environment, managing resources, upholding food safety standards, and ensuring social responsibility. Their overarching goal is to strike a harmonious equilibrium between the economic advantages of aquaculture and the preservation of natural ecosystems. Sustainable aquaculture policies typically accentuate practices that curtail environmental impact, minimize the usage of antibiotics and chemicals, and safeguard the well-being of industry personnel. Furthermore, an effective policy framework transcends national borders and may encompass international agreements and standards aimed at fostering responsible aquaculture on a global scale.

An increasingly critical consideration for aquaculturists and policymakers is the influence of environmental change, especially the compounded effects of multiple stressors stemming from climate change. In ecological terms, a stressor refers to any external factor or influence that disrupts the normal functioning or equilibrium of an organism or system. For organisms, this can lead to survival challenges or limitations in growth and reproduction (Alexander, 1999).

Aquatic environments where bivalves dwell are severely impacted by human activities and at risk of disturbance from climate change. Burning fossil fuels and deforestation has led to increased greenhouse gases levels in the atmosphere, making the worlds' oceans warmer, increasingly deoxygenated, and more acidic (Archer & Brovkin, 2008; Emanuel et al., 2020; Pauchauri & Meyer, 2014; Provoost et al., 2010). These changes also lead to increased frequency of harmful algal blooms, heatwaves, droughts and flooding of the coasts, causing unusually high salinity fluctuations in estuaries and nutrient/pollutant run-off (Claret et al., 2018; Kudela et al., 2015; Perkins-Kirkpatrick & Lewis, 2020; Peteiro et al., 2018).

These individual stressors can already pose significant challenges to the well-being of cultured species. Increased temperature is shown to change bivalves' energetic and metabolic patterns and decrease the energy available for growth (scope for growth), and body condition (Anestis et al., 2010; Guzmán-Agüero et al., 2013; A. , Lemasson et al., 2018; Sobral & Widdows, 1997). Small temperature increases are found to decrease fertilisation success of gametes, development and growth of embryos, survival, lipid synthesis, and increase abnormal morphology of bivalve

larvae (Parker et al., 2010; Talmage & Gobler, 2011a). Studies on the impact of ocean acidification on bivalves show a reduction in shell length, strength, and increase shell damage (Lemasson & Knights, 2021; Range et al., 2014; Welladsen et al., 2010). Furthermore, acidification affects fertilisation, sperm swimming speed, and sperm motility. It also causes decreased hatching, survival, size, development rate, shell shape, and metamorphosis to bivalve embryos and larvae (as reviewed by Gazeau et al., 2013). Another stressor, salinity irregularities during flooding or drought, is reported to compromise the immune status and histological structure of bivalves and decrease their scope for growth and activity (Carvalho et al., 2015; Dominguez et al., 2020; Woodin et al., 2020). Additionally, hypoxia alters feeding, behaviour, anaerobic and oxidative osmoregulation metabolism, expression of immune-related genes, reduce metabolism, and induce cellular damage in bivalves (Belivermis et al., 2020; Q. Li et al., 2019; Nie et al., 2020; Villnas et al., 2019).

What makes the situation more complex is the interaction and combination of multiple stressors. Compound stressors refer to the simultaneous presence and interaction of multiple stressors, leading to amplified and sometimes unpredictable impacts. In the context of climate change, stressors rarely act in isolation; instead, they intertwine in intricate ways, creating combined effects that can be more severe than the sum of their individual impacts (Heugens et al., 2001).

For instance, the rise in global temperatures can set off a chain reaction of events. As temperatures increase, glaciers melt at an accelerated rate, contributing to rising sea levels. This, in turn, exacerbates the impacts of coastal flooding during storms

and results in a decrease in water salinity (Griggs & Reguero, 2021). Another prominent illustration of this complex interaction is the connection between climate change and the emergence of harmful algal blooms (HABs) (Trainer et al, 2020). Climate change directly leads to elevated temperatures and a higher frequency of heatwaves. As temperatures rise, various ecological processes are accelerated, including the proliferation of microalgae. Planktonic microalgae, vital as the principal sustenance for suspension-feeding bivalves, exhibit a propensity for explosive proliferation. This phenomenon often culminates in the development of algal blooms, characterized by millions of cells per litre blooms (Lloyd et al., 2013).

Although these algal blooms may initially appear beneficial, as they provide a food source for aquatic life, they can transition into harmful algal blooms (HABs). HABs can cause significant economic damage to aquaculture, fisheries, and tourism industries, and they have detrimental effects on the environment, animal health, and human well-being (Turner et al., 2019).

Figure 1.2 vividly depicts the direct connection between climate change and rising temperatures, while also elucidating the indirect consequences of climate change, notably the emergence of harmful algal blooms as a result of the heightened temperature. Consequently, the concurrent presence of elevated temperatures and harmful algal blooms can serve as dual stressors to marine organisms, such as bivalves.

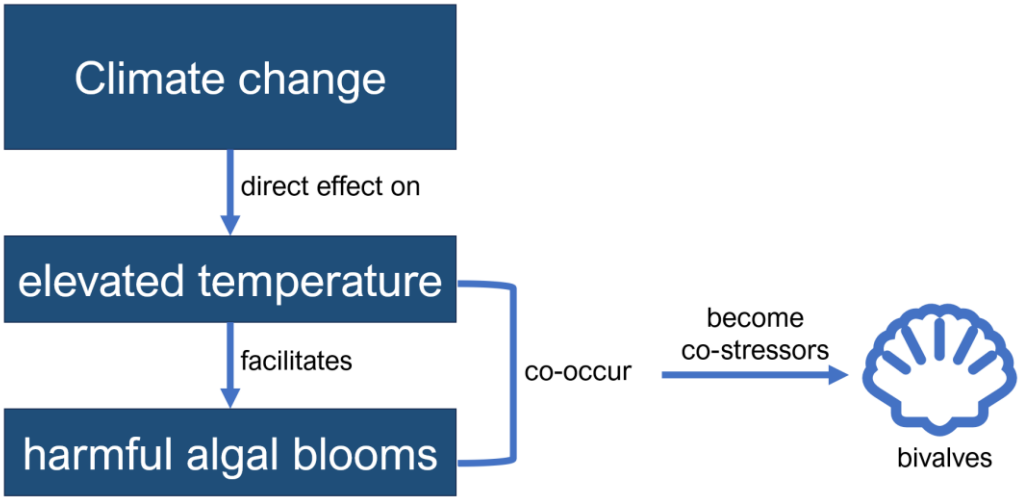


***Figure 1.2**. Illustration of the Relationship between Elevated Temperatures and Toxic Algal Blooms as Bivalve Co-Stressors. Climate change drives elevated temperatures, which in turn facilitate the development of harmful algal blooms.*

The escalation in both the intensity and frequency of harmful algal blooms (HABs) is influenced by a spectrum of factors associated with climate change, extending beyond just the warming and heatwave aspects. Coastal regions, which frequently witness the simultaneous presence of harmful algal blooms and heightened levels of CO_2, present distinctive challenges for aquaculture. The alteration in seawater chemistry, driven by elevated CO_2 concentrations, can deeply impact the physiology of harmful algae, potentially fostering their growth and the production of toxins. CO_2 plays a pivotal role in the process of algal photosynthesis, serving as a crucial resource for their metabolic functions. Notably, research suggests that specific harmful algal species exhibit accelerated growth rates under acidic conditions resulting from CO_2 absorption. This phenomenon contributes to the emergence and persistence of toxic algal blooms, further complicating matters for aquaculture practitioners (Brandenburg et al., 2019; Gao et al., 2021; Gobler et al., 2017; Hallegraeff, 2010; Moore et al., 2008).

During harmful algal blooms, the accumulation of phycotoxins in the tissues of filter-feeding bivalves can reach levels perilous to human health, prompting the closure of shellfish production areas. Several categories of human shellfish poisoning have been identified, including paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and azaspiracid shellfish poisoning (AZP) (Butzke et al., 2013). In the European Atlantic Arc region, the closure of shellfish production areas is predominantly caused by the regular occurrence of diarrhetic shellfish toxins (DST), specifically toxins within the OA group, particularly during the summer months (Fernandes-Salvador et al., 2021).

Stewart-Sinclair et al. (2020) created a global assessment of the vulnerability of global bivalve aquaculture to climate change and ocean acidification (CC-OA) from 2020 to 2100 under the highest baseline emissions scenario (RCP 8.5). The study's vulnerability assessment was conducted through a systematic approach. To begin, spatially explicit projections related to CC-OA were overlaid with national data on bivalve production, as well as socioeconomic factors. All spatial data were standardized to the World Geodetic System 1984 (WGS84) using R version 3.3.2 (R Core Team, 2013).

Within the study, various indices were generated, categorized into exposure, sensitivity, and adaptive capacity. These indices were applied to United Nations-recognized countries, including Antarctica, with each nation assigned World Bank

development classifications according to the system outlined by Prince and Fantom (2014).

To assess the impact, the numerical values derived from these indices were reclassified onto a scale from one to five, with five signifying the highest level of impact, following the method detailed by Handisyde et al. (2006). Since the data used in their study were diverse in terms of type, scale, and composition, the reclassification process was adapted on a case-by-case basis, guided by relevant literature. In cases where there was no established reclassification precedent, value-reclassification followed the approach outlined by Allison et al. (2009), involving normalization onto a scale from 0 to 1 and linear reclassification to the 1–5 risk score.

They found that exposure risk to CC-OA increases over time, with ten nations predicted to experience very high exposure to CC-OA in at least one decade during 2020-2100 (Fig 1.3). Seven of the countries that belong to the top fifteen largest producers of bivalves from aquaculture are at very high risk of exposure to CC-OA in 2100. These countries include China, Japan, South Korea, Vietnam, Italy, Taiwan, and North Korea. While the remainder of the largest producers of bivalve mariculture are in moderate risk of exposure in 2100, namely Chile, Spain, Thailand, France, New Zealand, Netherlands, and Peru.

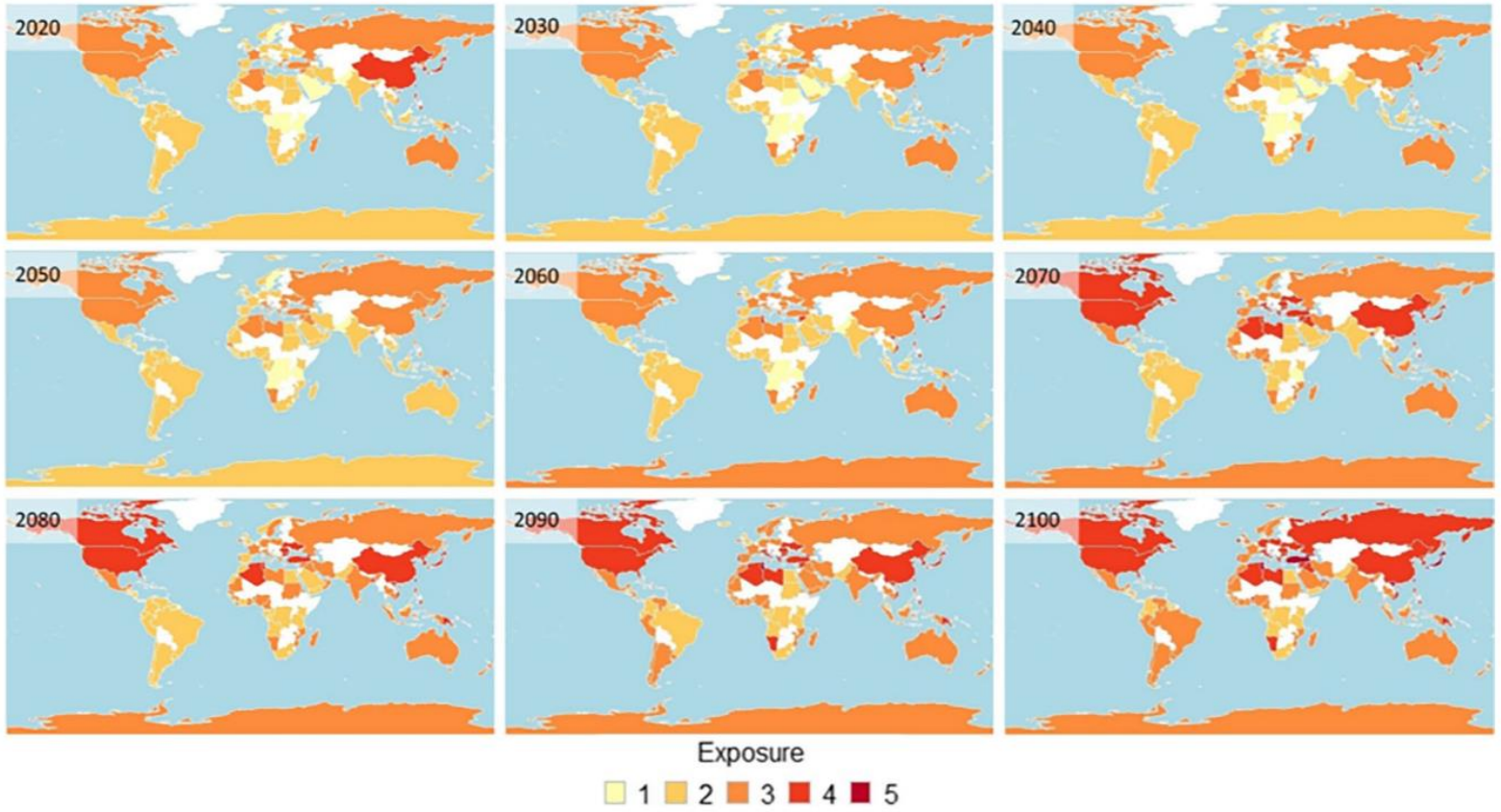


Figure 1.3 *Risk of exposure to climate change and ocean acidification for nations with existing bivalve aquaculture operations, measured as nation-specific mean of indices within the exposure sublayer in 2020 to 2100, for IPCC scenario RCP8.5. Colours represent exposure score (1 = very low, 2 = low, 3 = moderate, 4 = high, and 5 = very high), and white is for when no data could be obtained. Image taken from Stewart-Sinclair et al. (2020).*

With the increased vulnerability of bivalve aquaculture to CC-OA, understanding the interactions between multiple stressors allows for more precise predictions of their impacts on bivalves and aquatic ecosystems. This knowledge is vital for assessing risks and vulnerabilities and formulating effective adaptive strategies. Addressing only individual stressors may not be sufficient to safeguard bivalves and aquaculture systems. By considering compound stressors, policymakers and aquaculturists can develop comprehensive mitigation measures that address the multiple challenges faced by bivalves in changing environments.

Compound stressors can trigger feedback loops and cascading effects within ecosystems. By studying these interactions, conservationists can better comprehend the factors that contribute to ecosystem resilience or vulnerability in the face of climate change. To ensure the long-term sustainability of aquaculture, it is crucial to identify and manage the combined stressors that pose the greatest risks. Integrating this knowledge into aquaculture practices can help foster resilient and adaptive systems.

1.2. Contribution to scientific knowledge

This PhD thesis delves into the significant role that bivalve aquaculture can play in addressing global food insecurity in the future, particularly in the face of climate change challenges. Given the predictability of climate change impacts, this research

aims to anticipate and understand how these changes will affect bivalves through rigorous scientific investigation.

The primary objective of this thesis is to enhance our knowledge regarding the vulnerabilities of bivalves under future climate change scenarios and to pinpoint the adaptations that aquaculturists, policymakers, and conservation managers should implement to achieve optimal success in bivalve farming. Within this scope, the research acknowledges two critical concerns: the increasing frequency of heatwaves and the proliferation of harmful algal blooms, both of which pose significant threats to bivalve production and health. These concerns serve as the focal points of this thesis.

To accomplish these goals, the thesis is organized into four distinct data chapters (Chapters 2-5), each tailored and formatted for scholarly publication. The thesis culminates in Chapter 6, which serves as a General Discussion and Conclusion These chapters are designed with specific research objectives in mind, which can be summarized as follows:

- **Chapter 2** reflects the outcomes of my work during the first year of my PhD, where I undertook a systematic review aimed at identifying the gaps in research on bivalve and multiple stressors. The chapter presents the results of this thorough investigation, shedding light on areas where further research is needed to gain a comprehensive understanding of the effects of multiple stressors on bivalve species.

This chapter commences by providing an introductory exploration of the concept of stressors and their intricate impact on organisms in the context of multiple climate change stressors. Subsequently, Chapter 2, unveils the nations at the forefront of multiple stressor studies, and elucidates the specific bivalve species, climate change co-stressors, stressor combinations, and responses that have been the central focus of existing literature. It summarises the current knowledge gaps and identifies further research directions. The findings of this chapter paved the way into the research direction of this thesis, focusing on two less-studied stressor combinations: heatwaves and toxic algal blooms.

- **Chapter 3** presents a series of preliminary experiments I conducted, leveraging my newly acquired expertise in respirometry techniques. The focus of this chapter is to explore the metabolic responses of bivalves, with particular attention to European native oysters (*Ostrea edulis*), when subjected to a single stressor of elevated temperatures. The experiments aim to simulate short-term summer maximum temperature periods, akin to heatwaves, in a laboratory setting. Through respirometry and heart rate measurements, I delve into how these temperature stressors impact the metabolic processes and condition index of *O. edulis*, providing crucial insights into the physiological implications of warming on these organisms.

  Furthermore, the chapter examines the onset of thermal stress in *O. edulis*, helping to identify the temperature thresholds at which these oysters are

significantly affected by warming. The chapter concludes with practical recommendations for selecting suitable production or restoration sites for *O. edulis,* taking into account the implications of warming on their overall health and well-being.

- **Chapter 4** holds significant importance in this thesis as it serves as the primary data chapter. Within this chapter, the results of a crucial experiment are presented, focusing on the combined effects of two stressors: heatwaves and toxic algal bloom. As of this writing, this chapter has already been published in the esteemed journal Science of the Total Environment, further validating the significance and impact of the research findings (Funesto et al, 2023).

  This chapter starts with an introduction about harmful algal blooms, and how they should be considered as a climate change-co-stressor. This chapter conveys the result of a laboratory experiment that assessed the combined immediate and delayed effects of a heatwave and *Prorocentrum lima* bloom, which is a diarrhetic shellfish toxin producer, on the toxin accumulation, metabolism, immune response, and condition index of the widely cultured and commercially important Pacific rock oyster (*Magallana gigas*). This chapter concludes with a discussion on how shellfish producers and policy makers should adapt to the predicted vulnerability of rock oysters to increased frequency of heatwaves and toxic algal blooms and the likelihood of more toxic shellfish events in a warming world.

- The final data chapter, **Chapter 5**, serves as a practical and crucial component of this thesis. It commences with a discussion about the concerning issue of diarrhetic shellfish toxins, emphasizing their detrimental impact on both shellfish production and human health. Building upon the earlier findings presented in Chapter 4, this chapter then proceeds to unveil the results of experiments designed to examine whether the UK threshold levels (10^2 cells L^{-1}) and high concentration (10^6 cells L^{-1}) of *P. lima* induce valve behaviour changes in *M. gigas.*

  The significance of this investigation lies in the potential consequences of combined warming and toxic algal blooms. Given the potential risks of increased toxicity in shellfish, there is a growing need for reliable and sensitive biosensors. Consequently, this chapter delves into exploring the possible utilization of *M. gigas* as a biosensor to detect the presence of *P. lima* in the field. The findings from these experiments hold immense practical value, as biosensors can play a pivotal role in monitoring and mitigating the risks posed by toxic algal blooms and their impact on shellfish.

- **Chapter 6** encompasses a multifaceted exploration of my research findings and their implications in the context of bivalve aquaculture and climate change resilience. I delve into key discoveries and engage in an insightful discussion, drawing connections between chapters, and revealing overarching themes and insights that offer a holistic perspective. Policy management and implications come into focus, emphasizing the real-world applications of this work for aquaculturists, policymakers, and conservationists.

Within this chapter, I candidly address research limitations and propose future research directions, underlining the need for continued investigations in this critical domain. Finally, this journey concludes with a comprehensive summary, encapsulating the significance of this work, its impact on global food security and ecosystem resilience, and its contributions to a sustainable future for bivalve aquaculture and marine ecosystems.

1.3. References

Alexander, D. E. (1999). Ecological stress. In Environmental Geology. Springer Netherlands. 159–160. https://doi.org/10.1007/1-4020-4494-1_94

Allison, E.H., Beveridge, M.C.M., van Brakel, M. (2009). Climate change small-scale fisheries and smallholder aquaculture. In: P. Wramner, M. Cullberg, & H. Ackefors (Eds.), Fisheries sustainability and development. Stockholm: Royal Swedish Academy of Agriculture and Forestry. 109-122.

Anestis, A., Pörtner, H. O., Karagiannis, D., Angelidis, P., Staikou, A., & Michaelidis, B. (2010). Response of *Mytilus galloprovincialis* (L.) to increasing seawater temperature and to marteliosis: Metabolic and physiological parameters. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 156(1), 57–66. https://doi.org/10.1016/j.cbpa.2009.12.018

Archer, D., & Brovkin, V. (2008). The millennial atmospheric lifetime of anthropogenic CO_2. Climatic Change, 90(3), 283–297. https://doi.org/10.1007/s10584-008-9413-1

Azra, M. N., Okomoda, V., Tabatabaei, M., Hassan, M., & Ikhwanuddin, M. (2021). The Contributions of Shellfish Aquaculture to Global Food Security: Assessing Its Characteristics from a Future Food Perspective. Frontiers in Marine Science, 8, 654897. https://doi.org/10.3389/fmars.2021.654897

Belivermis, M., Swarzenski, P. W., Oberhansli, F., Melvin, S. D., & Metian, M. (2020). Effects of variable deoxygenation on trace element bioaccumulation and resulting metabolome profiles in the blue mussel (*Mytilus edulis*). Chemosphere, 250, 126314. https://doi.org/10.1016/j.chemosphere.2020.126314

Beukema, J. J., & Dekker, R. (2014). Variability in predator abundance links winter temperatures and bivalve recruitment: Correlative evidence from long-term data in a tidal flat. Marine Ecology Progress Series, 513, 1–15. https://doi.org/10.3354/meps10978

Brandenburg, K. M., Velthuis, M., & Van de Waal, D. B. (2019). Meta-analysis reveals enhanced growth of marine harmful algae from temperate regions with warming and elevated CO2 levels. Global Change Biology, 25(8), 2607–2618. https://doi.org/10.1111/gcb.14678

Carvalho, Y. B. M., Romano, L. A., Poersch, L. H. S., Carvalho, Y. B. M., Romano, L. A., & Poersch, L. H. S. (2015). Effect of low salinity on the yellow clam *Mesodesma mactroides*. Brazilian Journal of Biology, 75(1), 8–12. https://doi.org/10.1590/1519-6984.03213

Chen, Y. (2022). Press Release: As the world's population hits 8 billion people, UN calls for solidarity in advancing sustainable development for all. United Nations Sustainable Development. Retrieved, December 10, 2022, from https://www.un.org/sustainabledevelopment/blog/2022/11/press-release-as-the-

worlds-population-hits-8-billion-people-un-calls-for-solidarity-in-advancing-sustainable-development-for-all/

Claret, M., Galbraith, E.D., Palter, J.B. et al. Rapid coastal deoxygenation due to ocean circulation shift in the northwest Atlantic. Nature Clim Change 8, 868–872 (2018). https://doi.org/10.1038/s41558-018-0263-1

Daily, G. C. (1997). Nature's services. Societal dependence on natural ecosystems. Island Press, Washington, DC. 392 pp. ISBN 1-55963-475-8

Darrigran, G., & Damborenea, C. (2011). Ecosystem Engineering Impact of *Limnoperna fortunei* in South America. Zoological Science, 28(1), 1–7. https://doi.org/10.2108/zsj.28.1

Dominguez, R., Vazquez, E., Woodin, S. A., Wethey, D. S., Peteiro, L. G., Macho, G., & Olabarria, C. (2020). Sublethal responses of four commercially important bivalves to low salinity. Ecological Indicators, 111, 106031. https://doi.org/10.1016/j.ecolind.2019.106031

Ellis, T., Gardener, R., Gubbins, M., Reese, A., & Smith, D. (2015). Aquaculture statistics for the UK, with a focus on England and Wales 2012. Centre for Environment, Fisheries and Aquaculture Science.

Emanuel, M., Pillay, D., van der Merwe, M., & Branch, G. (2020). Interactive effects of pH and temperature on native and alien mussels from the west coast of South Africa. African Journal of Marine Science, 42(1), 1–12. https://doi.org/10.2989/1814232X.2019.1699162

FAO (2016). The State of Food and Agriculture (2016): Climate Change, Agriculture and Food Security - World | ReliefWeb. Retrieved December 10, 2022, from https://reliefweb.int/report/world/state-food-and-agriculture-2016-climate change-agriculture-and-food-security

FAO (2018). SOFIA 2018—State of Fisheries and Aquaculture in the world 2018. Retrieved January 11, 2023, from http://www.fao.org/state-of-fisheries-aquaculture

Ferreira-Rodriguez, N. (2019). Spatial aggregation of native with non-native freshwater bivalves and activity depletion under summer heat waves: "dangerous liaisons' in a climate change context. Hydrobiologia, 834(1), 75–85. https://doi.org/10.1007/s10750-019-3910-2

Funesto, E. G. M., Lewis, A. M., Turner, A. D., Cameron, T. C., & Steinke, M. (2023). Immediate and delayed effects of a heatwave and *Prorocentrum lima* ((Ehrenberg) Stein 1878) bloom on the toxin accumulation, physiology, and survival of the oyster *Magallana gigas* (Thunberg, 1793). Science of The Total Environment, 892, 164485. https://doi.org/10.1016/j.scitotenv.2023.164485

Gao, G., Zhao, X., Jiang, M., & Gao, L. (2021). Impacts of Marine Heatwaves on Algal Structure and Carbon Sequestration in Conjunction with Ocean Warming and Acidification. Frontiers in Marine Science, 8. https://www.frontiersin.org/articles/10.3389/fmars.2021.

Gazeau, F., Parker, L. M., Comeau, S., Gattuso, J.-P., O'Connor, W. A., Martin, S., Pörtner, H.-O., & Ross, P. M. (2013). Impacts of ocean acidification on marine shelled molluscs. *Marine Biology*, *160*(8), 2207–2245. https://doi.org/10.1007/s00227-013-2219-3

Gazeau, F., Parker, L. M., Comeau, S., Gattuso, J.-P., O'Connor, W. A., Martin, S., Pörtner, H.-O., & Ross, P. M. (2013). Impacts of ocean acidification on marine shelled molluscs. Marine Biology, 160(8), 2207–2245. https://doi.org/10.1007/s00227-013-2219-3

Gobler, C. J., Clark, H. R., Griffith, A. W., & Lusty, M. W. (2017). Diurnal Fluctuations in Acidification and Hypoxia Reduce Growth and Survival of Larval and Juvenile

Bay Scallops (*Argopecten irradians*) and Hard Clams (*Mercenaria mercenaria*). Frontiers in Marine Science, 3. https://doi.org/10.3389/fmars.2016.00282

Grebmer, K. von, Ringler, C., Rosegrant, M., Olofinbiyi, T., & Wiesmann, D. (2012). 2012 Global Hunger Index: The challenge of hunger: Ensuring sustainable food security under land, water, and energy stresses.

Griggs, G., & Reguero, B. G. (2021). Coastal Adaptation to Climate Change and Sea-Level Rise. *Water*, *13*(16), Article 16. https://doi.org/10.3390/w13162151

Guoliang J., Yun LIU, Mingyu D., Xiuqin K. (2003). Influences of oyster shell soil conditioner on soil and plant rhizospheric microorganisms. Journal of Ocean University of Qingdao 2: 230–232

Guzmán-Agüero, J., Nieves-Soto, M., Hurtado-Oliva, M., Piña-Valdez, P., & Garza, C. (2013). Feeding physiology and scope for growth of the oyster *Crassostrea corteziensis* (Hertlein, 1951) acclimated to different conditions of temperature and salinity. Aquaculture International, 21, 283–297. https://doi.org/10.1007/s10499-012-9550-4

Gobler, C. J., Clark, H. R., Griffith, A. W., & Lusty, M. W. (2017). Diurnal Fluctuations in Acidification and Hypoxia Reduce Growth and Survival of Larval and Juvenile Bay Scallops (*Argopecten irradians*) and Hard Clams (*Mercenaria mercenaria).* Frontiers in Marine Science, 3. https://doi.org/10.3389/fmars.2016.00282

Handisyde, N., Telfer, T. C., & Ross, L. G. (2017). Vulnerability of aquaculture-related livelihoods to changing climate at the global scale. Fish and Fisheries, 18(3), 466–488. https://doi.org/10.1111/faf.12186

Heugens, E. H. W., Hendriks, A. J., Dekker, T., Straalen, N. M. van, & Admiraal, W. (2001). A Review of the Effects of Multiple Stressors on Aquatic Organisms

and Analysis of Uncertainty Factors for Use in Risk Assessment. *Critical Reviews in Toxicology*, *31*(3), 247–284. https://doi.org/10.1080/20014091111695

Hishamunda, N., Ridler, N., Bueno, P., Satia, B., Kuemlangan, B., Percy, D., Gooley, G., Brugere, C. & Sen, S. (2012). Improving aquaculture governance: what is the status and options? In R.P. Subasinghe, J.R. Arthur, D.M. Bartley, S.S. De Silva, M. Halwart, N. Hishamunda, C.V. Mohan & P. Sorgeloos, eds. Farming the Waters for People and Food. Proceedings of the Global Conference on Aquaculture 2010, Phuket, Thailand. 22–25 September 2010. pp. 233–264. FAO, Rome and NACA, Bangkok.

Kudela, R. M., Bickel, A., Carter, M. L., Howard, M. D. A., & Rosenfeld, L. (2015). Chapter 5 - The Monitoring of Harmful Algal Blooms through Ocean Observing: The Development of the California Harmful Algal Bloom Monitoring and Alert Program. In Y. Liu, H. Kerkering, & R. H. Weisberg (Eds.), Coastal Ocean Observing Systems (pp. 58–75). Academic Press, USA. https://doi.org/10.1016/B978-0-12-802022-7.00005-5

Lemasson, A. J., Hall-Spencer, J. M., Fletcher, S., Provstgaard-Morys, S., & Knights, A. M. (2018). Indications of future performance of native and non-native adult oysters under acidification and warming. Marine Environmental Research, 142, 178–189. https://doi.org/10.1016/j.marenvres.2018.10.003

Lemasson, A., & Knights, A. (2021). Differential responses in anti-predation traits of the native oyster *Ostrea edulis* and invasive *Magallana gigas* to ocean acidification and warming. https://doi.org/10.3354/meps13687

Li, Q., Sun, S., Zhang, F., Wang, M., & Li, M. (2019). Effects of hypoxia on survival, behavior, metabolism and cellular damage of Manila clam (*Ruditapes*

philippinarum). Plos One, 14(4), e0215158. https://doi.org/10.1371/journal.pone.0215158

Liu, W., & He, M. (2012). Effects of ocean acidification on the metabolic rates of three species of bivalve from southern coast of China. Chinese Journal of Oceanology and Limnology, 30(2), 206–211. https://doi.org/10.1007/s00343-012-1067-1

Lloyd, J. K., Duchin, J. S., Borchert, J., Quintana, H. F., & Robertson, A. (2013). Diarrhetic Shellfish Poisoning, Washington, USA, 2011. Emerging Infectious Diseases, 19(8), 1314–1316. https://doi.org/10.3201/eid1908.121824

Misra, A. K. (2014). Climate change and challenges of water and food security. International Journal of Sustainable Built Environment, 3(1), 153–165. https://doi.org/10.1016/j.ijsbe.2014.04.006

Moore, S. K., Trainer, V. L., Mantua, N. J., Parker, M. S., Laws, E. A., Backer, L. C., & Fleming, L. E. (2008). Impacts of climate variability and future climate change on harmful algal blooms and human health. Environmental Health, 7(2), S4. https://doi.org/10.1186/1476-069X-7-S2-S4

Morris JP, Backeljau T, Chapelle G (2018). Shells from aquaculture: a valuable biomaterial, not a nuisance waste product. Reviews in Aquaculture 1–16. https://doi.org/10.1111/raq. 12225

Nie, H., Wang, H., Jiang, K., & Yan, X. (2020). Transcriptome analysis reveals differential immune related genes expression in *Ruditapes philippinarum* under hypoxia stress: Potential HIF and NF-kappa B crosstalk in immune responses in clam. BMC Genomics, 21(1), 318. https://doi.org/10.1186/s12864-020-6734-6

Norkko, J., & Shumway, S. E. (2011). Bivalves as Bioturbators and Bioirrigators. In Shellfish Aquaculture and the Environment (pp. 297–317). John Wiley & Sons, Ltd.,USA. https://doi.org/10.1002/9780470960967.ch10

Olivier, A. van der S., Jones, L., Vay, L. L., Christie, M., Wilson, J., & Malham, S. K. (2020). A global review of the ecosystem services provided by bivalve aquaculture. Reviews in Aquaculture, 12(1), 3–25. https://doi.org/10.1111/raq.12301

Parker, L. M., Ross, P. M., & O'Connor, W. A. (2010). Comparing the effect of elevated pCO_2 and temperature on the fertilization and early development of two species of oysters. Marine Biology, 157(11), 2435–2452. https://doi.org/10.1007/s00227-010-1508-3

Pauchauri, R. K., & Meyer, L. A. (2014). PCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (p. 151). IPCC.

Perkins-Kirkpatrick, S. E., & Lewis, S. C. (2020). Increasing trends in regional heatwaves. Nature Communications, 11(1), Article 1. https://doi.org/10.1038/s41467-020-16970-7

Peteiro, L. G., Woodin, S. A., Wethey, D. S., Costas-Costas, D., Martínez-Casal, A., Olabarria, C., & Vázquez, E. (2018). Responses to salinity stress in bivalves: Evidence of ontogenetic changes in energetic physiology on *Cerastoderma edule*. Scientific Reports, 8(1), 1–9. https://doi.org/10.1038/s41598-018-26706-9

Prince, W., & Fantom, N. (2014). World development indicators 2014 (English).

Provoost, P., van Heuven, S., Soetaert, K., Laane, R. W. P. M., & Middelburg, J. J. (2010). Seasonal and long-term changes in pH in the Dutch coastal zone. Biogeosciences, 7(11), 3869–3878. https://doi.org/10.5194/bg-7-3869-2010

Range, P., Chícharo, M. A., Ben-Hamadou, R., Piló, D., Fernandez-Reiriz, M. J., Labarta, U., Marin, M. G., Bressan, M., Matozzo, V., Chinellato, A., Munari, M., El Menif, N. T., Dellali, M., & Chícharo, L. (2014). Impacts of CO_2-induced seawater acidification on coastal Mediterranean bivalves and interactions with other climatic stressors. Regional Environmental Change, 14(1), 19–30. https://doi.org/10.1007/s10113-013-0478-7

R Core Team.(2013). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Shumway, S., Davis, C., Downey, R., Karney, R., Kraeuter, J., Parsons, J., Rheault, R., & Wikfors, G. (2012). Guest Editorial Shellfish aquaculture—In praise of sustainable economies and environments. World Aquaculture.

Sobral, P., & Widdows, J. (1997). Effects of elevated temperatures on the scope for growth and resistance to air exposure of the clam *Ruditapes decussatus* (L.), from southern Portugal. Scientia Marina.

Stewart-Sinclair, P. J., Last, K. S., Payne, B. L., & Wilding, T. A. (2020). A global assessment of the vulnerability of shellfish aquaculture to climate change and ocean acidification. Ecology and Evolution, 10(7), 3518–3534. https://doi.org/10.1002/ece3.6149

Sylvester, F., Boltovskoy, D., & Cataldo, D. H. (2007). Fast response of freshwater consumers to a new trophic resource: Predation on the recently introduced Asian bivalve *Limnoperna fortunei* in the lower Paraná river, South America.

Austral Ecology, 32(4), 403–415. https://doi.org/10.1111/j.1442-9993.2007.01707.x

Talmage, S. C., & Gobler, C. J. (2011). Effects of Elevated Temperature and Carbon Dioxide on the Growth and Survival of Larvae and Juveniles of Three Species of Northwest Atlantic Bivalves. PLoS ONE, 6(10). https://doi.org/10.1371/journal.pone.0026941

Tan, K., Zhang, H., & Zheng, H. (2020). Selective breeding of edible bivalves and its implication of global climate change. Reviews in Aquaculture, 12(4), 2559–2572. https://doi.org/10.1111/raq.12458

Trainer, V. L., Moore, S. K., Hallegraeff, G., Kudela, R. M., Clement, A., Mardones, J. I., & Cochlan, W. P. (2019). Pelagic harmful algal blooms and climate change: Lessons from nature's experiments with extremes. *Harmful Algae*, *91*, 101591. https://doi.org/10.1016/j.hal.2019.03.009

Troell, M., Naylor, R. L., Metian, M., Beveridge, M., Tyedmers, P. H., Folke, C., Arrow, K. J., Barrett, S., Crépin, A.-S., Ehrlich, P. R., Gren, Å., Kautsky, N., Levin, S. A., Nyborg, K., Österblom, H., Polasky, S., Scheffer, M., Walker, B. H., Xepapadeas, T., & de Zeeuw, A. (2014). Does aquaculture add resilience to the global food system? Proceedings of the National Academy of Sciences, 111(37), 13257–13263. https://doi.org/10.1073/pnas.1404067111

Turner, L. M., Havenhand, J. N., Alsterberg, C., Turner, A. D., Girisha, S. K., Rai, A., Venugopal, M. N., Karunasagar, I., & Godhe, A. (2019). Toxic Algae Silence Physiological Responses to Multiple Climate Drivers in a Tropical Marine Food Chain. Frontiers in Physiology, 10, 373. https://doi.org/10.3389/fphys.2019.00373

Vaughn, C. C., & Hakenkamp, C. C. (2001). The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology, 46(11), 1431–1446. https://doi.org/10.1046/j.1365-2427.2001.00771.x

Villnas, A., Norkko, A., & Lehtonen, K. K. (2019). Multi-level responses of *Macoma balthica* to recurring hypoxic disturbance. Journal of Experimental Marine Biology and Ecology, 510, 64–72. https://doi.org/10.1016/j.jembe.2018.10.005

Welladsen, H. M., Southgate, P. C., & Heimann, K. (2010). The effects of exposure to near-future levels of ocean acidification on shell characteristics of *Pinctada fucata* (Bivalvia: Pteriidae). Molluscan Research, 30(3), 125–130.

Wheeler, T., & von Braun, J. (2013). Climate Change Impacts on Global Food Security. Science, 341(6145), 508–513. https://doi.org/10.1126/science.1239402

Woodin, S. A., Wethey, D. S., Olabarria, C., Vazquez, E., Dominguez, R., Macho, G., & Peteiro, L. (2020). Behavioral responses of three venerid bivalves to fluctuating salinity stress. Journal of Experimental Marine Biology and Ecology, 522, 151256. https://doi.org/10.1016/j.jembe.2019.151256

CHAPTER TWO

Knowledge gaps on the impacts of multiple climate change-stressors on bivalves: a 21- year review

Abstract

This systematic review evaluates the knowledge gaps on the impact of multiple climate change stressors on bivalves through controlled, manipulative multi-stressor experiments. Among 69 eligible studies, we observe a focus on a limited subset of bivalve species, primarily *Crassostrea virginica* and *Mytilus galloprovincialis* (12 studies each), with adult stages receiving predominant attention (57%). Few studies address embryonic stages (3%), and only two consider stressor fluctuations. Key response parameters include survival rates, biochemical indicators (e.g., antioxidant enzymes, energy-related markers), and heavy metal accumulation. To enhance research comprehensiveness, future efforts should broaden species coverage to include economically valuable but lesser-studied bivalves (e.g., giant clams, cockles, European native oysters, Pacific rock oysters) and expand investigations to encompass early life stages. Additionally, addressing underexplored climate change stressors like harmful algal blooms and replicating realistic environmental fluctuations in laboratory settings is crucial. Further research should delve into bivalves' reproductive traits and behaviours essential for ecosystem resilience. Collaborative engagement among stakeholders (scientists, policymakers, industry leaders, communities) and equitable resource allocation are warranted to facilitate research in low-income nations. Understanding bivalve vulnerability across life stages and their responses to climate change stressors will facilitate sustainable aquaculture practices, population management, and risk mitigation. In this era of climate change, the collective dedication to research, collaboration, and sustainable stewardship will ensure the continued contribution of these organisms in evolving ecosystems.

2.1. Introduction

2.1.1. What is a stressor?

Understanding how organisms respond to environmental stress is becoming increasingly critical as the planet undergoes rapid climate change due to human activities. The term 'environmental stress' is used in multiple fields of biology, but its definition needs to be clarified, leading to confusion when attempting to understand organisms' responses to environmental changes. In their work, Schulte (2014) examines and consolidates various definitions of stress and related concepts in biology. They propose a specific definition for stressors, framing them as environmental changes that can diminish an organism's overall performance or fitness. By suggesting this definition, Schulte emphasises the importance of understanding how these external environmental factors can directly impact an organism's ability to function optimally and thrive within its ecological context.

This proposed definition of stressors highlights the crucial link between the environment and an organism's well-being. Stressors are not limited to isolated events but encompass various environmental changes that can persist or occur repeatedly. These changes can include fluctuations in temperature, altered nutrient availability, exposure to toxins, variations in humidity or salinity, and shifts in predator-prey dynamics, among others.

By emphasising the reduction in performance or fitness because of stressors, it becomes clear that the impact of environmental changes extends beyond mere inconvenience or discomfort. Instead, stressors present tangible challenges that constrain an organism's ability to survive, reproduce, and thrive in its environment. These challenges can arise at various levels, including cellular and molecular processes, organism-level performance, and population dynamics.

2.1.2. Interplay and co-occurrence of environmental stressors

Schulte's proposed definition of stressors as environmental changes that impact an organism's performance or fitness is crucial for understanding and quantifying the effects of environmental stress. This broader definition allows researchers to investigate the underlying mechanisms and the evolutionary implications of stress response dynamics. Moreover, it enables the development of strategies for managing and mitigating stressors in rapidly changing environments amid climate change. The high concentration of greenhouse gases in the atmosphere that has been accelerating since the Industrial Revolution has resulted in a phenomenal and unprecedented temperature increase. In the last four decades, temperatures have been successively warmer than any previous decade, and this trend is expected to continue to intensify, leading to significant ecological changes and the loss of coastal and estuarine ecosystems (IPCC, 2007, 2021).

Other than increasing temperatures, climate change scenarios would have far-reaching impacts on ecosystems and environmental conditions, including sea-level rise, changes in rainfall patterns, and increased intensity of storms. The resulting incremental changes in environmental factors will significantly worsen the already sensitive ecosystems. Furthermore, the rise in temperature in coastal zones will contribute to a further decline in oxygen concentration, along with increasing ocean acidity due to the absorption of carbon dioxide in seawater, further worsening ecological problems.

Anticipated climate change brings forth a complex array of environmental shifts, encompassing adjustments in conditions and heightened occurrence and intensity of environmental stressors. These changes hold profound consequences for biodiversity. In particular, the intricate interplay and interactions among co-occurring multiple stressors necessitate a comprehensive grasp of organisms' reactions to such challenges. Understanding these responses and their evolution is paramount, especially when considering the anticipation and management of climate change impacts on diverse species.

2.1.3. The effect of multiple stressors on organism performance

Theoretical considerations suggest that simultaneous exposure to multiple stressors could lead to additive, antagonistic, or synergistic effects on organism performance

(Fig 2.1) (Gunderson et al., 2016; Todgham & Stillman, 2013). An additive effect manifests when the cumulative impact of these stressors aligns with the sum of their individual effects. This phenomenon is exemplified in bivalves like *Saccostrea glomerata* (Sydney rock oyster) and *Magallana gigas* (Pacific rock oyster). These species exhibit reduced gamete fertilisation success, embryo development, larval size, and spat size in suboptimal temperatures alongside elevated pCO_2 (Parker et al., 2010). An escalation in abnormal larval morphology is also noted. In a similar vein, Talmage & Gobler (2011) delve into responses among larval and juvenile stages of three bivalve species (*Mercenaria mercenaria, Crassostrea virginica,* and *Argopecten irradians*) when exposed to variations in temperature and CO_2 concentrations. They conclude that elevated temperature and CO_2 levels significantly diminish survival rates, development, growth, and lipid synthesis in *M. mercenaria* and *A. irradians* larvae.

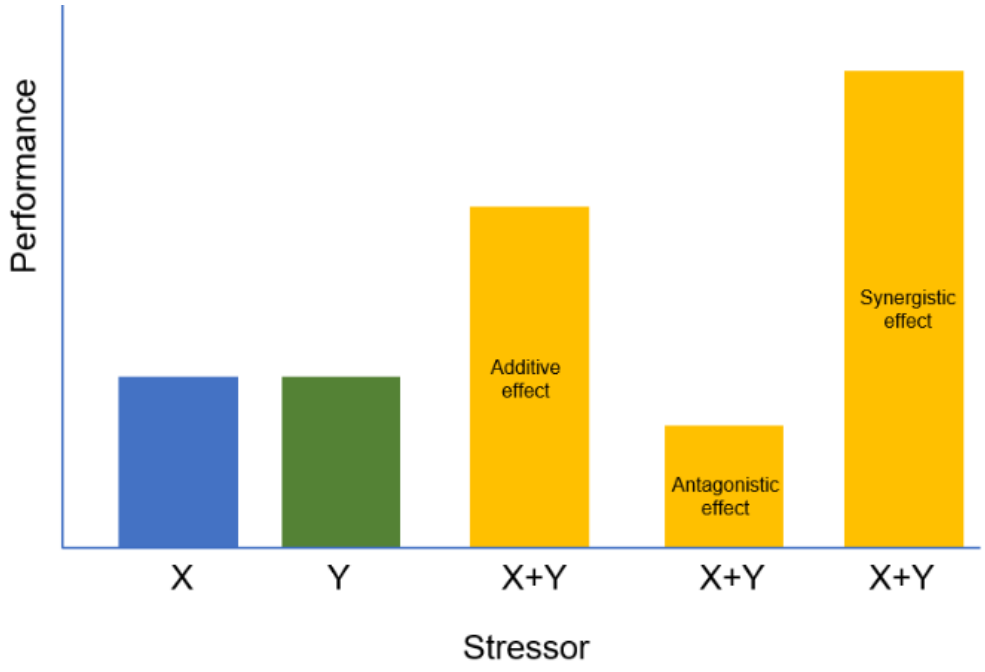


Figure 2.1 *Theoretical interactive effects of two stressors on physiological performance (Adapted with modifications from Gunderson et al., 2016).*

An antagonistic effect arises when the cumulative impact of multiple stressors falls short of the expected additive effect. For instance, such effects have been observed in bay scallops *A. irradians*, Eastern oysters *Crassostrea virginica,* blue mussels *Mytilus edulis,* and hard clams *M. mercenaria* when exposed to a combination of low dissolved oxygen and low pH. Surprisingly, the dual stressors result in higher growth rates than predicted by the individual stressors alone. This suggests that certain anaerobic metabolic pathways might function optimally under increased CO_2 levels, as indicated by the work of Stevens & Gobler (2018).

Conversely, a synergistic effect manifests when the combined impact of multiple stressors exceeds the projected additive effect (Gunderson et al., 2016). Such an effect is observable in bivalves confronted with high temperatures and low salinity. The Asian green mussel *Perna viridis*, for instance, can tolerate temperatures ranging from 9 to 35°C when the salinity is 35-37. However, as salinity decreases, the thermal survival range for *P. viridis* becomes narrower. Similarly, the adult and juvenile Charru mussel *Mytella charruana* exhibit broad salinity tolerance, ranging from 5 to 40 at 20°C. Nevertheless, this salinity tolerance range contracts as temperatures decrease or increase (Yuan et al., 2016).

2.1.4. Chapter Rationale and Aims

Multiple stressor research is an ever-expanding domain aimed at deciphering and ultimately predicting interactions between various stressors. Previous reviews and meta-analyses on multiple stressors have delved into various aspects of aquatic organisms (Heugens et al., 2001), marine infauna (Ellis et al., 2017), marine embryos and larvae (Przeslawski et al., 2015), and the dynamics of coral reefs (Harborne et al., 2017). However, there is a conspicuous gap in the literature regarding a comprehensive review or meta-analysis consolidating the existing knowledge regarding the impact of multiple stressors on bivalves. This omission is noteworthy because bivalves are one of the groups profoundly affected by these multifaceted stressors. Their sessile nature and residence in coastal environments, which are subject to constant change and proximity to anthropogenic stressors, make them a particularly pertinent subject for such a study. In this context, this chapter takes on the form of a systematic review, with the following specific aims: (1) assess publication performance and trends, and (2) identify research gaps and propose strategies to improve and advance research concerning the effects of multiple climate change stressors on bivalves.

Building upon the objectives outlined above, it is hypothesised that (1) there exists a discernible pattern in the publication performance and trends related to multiple climate change stressors' impacts on bivalves, (2) an analysis of existing literature will reveal critical research gaps in understanding the cumulative effects of multiple

stressors on bivalves. Moreover, this study will propose actionable strategies to mitigate these knowledge gaps and pave the way for a more resilient and sustainable bivalve aquaculture industry, thus contributing to broader climate change mitigation and adaptation efforts.

2.1.5. Limitations

Initiated in 2020 at the outset of my doctoral journey, this study was designed to serve as a foundational cornerstone guiding my research pursuits by giving me a good understanding of what has already been studied and what areas still need exploration. The selection of keywords was executed to ensure the inclusion of as many relevant studies as possible, all sourced from the Web of Science database.

Nevertheless, it is acknowledged that the review's scope may only encompass some pertinent studies within this vast field. Expressly, it does not incorporate research that did not utilise the keywords I employed or studies that were absent from the Web of Science database at the time of my search.

Furthermore, it is imperative to underscore that this review represents the state of knowledge up to 2020 and does not encompass any subsequent updates or advancements in the field that may have transpired since that time up to the current year of 2023.

2.2. Methods

2.2.1. Data Compilation and Literature Review

A database was compiled containing data from research papers concerning the impacts of various climate change stressor combinations on bivalves. The literature review utilised the Web of Science database (www.webofknowledge.com) due to its reputation for comprehensive scholarly coverage. On June 4, 2020, a search was conducted using the query "('bivalve*' OR 'shellfish*') AND ('climate change' OR 'global warming' OR 'global climate change')," resulting in a total of 916 results.

The selection of keywords was deliberate, to specifically target research studies that investigate the effects of climate change on bivalves. This precision allowed for retrieving studies highly pertinent to the research objectives. A screening process was applied to ensure the database's quality and relevance, focusing exclusively on studies featuring controlled, manipulative multiple stressor experiments while deliberately excluding those concentrating solely on single stressors or field-based investigations. Following this screening, 69 publications were retained in the database. The bibliographical details of these publications can be found in Appendix A.

2.2.2. Data Collection

From the compiled database, the following data were gathered to assess performance and trends and identify knowledge gaps regarding the effects of multiple climate change stressors in bivalves:

1. The year of publication
2. The country where the study was conducted
3. The bivalve species and life stages studied
4. The stressors employed
5. The responses observed
6. The fluctuation of stressors during exposure

2.2.3. Statistical Analyses

Statistical analysis was made to discern whether economic factors affected the number of published articles in the country where the research was conducted. A generalized linear model (GLM) with a quasi-Poisson distribution was employed to investigate this. In this context, Gross Domestic Product (GDP) was considered the explanatory variable, while the number of published papers served as the response variable. GDP data was obtained from the World Bank database (http://data.worldbank.org). Adopting the quasi-Poisson distribution

was necessary due to observed overdispersion, which rendered a Poisson distribution unsuitable for the analysis (Dunn & Smith, 2018).

Additionally, given that the vast majority of marine bivalve production, approximately 89%, is attributed to aquaculture practices, as opposed to the 11% sourced from wild fisheries (Wijsman et al., 2019), further investigation was conducted to explore any potential correlation between the volume of published papers and the output of the aquaculture industry in each respective country. To examine this relationship, a Generalized Linear Model (GLM) with a quasi-Poisson distribution was applied, utilising Aquaculture Production (metric tons) as the independent variable and the Number of Published Papers as the dependent variable. Like the GDP data, the aquaculture production data was sourced from the World Bank database and focused on the final harvest and consumption output from aquaculture activities. All analyses were done using R version 4.3.0.

2.3. Results

2.3.1. Number of publications per year

The first multi-stressor study recorded in 1998 investigated the effects of elevated temperature and suspended sediments on the growth and survival of two mussel species (Thorp et al., 1998). No studies were recorded from 1999-2003, but there was a steady increase in the number of studies starting in 2004. The highest count was in 2018, with 14 published studies (Fig 2.2).

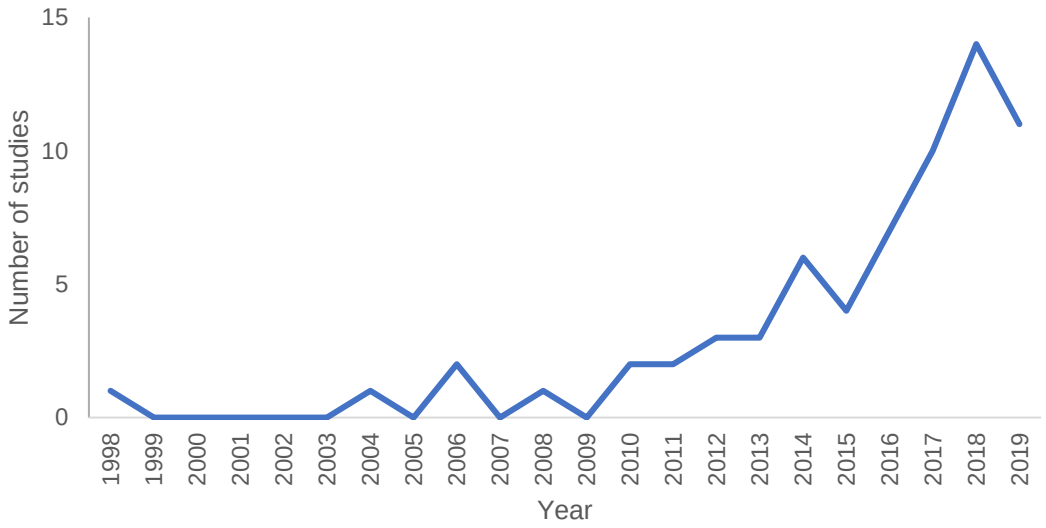


Figure 2.2 *Number of controlled, manipulative, multi-stressor studies on the effects of climate change in bivalves per year, listed on the Web of Science from 1998-2019, using the query "("bivalve* "OR "shellfish*") AND ("climate change" OR "global warming" OR "global climate change")" on 4 June 2020.*

2.3.2. Species of bivalves studied

Table 2.1 provides an overview of the number of studies conducted on various bivalve species, reflecting the degree of scientific interest and research focus across different species. The species listed in the table range from well-known and extensively studied species to those with limited research attention. Notably, *Crassostrea virginica* and *Mytilus galloprovincialis* have each been the subject of 12 studies, indicating a substantial scientific interest in these species. *Mercenaria mercenaria* follows closely with eight studies, underscoring its significance in marine research. *Mytilus edulis* and *Ruditapes philipinarum* are subjects of seven studies each, suggesting a similar level of attention in the scientific community.

Species such as *Argopecten irradians* and *Magallana gigas* have been investigated in five studies each, indicating moderate research activity. In contrast, numerous species have been the focus of only one or two studies, including less-studied species like *Anadara trapezia, Arctica islandica*, and *Dreissena polymorpha*. Species with a single study, such as *Brachidontes pharaonis, Gaimardia trapesina*, and *Pinctada fucata,* are less commonly researched and represent areas where additional scientific investigation may be warranted.

Table 2.1 *Number of Studies Examining Each Bivalve Species in Controlled Multiple Stressor Climate Change Research, Indexed in the Web of Science as of June 4, 2020.*

Species Name	Number of Studies
1. Crassostrea virginica	12
2. Mytilus galloprovincialis	12
3. Mercenaria mercenaria	8
4. Mytilus edulis	7
5. Ruditapes philipinarum	7
6. Argopecten irradians	5
7. Magallana gigas	5
8. Laternula elliptica	3
9. Ostrea edulis	3
10. Pecten maximus	3
11. Cerastoderma edule	2
12. Mya arenaria	2
13. Mytilus coruscus	2
14. Anadara trapezia	1
15. Arctica islandica	1
16. Argopecten purpuratus	1
17. Brachidontes pharaonis	1
18. Chamelea gallina	1
19. Crassostrea angulata	1
20. Dreissena bugensis	1
21. Dreissena polymorpha	1
22. Flexopecten glaber	1
23. Gaimardia trapesina	1
24. Meretrix meretrix	1
25. Mytilus chilensis	1
26. Mytilus spp*	1
27. Patinopecten yessoensis	1
28. Perna viridis	1
29. Pinctada fucata	1
30. Pinna nobilis	1
31. Ruditapes decussatus	1
32. Scrobicularia plana	1
33. Tridacna squamosa	1

*One of the studies did not specify the species of *Mytilus* studied.

2.3.3. Countries producing multiple stressor studies

The multiple stressor studies gathered were performed in 15 countries (Fig 2.3). The United States produced the most publications (16 studies), followed by Portugal (13 studies), Italy (9 studies), Germany (6 studies), the United Kingdom (5 studies), China (4 studies); Australia, and Spain (3 studies each), Sweden, Norway, and Chile (2 studies each), and finally, New Zealand, Hong Kong, France, and Finland (1 study each).

The analysis observed a positive and statistically significant relationship between a country's Gross Domestic Product (GDP) and the number of studies it makes. The intercept, representing the expected number of studies when GDP is zero, was estimated at approximately 1.216 ($p < 0.001$). A small but statistically significant increase in the expected number of studies produced was found for each additional unit of GDP increase, with a coefficient estimate of 5.298e-08 ($p = 0.039$). These results indicate that as a country's economy grows, empirical evidence suggests a corresponding increase in research output for the effects of multiple climate change stressors on bivalves.

Moreover, the model's goodness of fit was assessed using the residual deviance, which was found to be 38.329. This value suggests a relatively low level of unexplained variability in the data. Lower residual deviance indicates a better fit of the model to the data, further supporting the notion that the GDP variable explains a

significant portion of the variation in the number of studies produced by different countries. Therefore, these results establish a positive relationship between GDP and research output and demonstrate that the model adequately captures the observed patterns in the data. This underscores the practical implication that fostering economic growth can be associated with a measurable increase in research productivity.

In analysing of the relationship between aquaculture output and the number of studies produced, the results revealed that the intercept, representing the expected number of studies when aquaculture output is zero, was estimated at approximately 1.537 ($p < 0.001$). However, the coefficient estimate for aquaculture output was approximately -2.264e-09, and the associated p-value was 0.889, indicating no statistically significant relationship between aquaculture output and the number of studies produced.

Most studies published in the USA studied *C. virginica, M. mercenaria,* and *A. irradians.* Most o the studies in Portugal were on *M. galloprovincialis, R. philipinarum,* and *M. gigas.* In Italy, the most-studied species were *M. galloprovincialis, R. philipinarum,* and *M. mercenari*a.

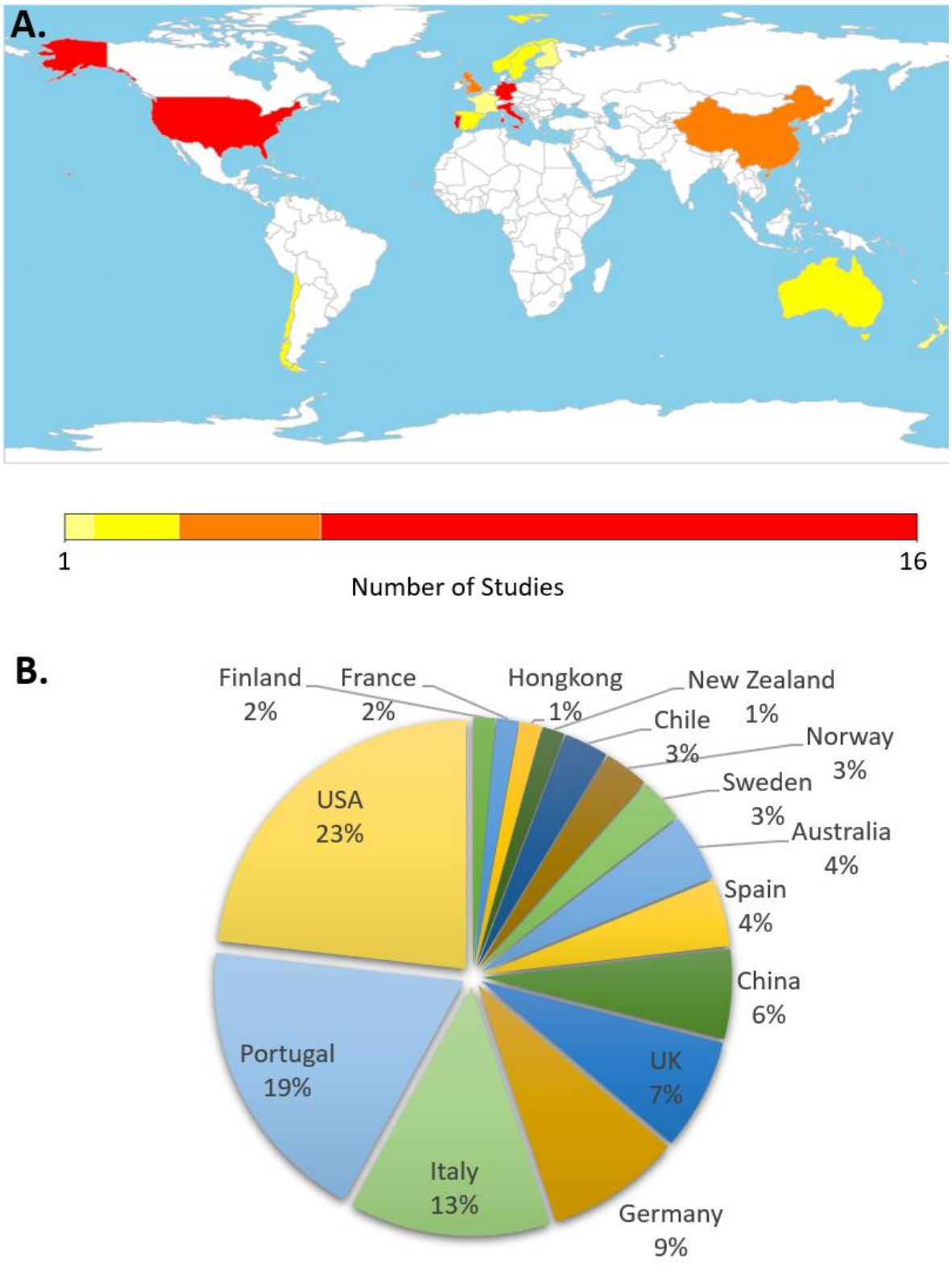


Figure 2.3 *Map (**A**) and a pie chart (**B**) showing countries recorded as study sites for the controlled, manipulative, multi-stressor studies on the effects of climate change in bivalves, listed on the Web of Science as of 4 June 2020.*

2.3.4. Life stages

Over half of the studies (57%) focused on climate change stressors' impact on adult bivalves. Nineteen per cent of the research investigated juvenile bivalves, while 10% examined both larvae and juveniles. Four per cent specifically studied larvae, 3% focused on embryos and larvae, and 7% did not specify the life stage under investigation (Fig. 2.4).

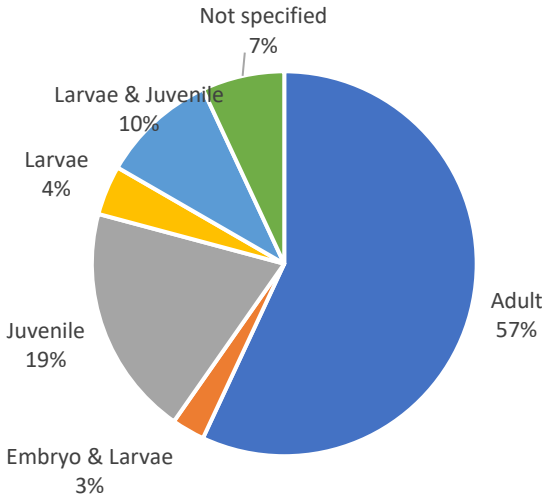


Figure 2.4 *Percentage of studies that investigated bivalve adults, juveniles, larvae and embryos/fertilisation among the multi-stressor studies gathered listed on the Web of Science as of 4 June 2020.*

2.3.5. Stressors

Seventeen potential stressors were studied by the publications collected (Figure 2.5). Most of the publications (45 out of 69 papers) included elevated temperature as a stressor. This was followed by acidification (38 papers) and the presence of heavy

metals (15 articles). The least-studied stressors are not directly climate change related, including presence of carbon nanomaterial, competitors, herbicides, caffeine, parasitic trematode worms, turbid waters, and the presence of viruses (1 study each). Shown in Fig 2.6 are the potential stressors studied by year, showing that early multiple stressor laboratory experiments focused on elevated temperature and the presence of heavy metal. Interest in the effects of acidification started in 2012, and more studies that include acidification as a co-stressor have been published since, along with other stressors (Fig 2.6).

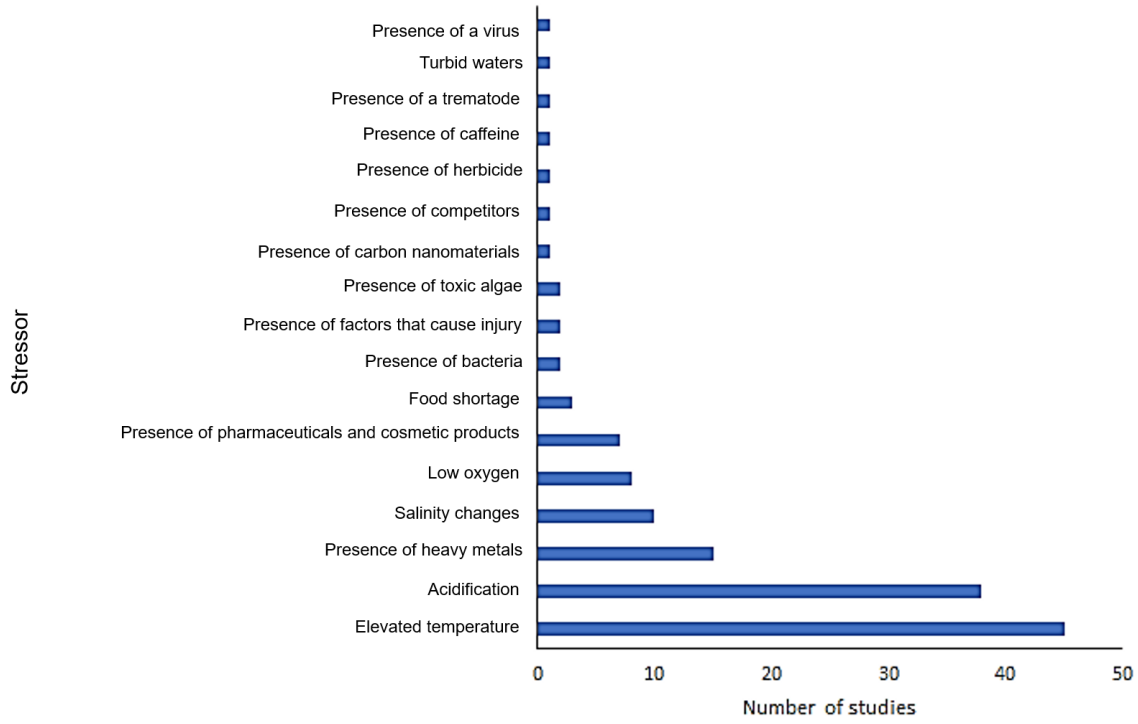


Figure 2.5 *The number of studies for each stressor investigated in the multi-stressor studies on bivalves listed on the Web of Science as of 4 June 2020.*

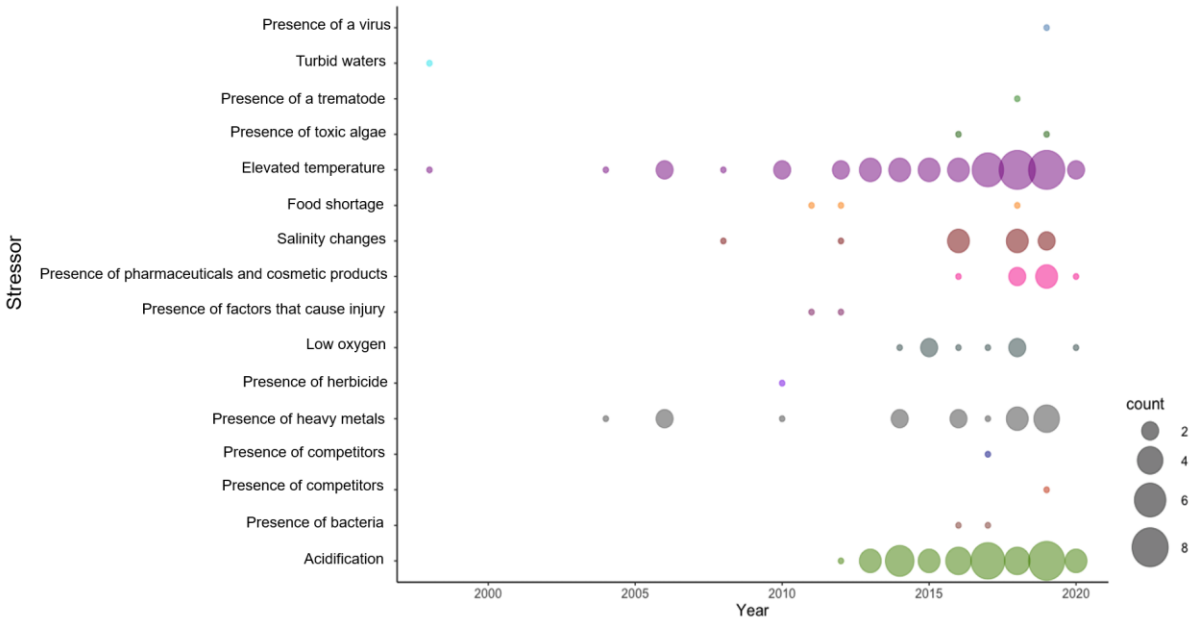


Figure 2.6 *The temporal distribution of stressors employed in controlled, manipulative multi-stressor studies on bivalves from 1998-2020, listed on the Web of Science as of 4 June 2020.*

2.3.6. Stressor combinations

Fifty-four of the 69 studies involved a combination of two stressors. Most of them studied the combination of acidification and elevated temperature (19 studies) (Fig 2.7). This combination was followed by elevated temperature and presence of heavy metals (7 studies); acidification and low oxygen (6 studies); elevated temperature and salinity changes (4 studies); acidification and presence of heavy metals (3 studies); acidification and presence of pharmaceutical & personal care products (3 studies); elevated temperature and salinity changes (3 studies); food shortage and presence of factor that cause injury (3 studies); acidification and salinity changes (2 studies); and temperature and low oxygen (2 studies). The rest of the two-stressor combinations had only one study each (Fig 2.7).

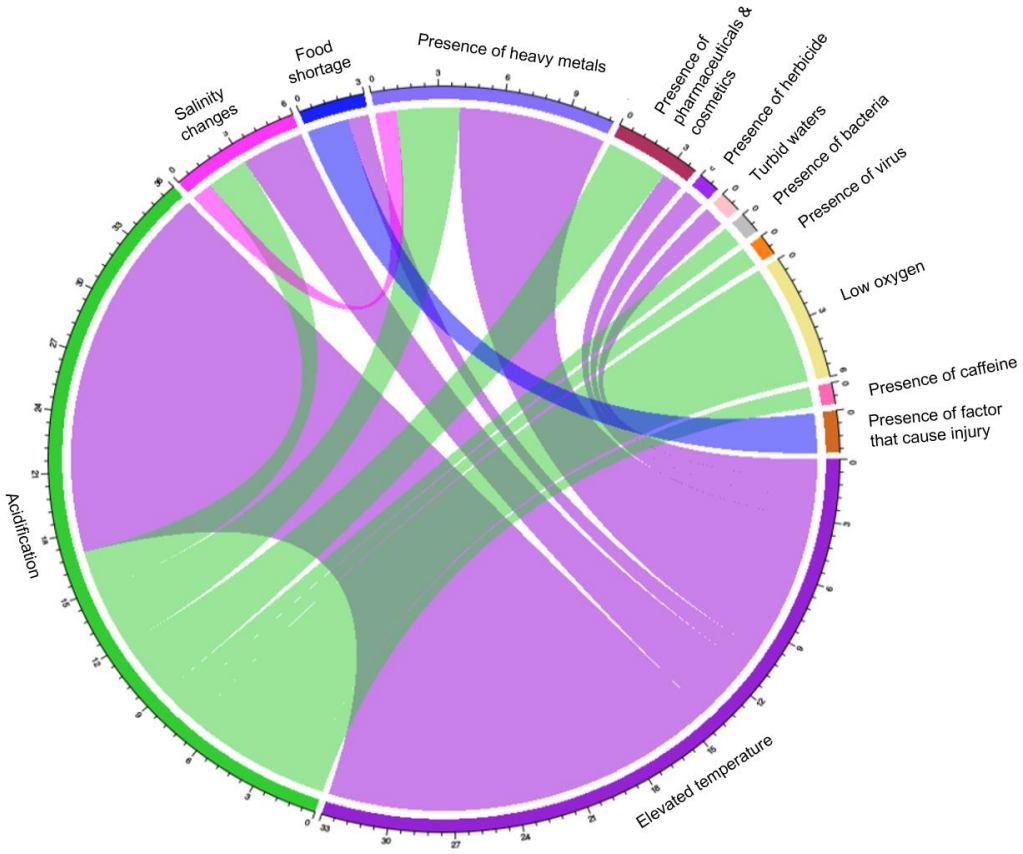


Figure 2.7 *The number of studies per two-stressor combinations in the publications gathered that employed in controlled, manipulative, mulltiple stressor experiments on bivalves, listed on the Web of Science as of 4 June 2020.*

Thirteen of the studies had three-stressor combinations (Fig 2.8); the highest count (3) investigated the effects of temperature, salinity, and pharmaceutical or personal care products. As for four-stressor combinations, only two studies were recorded, and the stressor combinations are shown in Fig 2.8.

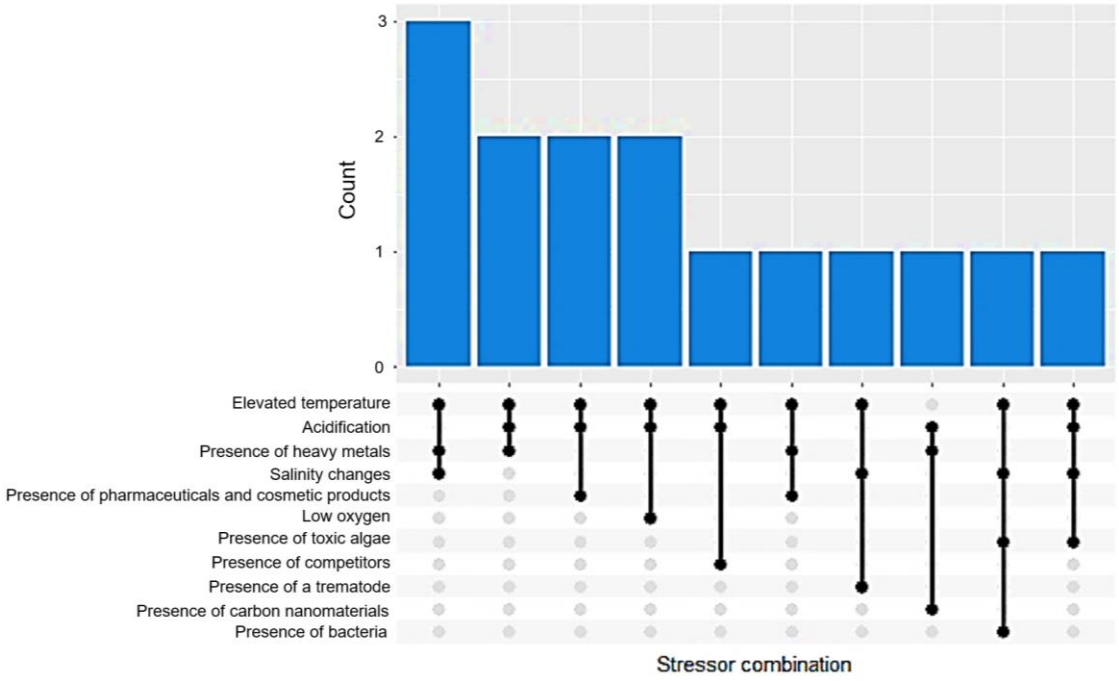


Figure 2.8 *The number of bivalve studies that involved three and four stressor combinations in the publications that employed controlled, manipulative, multi-stressor experiments, listed on the Web of Science as of 4 June 2020.*

2.3.7. *Responses*

A total of 61 responses were recorded in the multiple stressor studies. The chart in Figure 2.9 shows the top 20 most observed responses. After being exposed to the multiple stressors, survival was most observed, a critical concern in aquaculture. This was followed by antioxidant and biotransformation enzymes (catalase, superoxide dismutase, glutathione-S-transferase, glutathione peroxidase), which play crucial roles in maintaining the health and performance of bivalves. Third on the list are energy-related biomarkers (electron transport activity, protein levels, glycogen, ATP, ADP and AMP), which have an an important role in determining these organisms' energy status, metabolism, and overall health. Indicators of cellular

damage, heavy metal accumulation, immunological responses, shell calcification rate, and respiration rate follow these responses. (See Appendix B for a complete list of responses).

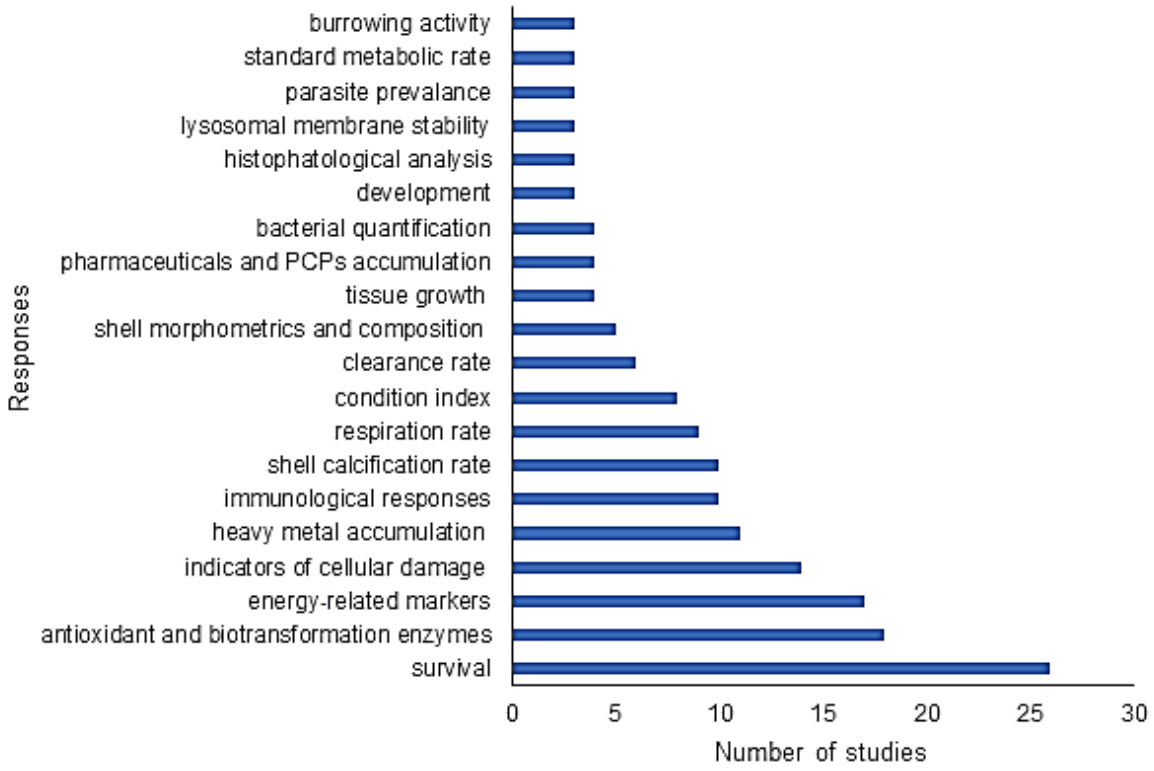


Figure 2.9 *The top 20 responses observed in the studies that investigated multiple climate change stressors on bivalves, listed on the Web of Science as of 4 June 2020.*

2.3.8. Fluctuation of Stressors

A mere two stood out by investigating the effects of fluctuating stressors, while the vast majority, 67 studies (accounting for a substantial 97%), exposed bivalves to a constant stress levelthroughout their experiments .

One of these studies was conducted by Gobler et al. (2014), who delved into the influence of diurnal fluctuations in pH and dissolved oxygen (DO) on bay scallops and hard clams, both native to Northeast US estuaries. Their research involved two sets of experiments: one examining the impact of varying pH levels on larval and juvenile bivalves, and the other exploring how larval bivalves coped with different pH and DO levels.

In a complementary study, Khan et al. (2018) focused on the effects of hypoxia and rising sea temperatures on oyster microbiomes. Throughout their laboratory experiments conducted in 2010 and 2011, they commenced with oysters from Bogue Sound, North Carolina, initially subjected to normal oxygen levels. After a brief acclimation period in the lab lasting 5-7 days, the oysters were exposed to various conditions for either 4 or 8 days. These conditions encompassed scenarios of normal oxygen levels, daily oxygen fluctuations characterised by diurnal cycles of hypoxia,

and extended episodes of continuous oxygen deficiency, known as prolonged continuous hypoxia.

2.4. Discussion

2.4.1. Multiple stressor studies are appropriate for studying climate change effect

The increase in the number of publications on the effects of multiple climate change stressors on bivalves shows a growing acknowledgement in the scientific community that multiple stressor experiments are appropriate to investigate the impact of climate change in aquatic habitats. Climate biologists have primarily conducted controlled laboratory experiments that employ a single environmental variable to measure organisms' performance under changing conditions (Gunderson et al., 2016). However, all organisms live in a multi-stressor world, the effects of which are worsened by global climate change (Byrne & Przeslawski, 2013). For example, mussels (*Mytilus* spp.) and Pacific rock oysters *(Magallana gigas),* the primary produce of the UK molluscan shellfish industry (95% and 4% tonnage, respectively; 82% and 15% of attributed value), dwell in the coastal zone where environmental variables that affect biological and ecological responses to climate change include temperature, wave energy, upwelling events, and freshwater inputs. These factors act and interact at various spatial and temporal scales and affect the physiology, growth, survival, and reproduction of bivalves (Ellis et al., 2017; Hewitt et al., 2016).

2.4.2. Climate Change Research and Bivalves in Low and High-Income Nations

A notable concentration of research on the impacts of multiple stressors on bivalves is observed in high-income countries, particularly the United States, Portugal, and Italy (Almeida et al., 2018; Britto et al., 2019; Cherkasov et al., 2006; Coppola et al., 2018; Costa et al., 2020; Dickinson et al., 2013; Freitas et al., 2019; Gobler et al., 2014; Gobler et al., 2017; Goetze et al., 2014; Goetze et al., 2020; Griffith & Gobler, 2017; Hiebenthal et al., 2013; Ivanina et al., 2008; Ivanina et al., 2013; Ivanina et al., 2016; Ivanina et al., 2016; Lesser et al., 2019; Magalhaes et al., 2018; Matoo et al., 2013; Matozzo et al., 2012; Matozzo et al., 2013; Matozzo et al., 2013; Maulvault et al., 2018; Moreira et al., 2018; C. Munari, 2011; Munari et al., 2019; Range et al., 2014; Serra-Compte et al., 2018; Speights et al., 2017; Stevens & Gobler, 2018; Talmage & Gobler, 2011; Thorp et al., 1998; Turner et al., 2016, 2019; Velez et al., 2016). Additionally, the analysis revealed a significant and positive correlation between a country's GDP and its research output concerning bivalves. Each incremental increase in GDP was associated with a modest yet statistically significant uptick in research output, with a coefficient estimate of 5.298e-08 ($p = 0.039$). These findings provide empirical evidence that as a nation's economy expands, there is a concurrent increase in research productivity in bivalve studies.

However, it is crucial to recognise that various interconnected factors, such as such as the availability of research funding, institutional capacities, and the ecological importance of a region may influence these observations. These factors should be considered when interpreting the geographical distribution of research efforts and its correlation with GDP.

One significant consideration is that countries with higher GDPs typically allocate more excellent resources to scientific research, encompassing studies related to environmental changes. As a result, this financial backing could contribute to a higher number of studies concentrating on the effects of climate change, with a particular emphasis on bivalves due to their ecological and economic importance.

This result suggests that constrained by budgetary limitations, low-income countries may allocate fewer resources to scientific research (Acharya & Pathak, 2019). Consequently, there could be an information gap concerning native bivalve species in low-income nations, particularly those that have not experienced invasive status or extensive cultivation, in contrast to species such as *Magallana gigas*, which have become invasive (Lemasson et al., 2018). These non-invasive native species face complex challenges, notably including the impacts of climate change.

An illustrative example is the recent identification of an endemic oyster species known as "tikod amo" in the Philippines, belonging to the genus *Spondylus* (dela Cruz, 2016). To the best of my knowledge, no published studies explicitly addressing tikod amo have been identified at this time. Despite its limited international

recognition, tikod amo has gained local and international popularity due to its unique and appealing taste. This increased demand has transformed tikod amo into a sought-after seafood delicacy and a significant source of income for marginalised local fishermen (dela Cruz, 2016). However, this newfound popularity has brought about a set of challenges. The natural stocks of tikod amo in the wild are now under severe threat due to overharvesting, exacerbated by unsustainable practices such as collecting juvenile oysters (spat) from their natural habitats (dela Cruz, 2016). The significant decline in oyster catches between 2006 and 2008, ranging from 40 to 60 per cent, starkly illustrates the pressing need for sustainable management and conservation efforts, which should be backed up by research on the ecological requirements of this species.

It is important to emphasise that this thesis chapter did not specifically address research on native or endemic bivalve species. However, in the above discussion, it is presumed that low-income countries, constrained by limited financial resources, may allocate comparatively fewer resources to comprehensive studies on their native non-invasive bivalve species.

Global population projections indicate significant growth, with estimates reaching 9.7 billion by 2050 and potentially 11 billion by 2100 (UN, 2015). This population surge, combined with an increasing demand for high-quality food, necessitates a 50% increase in food production. However, urbanisation, soil degradation, water scarcity, and climate change impact arable land availability.

Remarkably, a significant portion of the global population resides in coastal areas, highlighting the potential of marine ecosystems to meet food production needs. Unfortunately, many low-income nations lack the financial resources and essential infrastructure, including research facilities, to study their local non-invasive bivalve species.

This underscores the need for increased investment in research on the effects of climate change on native, non-invasive bivalve species in low-income nations. Such research is crucial to understanding and mitigating climate change's environmental and economic consequences on these vital species and the communities that depend on them. Moreover, this situation presents an opportunity for developed countries to support research on local bivalve species in developing nations while potentially acquiring new food sources for their citizens.

2.4.3. Investigating understudied ecologically important bivalve species

While it is good that the number of studies increased from when it was first recorded in 1998, 67% of these studies focused only on five bivalve species, namely *C. virginica, M. galloprovincialis, M. mercenaria*, *M. edulis,* and *R. philipinarum*. All five species are reported to be among the UK's top valued and produced shellfish in the most recent Aquaculture Statistics for the UK of the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) (Ellis et al., 2015). *M. galloprovincialis*

stands out as the dominant farmed species in the top three aquaculture-producing nations, namely South Africa, Spain and Italy. In 2017, the total value of *M. galloprovincialis* cultivation in these regions reached an impressive £254,834.25 (see Table 1.1). It can be deduced that these species are well-studied because of their market value in developed countries. Recent studies on cultured bivalve species show that some of the non-market values (ecosystem services) of shellfish are theoretically worth at least 50% in addition to the global production value. These studies acknowledge that actual non-market values are possibly much higher but not easily assessed (Carss et al., 2020; Clements & Comeau, 2019; Coen et al., 2007; Gentry et al., 2020; Grabowski & Peterson, 2007; Olivier et al., 2020).

Another possible explanation for the interest in the five bivalve species mentioned is that these species are found, cultured, and widely consumed in countries where multiple stressor studies are done (Eurofish, 2016; FAO, 2018; GAA, 2016). *C*. virginica is found in the Atlantic Ocean and the Eastern Pacific. *M. galloprovincialis* is distributed in the Northeast Atlantic, the Mediterranean, and the Black Sea and it has been introduced in the Arctic, Indian, and Pacific Oceans. *M. mercenaria* is found in the Atlantic and the Mediterranean. *M. edulis* occurs in the Atlantic Ocean and the southwest Pacific. Finally, *R. philipinarum* is distributed in the Indo-Pacific and the Mediterranean and introduced in the Northeast Atlantic (FishBase, 2020).

There is a need to study other bivalve species that play essential functions in the ecosystem and have the potential to provide economic products and benefits in the

future. For example, only two studies were recorded on the giant clam *Tridacna squamosa,* which plays critical ecological roles in the coral reef ecosystems. The tissues of the giant clam are food for various predators and scavengers. The live zooxanthellae that the clams release into the water, faeces, and gametes are food to opportunistic feeders. Their shells provide a substrate for epibionts, and their mantle cavities shelter commensal and ectoparasitic organisms. The giant clams increase the topographic heterogeneity of the reef, function as a reservoir for zooxanthellae (*Symbiodinium* spp.) and reduce eutrophication through water filtering. Dense populations of giant clams produce large quantities of calcium carbonate shell material that are eventually incorporated into the reef framework (Neo et al., 2015)

Another example of an under-studied (two publications among the 68 studies gathered) but ecologically important bivalve species is the wild-harvested common cockle *Cerastoderma edule.* Cockles provide supporting services to the ecosystem by filtering water, modifying and creating habitat, biogeochemical cycling, and supporting biodiversity. Cockles provide meat and shell products with a potential value of £10.1 million. They also remove nitrogen, phosphorus, and carbon from the marine and estuarine environment. They have a strong cultural influence in the countries along the Atlantic coast and can bridge ecosystem function and cultural values in coastal areas (Carss et al., 2020).

Ostrea edulis and *Magallana gigas*, the two oyster species focused on in this thesis, only had three and five publications each, respectively. *O. edulis* and *M. gigas* are both commercially exploited in Europe. Both species form habitats that provide

associated ecosystem services, including the reef formation, cycling and water purification, and food provision (Gilson, 2021). *M. gigas* particularly have positive traits favourable for aquaculture and as a reliable food source. They grow fast, have high reproductive performance, highly adaptive physiology, and low disease susceptibility (Stagličić, 2020).

2.4.4. Investigating Understudied Bivalve Life Stages

Studies gathered in this review have predominantly focused on adult stages, potentially due to their prominence in human consumption and the ease of laboratory maintenance and observation. However, it is essential to recognise that the early life stages of bivalves play a critical role in their overall population dynamics.

Bivalves exhibit heightened sensitivity during fertilisation and the pelagic larval phase compared to their later life stages. Their soft larval shells offer limited protection (Verween et al., 2007). This early phase is pivotal because any setbacks in growth or survival can have profound repercussions on the future adult population (Talmage & Gobler, 2011). Despite this significance, research efforts have disproportionately favoured the effects of climate change and multiple stressors on adult bivalves, with relatively few studies dedicated to embryos and larval bivalves.

Many of these multiple stressors, such as temperature and salinity, are crucial determinants of bivalve settlement, morphogenesis, survival, activity, and

distribution. Optimal growth and normal physiological development of organisms typically occur within a specific temperature range, narrower than their tolerance range. Larval bivalves exhibit maximum growth and survival rates under ideal temperature conditions, making them particularly sensitive to even slight temperature increases beyond this threshold. Bivalve gametogenesis and spawning are also temperature-dependent processes (Hassan, 2018; Talmage & Gobler, 2011). Salinity changes also exert significant effects, with each developmental stage demonstrating distinctive phenotypic plasticity, distinct from other stages (Peteiro et al., 2018). Furthermore, environmental tolerance may differ between gametes and adult bivalves (Verween et al., 2007).

Understanding the early life stages of bivalves has profound implications for bivalves' aquaculture and global food production. Aquaculturists can enhance their ability to cultivate bivalves efficiently and sustainably by focusing on the less-studied early life stages. Effective managing bivalve populations, from larvae to adults, is critical for ensuring stable production levels. In-depth knowledge of how environmental factors impact early life stages is instrumental in optimising hatchery conditions and maximising larval survival rates.

2.4.5. Challenges and Implications of Multiple Stressor Studies in Bivalve Research

While exploring multiple stressors in bivalve research, this chapter has brought to light several significant challenges and implications. Notably, certain stressors, such as harmful algal blooms (HAB), have garnered limited attention within the reviewed literature, with only two studies addressing their impact on aquatic organisms (Turner et al., 2016, 2019). While the effects of climate change on HAB growth have been well-researched, the combined impacts of climate-change stressors and HAB on aquatic organisms remain inadequately understood (Griffith & Gobler, 2017). As discussed in Section 1.1.3 and further elaborated upon in Section 1.2 of this thesis, HAB can act as an indirect climate change stressor alongside factors like elevated temperature.

The consequences of HAB events can significantly affect aquaculture operations, contaminating bivalve species cultivated in affected areas. This contamination poses a unique threat as many HAB species produce toxins that can accumulate in bivalve tissues, potentially causing harm to consumers, including poisoning and even death (Griffith & Gobler, 2017).

Moreover, the contamination of bivalve aquaculture products carries severe economic repercussions. Contamination incidents may necessitate production

closures, resulting in substantial losses for aquaculture companies, disruption of supply chains, damage to market reputation, and financial setbacks for the industry (Fernandes-Salvador et al., 2021).

Among the publications gathered, two studies investigated fluctuating stressors, while the majority subjected bivalves to a constant stress level throughout their experiments. Gobler et al. (2017) examined diurnal fluctuations in pH and dissolved oxygen (DO) on bay scallops and hard clams, while Khan et al. (2018) focused on the effects of hypoxia and rising sea temperatures on oyster microbiomes.

A common challenge in multi-stressor experiments is the need for ecologically realistic approaches. While researchers should consider the timescales and synchronicity of stressor fluctuations, implementing these aspects in laboratory experiments can be cost-prohibitive and technically demanding. This poses a significant hurdle for researchers aiming to recreate realistic environmental conditions.

Nonetheless, it's worth noting that not all stressors require such fluctuations. For instance, ocean acidification, driven by increased atmospheric carbon dioxide (CO_2) levels, does not necessarily fluctuate in short-term laboratory experiments. Researchers often maintain a consistent and elevated CO_2 concentration in experiments to reflect the long-term trend of ocean acidification associated with climate change. This approach aligns with the ecological relevance of the stressor despite the lack of short-term fluctuations during the study.

2.4.6. *Exploring the effects of climate change on reproductive traits and behavioural changes*

The responses observed in the gathered studies hold significant relevance to bivalve aquaculture. Most of these studies have primarily focused on assessing survival and biochemical responses, crucial indicators of the overall health and resilience of bivalves in aquaculture systems. However, it is worth noting that a comprehensive understanding of bivalve aquaculture's sustainability and long-term success requires a more nuanced examination of reproductive traits such as fertilisation and gonadal development.

Of the 69 publications gathered, only one delved into assessing these vital reproductive responses. This gap in research is noteworthy because environmental factors substantially impact the reproductive capabilities and fitness of bivalve populations. Successful reproduction contributes to population growth, reduces the risk of extinction, and enhances the adaptive potential, divergence, and speciation of distinct populations.

Conversely, failure in reproduction can lead to low population growth, resulting in diminished population sizes and increased vulnerability to extinction. Therefore, it is imperative to redirect attention towards investigating the effects of climate change and multiple stressors on bivalves' reproductive traits and potential. Such research endeavours can provide invaluable insights into the short and long-term

consequences of climate change on bivalve aquaculture, ultimately guiding strategies for the sustainable management and conservation of these critical species (Grazer & Martin, 2012).

Furthermore, it is crucial to expand our investigations into behavioural responses within the context of bivalve aquaculture. Surprisingly, only two studies have delved into this aspect, focusing on burrowing activity. Behavioural responses encompass a wide array of actions, including predator avoidance, competition, and feeding, all of which play pivotal roles in shaping population and community interactions within bivalve aquaculture systems.

These behavioural traits also hold a special significance as they contribute to the functions of bivalves as ecosystem engineers. Bivalves can remarkably modify their surroundings, thereby shaping the abiotic environment and exerting influence over the function and dynamics of their habitats. Understanding how bivalves behave in response to various environmental conditions is not only scientifically intriguing but also practically indispensable in bivalve aquaculture.

In this regard, it is imperative to gain insights into the behavioural responses of these species and their feedback processes in the face of changing environmental conditions. Such knowledge provides a foundational understanding of the mechanisms that bolster or diminish their resilience to the consequences of climate change. This holistic approach to studying bivalve behaviour and its interplay with

the environment is essential to advancing our understanding and, consequently, the sustainability of bivalve aquaculture (Van Colen et al., 2020).

2.5. Conclusion

As discussed in this review, the exploration of multiple climate change stressor effects on bivalves underscores several critical themes that warrant further attention and consideration in the scientific community.

The increasing recognition of the appropriateness of multiple stressor experiments is a significant milestone in climate change research. Bivalves, vital components of aquatic ecosystems, interact with many stressors, which necessitates comprehensive investigations to understand their responses to a changing environment. These studies provide essential insights into the physiology, growth, survival, and reproduction of bivalves, helping us anticipate and mitigate the impacts of climate change on these ecologically and economically important organisms.

An observable concentration of research efforts in high-income countries, correlated with GDP, highlights the influence of financial resources on scientific investigations. However, this geographic bias potentially leaves crucial knowledge gaps in low-income nations, particularly concerning native non-invasive bivalve species. Addressing this disparity in research allocation is essential for ensuring the resilience

of these species and the communities that depend on them in the face of climate change.

While *C. virginica, M. galloprovincialis, M. mercenaria*, *M. edulis,* and *R. philipinarum* garner substantial research attention potentially due to their economic value, it is imperative to extend our focus to less-studied species that play pivotal roles in ecosystems and possess untapped economic potential. Species like the giant clam, common cockle, European native oysters, and Pacific rock oysters offer valuable ecosystem services, cultural significance, and economic benefits, making them deserving subjects of future research efforts.

More than half of the studies gathered in this review focused on adult bivalves. Acknowledging the vulnerability of early life stages in bivalves is essential for a comprehensive understanding of population dynamics and sustainable aquaculture practices. These stages are sensitive to environmental fluctuations, and their survival and growth significantly impact adult populations. More emphasis on studying embryos and larval bivalves is vital for optimising hatchery conditions and enhancing bivalve aquaculture sustainability.

Elevated temperature, acidification, and the presence of heavy metals were the most studied stressors among the publications gathered in this review. The challenges of certain stressors, such as harmful algal blooms, call for more research attention. Understanding the combined impacts of climate-change stressors and HABs on aquatic organisms is critical for ecological reasons and safeguarding the economic

viability of bivalve aquaculture. Research on the combined impacts of climate-change stressors and HABs informs aquaculture management practices and helps protect consumers from potential harm.

Research on bivalves should extend beyond survival and biochemical responses to encompass reproductive traits and behavioural changes. A comprehensive understanding of these aspects is vital for supporting ecosystem resilience and ensuring the continued provision of ecosystem services. These aspects are fundamental to population dynamics, ecosystem interactions, and the overall resilience of bivalves in the face of environmental change. Investigating these facets can provide invaluable insights into climate change's short- and long-term consequences on bivalve aquaculture.

Recognising the far-reaching implications of bivalve research, it is imperative to engage various stakeholders, including scientists, policymakers, aquaculture industry leaders, and local communities, in the dialogue surrounding climate change effects on bivalves. Collaborative efforts that transcend geographical boundaries and incorporate diverse perspectives are essential for developing holistic strategies to address the challenges identified in this review.

In this era of climate change, the future of bivalves, their ecosystems, and the communities reliant upon them hinges on our collective commitment to research, collaboration, and sustainable management. By engaging stakeholders, promoting equitable resource allocation, and fostering a holistic understanding of bivalve

biology, we can navigate the challenges of a changing world while preserving the invaluable contributions of these remarkable organisms.

2.6. References

Acharya, K. P., & Pathak, S. (2019). Applied Research in Low-Income Countries: Why and How? Frontiers in Research Metrics and Analytics, 4, 3. https://doi.org/10.3389/frma.2019.00003

Almeida, A., Freitas, R., Calisto, V., Esteves, V. I., Schneider, R. J., Soares, A. M. V. M., Figueira, E., Campos, B., & Barata, C. (2018). Effects of carbamazepine and cetirizine under an ocean acidification scenario on the biochemical and transcriptome responses of the clam *Ruditapes philippinarum*. Environmental Pollution, 235, 857–868. https://doi.org/10.1016/j.envpol.2017.12.121

Britto, R. S., Nascimento, J. P., Serode, T., Santos, A. P., Soares, A. M. V. M., Figueira, E., Furtado, C., Lima-Ventura, J., Monserrat, J. M., & Freitas, R. (2019). The effects of co-exposure of graphene oxide and copper under different pH conditions in Manila clam *Ruditapes philippinarum*. Environmental Science and Pollution Research. https://doi.org/10.1007/s11356-019-06643-4

Byrne, M., & Przeslawski, R. (2013). Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. Integrative and Comparative Biology, 53(4), 582–596. https://doi.org/10.1093/icb/ict049

Carss, D. N., Brito, A. C., Chainho, P., Ciutat, A., de Montaudouin, X., Fernández Otero, R. M., Filgueira, M. I., Garbutt, A., Goedknegt, M. A., Lynch, S. A., Mahony, K. E., Maire, O., Malham, S. K., Orvain, F., van der Schatte Olivier, A.,

& Jones, L. (2020). Ecosystem services provided by a non-cultured shellfish species: The common cockle *Cerastoderma edule*. Marine Environmental Research, 158, 104931. https://doi.org/10.1016/j.marenvres.2020.104931

Cherkasov, A. S., Ringwood, A. H., & Sokolova, I. M. (2006). Combined effects of temperature acclimation and cadmium exposure on mitochondrial function in eastern oysters *Crassostrea virginica* gmelin (Bivalvia: Ostreidae). Environmental Toxicology and Chemistry, 25(9), 2461–2469. https://doi.org/10.1897/05-584R.1

Clements, J. C., & Comeau, L. A. (2019). Nitrogen removal potential of shellfish aquaculture harvests in eastern Canada: A comparison of culture methods. Aquaculture Reports, 13, 100183. https://doi.org/10.1016/j.aqrep.2019.100183

Coen, L. D., Brumbaugh, R. D., Bushek, D., Grizzle, R., Luckenbach, M. W., Posey, M. H., Powers, S. P., & Tolley, S. G. (2007). Ecosystem services related to oyster restoration. Marine Ecology Progress Series, 341, 303–307. https://doi.org/10.3354/meps341303

Coppola, F., Almeida, A., Henriques, B., Soares, A. M. V. M., Figueira, E., Pereira, E., & Freitas, R. (2018). Biochemical responses and accumulation patterns of *Mytilus galloprovincialis* exposed to thermal stress and Arsenic contamination. Ecotoxicology and Environmental Safety, 147, 954–962. https://doi.org/10.1016/j.ecoenv.2017.09.051

Costa, S., Coppola, F., Pretti, C., Intorre, L., Meucci, V., Soares, A. M. V. M., Freitas, R., & Sole, M. (2020). The influence of climate change related factors on the response of two clam species to diclofenac. Ecotoxicology and Environmental Safety, 189, 109899. https://doi.org/10.1016/j.ecoenv.2019.109899

Dickinson, G. H., Matoo, O. B., Tourek, R. T., Sokolova, I. M., & Beniash, E. (2013). Environmental salinity modulates the effects of elevated CO2 levels on juvenile hard-shell clams, *Mercenaria mercenaria*. Journal of Experimental Biology, 216(14), 2607–2618. https://doi.org/10.1242/jeb.082909

Dunn, P. K., & Smyth, G. K. (2018). Generalized linear models with examples in R. New York: Springer.tential economic factors influencing research output in this domain.

Ellis, J. I., Clark, D., Atalah, J., Jiang, W., Taiapa, C., Patterson, M., Sinner, J., & Hewitt, J. (2017). Multi-stressor effects on marine infauna: Responses of estuarine taxa and functional traits to sedimentation, nutrient and metal loading. Scientific Reports, 7(1), 12013. https://doi.org/10.1038/s41598-017-12323-5

Ellis, T., Gardener, R., Gubbins, M., Reese, A., & Smith, D. (2015). Aquaculture statistics for the UK, with a focus on England and Wales 2012. Centre for Environment Fisheries and Aquaculture.

Eurofish (2016). Italy—Eurofish.dk. Overview of the Italian Fisheries and Aquaculture Sector. Retrieved August 11, 2020, http://eurofish.dk/italy

Fernandes-Salvador, J. A., Davidson, K., Sourisseau, M., Revilla, M., Schmidt, W., Clarke, D., Miller, P. I., Arce, P., Fernández, R., Maman, L., Silva, A., Whyte, C., Mateo, M., Neira, P., Mateus, M., Ruiz-Villarreal, M., Ferrer, L., & Silke, J. (2021). Current Status of Forecasting Toxic Harmful Algae for the North-East Atlantic Shellfish Aquaculture Industry. Frontiers in Marine Science, 8. https://www.frontiersin.org/article/10.3389/fmars.2021.666583

FAO (2018). SOFIA 2018—State of Fisheries and Aquaculture in the world 2018. Retrieved August 11, 2020, from http://www.fao.org/state-of-fisheries-aquaculture

FishBase (2020). SeaLifeBase. Retrieved January 11, 2023,https://www.sealifebase.ca/

Freitas, R., Coppola, F., Costa, S., Pretti, C., Intorre, L., Meucci, V., Soares, A. M. V. M., & Solé, M. (2019). The influence of temperature on the effects induced by Triclosan and Diclofenac in mussels. Science of The Total Environment, 663, 992–999. https://doi.org/10.1016/j.scitotenv.2019.01.189

GAA (2016). Mollusc culture in Portugal « Global Aquaculture Advocate. Global Aquaculture Alliance. Retrieved August 11, 2020, https://www.aquaculturealliance.org/advocate/mollusc-culture-portugal/

Gentry, R. R., Alleway, H. K., Bishop, M. J., Gillies, C. L., Waters, T., & Jones, R. (2020). Exploring the potential for marine aquaculture to contribute to ecosystem services. Reviews in Aquaculture, 12(2), 499–512. https://doi.org/10.1111/raq.12328

Gilson, A. R., Coughlan, N. E., Dick, J. T. A., & Kregting, L. (2021). Marine heat waves differentially affect functioning of native (*Ostrea edulis*) and invasive (*Crassostrea* [*Magallana*] *gigas*) oysters in tidal pools. Marine Environmental Research, 172, 105497. https://doi.org/10.1016/j.marenvres.2021.105497

Gobler, C. J., Clark, H. R., Griffith, A. W., & Lusty, M. W. (2017). Diurnal Fluctuations in Acidification and Hypoxia Reduce Growth and Survival of Larval and Juvenile Bay Scallops (*Argopecten irradians*) and Hard Clams (*Mercenaria mercenaria*). Frontiers in Marine Science, 3, UNSP 282. https://doi.org/10.3389/fmars.2016.00282

Gobler, C. J., DePasquale, E. L., Griffith, A. W., & Baumann, H. (2014). Hypoxia and Acidification Have Additive and Synergistic Negative Effects on the Growth,

Survival, and Metamorphosis of Early Life Stage Bivalves. PLoS ONE, 9(1). https://doi.org/10.1371/journal.pone.0083648

Goetze, S., Bock, C., Eymann, C., Lannig, G., Steffen, J. B. M., & Poertner, H.-O. (2020). Single and combined effects of the “Deadly trio” hypoxia, hypercapnia and warming on the cellular metabolism of the great scallop *Pecten maximus*. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, 243, 110438. https://doi.org/10.1016/j.cbpb.2020.110438

Grabowski, J. H., & Peterson, C. H. (2007). 15—Restoring Oyster Reefs to Recover Ecosystem Services. In K. Cuddington, J. E. Byers, W. G. Wilson, & A. Hastings (Eds.), Theoretical Ecology Series (Vol. 4, pp. 281–298). Academic Press. https://doi.org/10.1016/S1875-306X(07)80017-7

Grazer, V. M., & Martin, O. Y. (2012). Investigating Climate Change and Reproduction: Experimental Tools from Evolutionary Biology. Biology, 1(2), 411–438. https://doi.org/10.3390/biology1020411

Griffith, A. W., & Gobler, C. J. (2017). Transgenerational exposure of North Atlantic bivalves to ocean acidification renders offspring more vulnerable to low pH and additional stressors. Scientific Reports, 7, 11394. https://doi.org/10.1038/s41598-017-11442-3

Gunderson, A. R., Armstrong, E. J., & Stillman, J. H. (2016). Multiple Stressors in a Changing World: The Need for an Improved Perspective on Physiological Responses to the Dynamic Marine Environment. Annual Review of Marine Science, 8(1), 357–378. https://doi.org/10.1146/annurev-marine-122414-033953

Harborne, A. R., Rogers, A., Bozec, Y.-M., & Mumby, P. J. (2017). Multi-stressors and the Functioning of Coral Reefs. Annual Review of Marine Science, 9(1), 445–468. https://doi.org/10.1146/annurev-marine-010816-060551

Hassan, M. M., Qin, J. G., & Li, X. (2018). Gametogenesis, sex ratio and energy metabolism in *Ostrea angasi*: Implications for the reproductive strategy of spermcasting marine bivalves. Journal of Molluscan Studies, 84(1), 38–45. https://doi.org/10.1093/mollus/eyx041

Heugens, E. H. W., Hendriks, A. J., Dekker, T., Straalen, N. M. van, & Admiraal, W. (2001). A Review of the Effects of Multi-stressors on Aquatic Organisms and Analysis of Uncertainty Factors for Use in Risk Assessment. Critical Reviews in Toxicology, 31(3), 247–284. https://doi.org/10.1080/20014091111695

Hewitt, J. E., Ellis, J. I., & Thrush, S. F. (2016). Multi-stressors, nonlinear effects and the implications of climate change impacts on marine coastal ecosystems. Global Change Biology, 22(8), 2665–2675. https://doi.org/10.1111/gcb.13176

Hiebenthal, C., Philipp, E. E. R., Eisenhauer, A., & Wahl, M. (2013). Effects of seawater pCO_2 and temperature on shell growth, shell stability, condition and cellular stress of Western Baltic Sea *Mytilus edulis* (L.) and *Arctica islandica* (L.). Marine Biology, 160(8), 2073–2087. https://doi.org/10.1007/s00227-012-2080-9

IPCC (2007). Summary for Policymakers. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)].

IPCC (2021). Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)].

Ivanina, A. V., Dickinson, G. H., Matoo, O. B., Bagwe, R., Dickinson, A., Beniash, E., & Sokolova, I. M. (2013). Interactive effects of elevated temperature and CO2 levels on energy metabolism and biomineralization of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology, 166(1), 101–111. https://doi.org/10.1016/j.cbpa.2013.05.016

Ivanina, A. V., Habinck, E., & Sokolova, I. M. (2008). Differential sensitivity to cadmium of key mitochondrial enzymes in the eastern oyster, *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). Comparative Biochemistry and Physiology C-Toxicology & Pharmacology, 148(1), 72–79. https://doi.org/10.1016/j.cbpc.2008.03.009

Ivanina, A. V., Hawkins, C., & Sokolova, I. M. (2016). Interactive effects of copper exposure and environmental hypercapnia on immune functions of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. Fish & Shellfish Immunology, 49, 54–65. https://doi.org/10.1016/j.fsi.2015.12.011

Khan, B., Clinton, S. M., Hamp, T. J., Oliver, J. D., & Ringwood, A. H. (2018). Potential impacts of hypoxia and a warming ocean on oyster microbiomes. Marine Environmental Research, 139, 27–34. https://doi.org/10.1016/j.marenvres.2018.04.018

Lemasson, A., Hall-Spencer, J., Fletcher, S., Provstgaard-Morys, S., & Knights, A. M. (2018). Indications of future performance of native and non-native adult oysters under acidification and warming. https://doi.org/10.1016/j.marenvres.2018.10.003

Lesser, M. P., Thompson, M. M., & Walker, C. W. (2019). Effects of Thermal Stress and Ocean Acidification on the Expression of the Retrotransposon Steamer in

the Softshell *Mya Arenaria*. Journal of Shellfish Research, 38(3), 535–541. https://doi.org/10.2983/035.038.0304

Magalhaes, L., de Montaudouin, X., Figueira, E., & Freitas, R. (2018). Trematode infection modulates cockles biochemical response to climate change. Science of the Total Environment, 637, 30–40. https://doi.org/10.1016/j.scitotenv.2018.04.432

Mantay, K. (2013). Eastern oysters use sound to help them find the right place to settle down, a new study suggests. (Photo). Retrieved August 11, 2020, from https://www.sciencenews.org/blog/scicurious/reefs-are-alive-sound-oysters

Matoo, O. B., Ivanina, A. V., Ullstad, C., Beniash, E., & Sokolova, I. M. (2013). Interactive effects of elevated temperature and CO2 levels on metabolism and oxidative stress in two common marine bivalves (*Crassostrea virginica* and *Mercenaria mercenaria*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 164(4), 545–553. https://doi.org/10.1016/j.cbpa.2012.12.025

Matozzo, V., Chinellato, A., Munari, M., Bressan, M., & Marin, M. G. (2013). Can the combination of decreased pH and increased temperature values induce oxidative stress in the clam *Chamelea gallina* and the mussel *Mytilus galloprovincialis*? Marine Pollution Bulletin, 72(1), 34–40. https://doi.org/10.1016/j.marpolbul.2013.05.004

Matozzo, V., Chinellato, A., Munari, M., Finos, L., Bressan, M., & Marin, M. G. (2012). First Evidence of Immunomodulation in Bivalves under Seawater Acidification and Increased Temperature. PLoS ONE, 7(3). https://doi.org/10.1371/journal.pone.0033820

Maulvault, A. L., Camacho, C., Barbosa, V., Alves, R., Anacleto, P., Fogaca, F., Kwadijk, C., Kotterman, M., Cunha, S. C., Fernandes, J. O., Rasmussen, R. R., Sloth, J. J., Aznar-Alemany, O., Eljarrat, E., Barcelo, D., & Marques, A. (2018). Assessing the effects of seawater temperature and pH on the bioaccumulation of emerging chemical contaminants in marine bivalves. Environmental Research, 161, 236–247. https://doi.org/10.1016/j.envres.2017.11.017

Moreira, A., Freitas, R., Figueira, E., Volpi Ghirardini, A., Soares, A. M. V. M., Radaelli, M., Guida, M., & Libralato, G. (2018). Combined effects of arsenic, salinity and temperature on Crassostrea gigas embryotoxicity. Ecotoxicology and Environmental Safety, 147, 251–259. https://doi.org/10.1016/j.ecoenv.2017.08.043

Munari, C. (2011). Effects of the 2003 European heatwave on the benthic community of a severe transitional ecosystem (Comacchio Saltworks, Italy). Marine Pollution Bulletin, 62(12), 2761–2770. https://doi.org/10.1016/j.marpolbul.2011.09.011

Munari, M., Matozzo, V., Chemello, G., Riedl, V., Pastore, P., Badocco, D., & Marin, M. G. (2019). Seawater acidification and emerging contaminants: A dangerous marriage for haemocytes of marine bivalves. Environmental Research, 175, 11–21. https://doi.org/10.1016/j.envres.2019.04.032

Neo, M. L., Eckman, W., Vicentuan, K., Teo, S. L.-M., & Todd, P. A. (2015). The ecological significance of giant clams in coral reef ecosystems. Biological Conservation, 181, 111–123. https://doi.org/10.1016/j.biocon.2014.11.004

Olivier, A. van der S., Jones, L., Vay, L. L., Christie, M., Wilson, J., & Malham, S. K. (2020). A global review of the ecosystem services provided by bivalve aquaculture. Reviews in Aquaculture, 12(1), 3–25. https://doi.org/10.1111/raq.12301

Parker, L. M., Ross, P. M., & O'Connor, W. A. (2010). Comparing the effect of elevated pCO2 and temperature on the fertilization and early development of two species of oysters. Marine Biology, 157(11), 2435–2452. https://doi.org/10.1007/s00227-010-1508-3

Peteiro, L. G., Woodin, S. A., Wethey, D. S., Costas-Costas, D., Martínez-Casal, A., Olabarria, C., & Vázquez, E. (2018). Responses to salinity stress in bivalves: Evidence of ontogenetic changes in energetic physiology on *Cerastoderma edule.* Scientific Reports, 8(1), 1–9. https://doi.org/10.1038/s41598-018-26706-9

Przeslawski, R., Byrne, M., & Mellin, C. (2015). A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. Global Change Biology, 21(6), 2122–2140. https://doi.org/10.1111/gcb.12833

R Core Team. (2021). R: A language and environment for statistical computing.

Range, P., Chícharo, M. A., Ben-Hamadou, R., Piló, D., Fernandez-Reiriz, M. J., Labarta, U., Marin, M. G., Bressan, M., Matozzo, V., Chinellato, A., Munari, M., El Menif, N. T., Dellali, M., & Chícharo, L. (2014). Impacts of CO_2-induced seawater acidification on coastal Mediterranean bivalves and interactions with other climatic stressors. Regional Environmental Change, 14(1), 19–30. https://doi.org/10.1007/s10113-013-0478-7

Schulte, P. M. (2014). What is environmental stress? Insights from fish living in a variable environment | Journal of Experimental Biology | The Company of Biologists. Journal of Experimental Biology, 217(1), 23–24. https://doi.org/10.1242/jeb.089722

Serra-Compte, A., Maulvault, A. L., Camacho, C., Alvarez-Munoz, D., Barcelo, D., Rodriguez-Mozaz, S., & Marques, A. (2018). Effects of water warming and acidification on bioconcentration, metabolization and depuration of

pharmaceuticals and endocrine disrupting compounds in marine mussels (*Mytilus galloprovincialis*). Environmental Pollution, 236, 824–834. https://doi.org/10.1016/j.envpol.2018.02.018

Smaal A. C, Ferreira J.G., Grant J., Petersen J. K, & Strand Ø. (2019). Goods and Services of Marine Bivalves (1st ed. 2019.). Springer International Publishing, NY, USA. https://doi.org/10.1007/978-3-319-96776-9

Speights, C. J., Silliman, B. R., & McCoy, M. W. (2017). The effects of elevated temperature and dissolved ρCO_2 on a marine foundation species. Ecology and Evolution, 7(11), 3808–3814. https://doi.org/10.1002/ece3.2969

Stagličić, N., Šegvić-Bubić, T., Ezgeta-Balić, D., Bojanić Varezić, D., Grubišić, L., Žuvić, L., Lin, Y., & Briski, E. (2020). Distribution patterns of two co-existing oyster species in the northern Adriatic Sea: The native European native oyster *Ostrea edulis* and the non-native Pacific oyster *Magallana gigas*. Ecological Indicators, 113, 106233. https://doi.org/10.1016/j.ecolind.2020.106233

Stevens, A. M., & Gobler, C. J. (2018). Interactive effects of acidification, hypoxia, and thermal stress on growth, respiration, and survival of four North Atlantic bivalves. Marine Ecology Progress Series, 604, 143–161. https://doi.org/10.3354/meps12725

Talmage, S. C., & Gobler, C. J. (2011). Effects of Elevated Temperature and Carbon Dioxide on the Growth and Survival of Larvae and Juveniles of Three Species of Northwest Atlantic Bivalves. PLoS ONE, 6(10). https://doi.org/10.1371/journal.pone.0026941

Thorp, J. H., Alexander, J. E., Bukaveckas, B. L., Cobbs, G. A., & Bresko, K. L. (1998). Responses of Ohio River and Lake Erie dreissenid molluscs to changes

in temperature and turbidity. Canadian Journal of Fisheries and Aquatic Sciences, 55(1), 220–229. https://doi.org/10.1139/cjfas-55-1-220

Todgham, A., & Stillman, J. (2013). Physiological Responses to Shifts in Multiple Environmental Stressors: Relevance in a Changing World. Integrative and Comparative Biology, 53. https://doi.org/10.1093/icb/ict086

Turner, L. M., Alsterberg, C., Turner, A. D., Girisha, S. K., Rai, A., Havenhand, J. N., Venugopal, M. N., Karunasagar, I., & Godhe, A. (2016). Pathogenic marine microbes influence the effects of climate change on a commercially important tropical bivalve. Scientific Reports, 6(1), Article 1. https://doi.org/10.1038/srep32413

Turner, L. M., Havenhand, J. N., Alsterberg, C., Turner, A. D., Girisha, S. K., Rai, A., Venugopal, M. N., Karunasagar, I., & Godhe, A. (2019). Toxic Algae Silence Physiological Responses to Multiple Climate Drivers in a Tropical Marine Food Chain. Frontiers in Physiology, 10, 373.

UN (2015). Population. Retrieved August 11, 2020, from https://www.un.org/en/sections/issues-depth/population/

Velez, C., Figueira, E., Soares, A. M. V. M., & Freitas, R. (2016). Combined effects of seawater acidification and salinity changes in *Ruditapes philippinarum*. Aquatic Toxicology, 176, 141–150. https://doi.org/10.1016/j.aquatox.2016.04.016

Verween, A., Vincx, M., & Degraer, S. (2007). The effect of temperature and salinity on the survival of *Mytilopsis leucophaeata* larvae (Mollusca, Bivalvia): The search for environmental limits. Journal of Experimental Marine Biology and Ecology, 348(1), 111–120. https://doi.org/10.1016/j.jembe.2007.04.011

Wijsman, J. W. M., Troost, K., Fang, J., & Roncarati, A. (2019). Global Production of Marine Bivalves. Trends and Challenges. In A. C. Smaal, J. G. Ferreira, J. Grant,

J. K. Petersen, & Ø. Strand (Eds.), Goods and Services of Marine Bivalves (pp. 7–26). Springer International Publishing. https://doi.org/10.1007/978-3-319-96776-9_2

Yuan, W. S., Walters, L. J., Brodsky, S. A., Schneider, K. R., & Hoffman, E. A. (2016). Synergistic Effects of Salinity and Temperature on the Survival of Two Nonnative Bivalve Molluscs, *Perna viridis (*Linnaeus 1758) and *Mytella charruana* (d'Orbigny 1846). Journal of Marine Biology, 2016, e9261309. https://doi.org/10.1155/2016/9261309

CHAPTER THREE

Thermal Requirements and Physiological Responses of European Native Oysters: Insights for Conservation and Management in a Changing Climate

Abstract

In recent years, heatwaves have raised significant concerns regarding their impact on oysters and other bivalve populations. These occurrences have been directly associated with mass mortalities, which pose formidable challenges to the growth, reproduction, and overall survival of bivalves. As a result, the bivalve aquaculture industry has endured substantial economic losses due to these events. Understanding the thermal requirements and limitations of species like *Ostrea edulis* is crucial for assessing their vulnerability to heatwaves and implementing effective management strategies. This study aimed to evaluate *O. edulis'* thermal performance and gain insights into its thermal niche. Two experiments were conducted. In Experiment 1, oysters were exposed to a short-term warming protocol from 9°C to 36°C in 3°C increments while measuring their metabolic response through their oxygen consumption rate (MO_2). In Experiment 2, experiments started from 18°C to 32°C with a 2°C increment, with both heart rate and MO_2 assessed. MO_2 consistently increased with temperature, ranging from 0 to 1.61 mL O2 h^{-1} g^{-1} ash-free dry weight. A significant rise in MO_2 occurred at 24°C, indicating a response to thermal stress. An average Arrhenius breakpoint temperature of 24.88 ± 1.25°C was idented. Above this threshold, cardiac arrests increased, indicating *O. edulis'* sensitivity to higher temperatures. Mortalities were recorded at 27°C and 33°C, with most oysters dying within 21 hours of exposure to 36°C. Elevated temperatures negatively affected oyster health, with lower condition indexes compared to control groups. Effective conservation and management strategies are crucial for protecting vulnerable *O. edulis* populations. Monitoring and regulating water temperatures in bivalve aquaculture settings play a vital role. The research findings will inform further

studies and strategies to mitigate heatwave impacts. Understanding the effects of temperature changes on growth, reproduction, and disease resistance is essential for predicting and addressing heatwave impacts, safeguarding *O. edulis* populations, and preserving their ecological significance in marine ecosystems and aquaculture.

3.1. Introduction

3.1.1. The importance of temperature in biological processes

Temperature is a critical environmental factor in regulating biological processes in all living organisms, from bacteria to humans. Temperature affects biological processes by influencing the rate of chemical reactions (Boscolo-Galazzo et al., 2018), the stability of biological structures (Somero, 1978), and the behaviour of biological molecules (Daniel & Danson, 2010; Russell, 1984). Temperature is important in biological processes, including enzyme activity, protein structure and function, membrane fluidity, cellular signalling, and metabolism.

One of the most fundamental effects of temperature on biological processes is its influence on enzyme activity. Enzymes are proteins that function as catalysts by lowering the activation energy required for a reaction. The action of enzymes is highly dependent on temperature, with most enzymes exhibiting an optimal

temperature range for activity. Outside of this range, the activity of enzymes decreases, and the rate of reaction slows down, eventually leading to enzyme denaturation and loss of activity. Metabolic reactions become impaired, leading to a decline in metabolic rate and, ultimately, death. In bivalves, for example, a particular enzyme called carbonic anhydrase (CA) plays a role in keeping their body's acid and base levels in balance and creating their protective shells. In a study, the activity of the CA enzyme in all parts of the *M. edulis'* body increases when exposed to a sudden increase in temperature between 5-35°C (Matoo et al., 2021). The activation energy (Ea) values were similar in all tissues, which suggests that the whole body of the mussel was responding to the warming (Matoo et al., 2021).

Temperature is also crucial in determining the shape and function of proteins, which perform various functions such as structural support, catalysis, and signalling in cells. Different temperatures can cause proteins to adopt different shapes and structures, impacting their function and leading to cellular process changes. For example, in the mollusc *Rapana thomasiana,* the protein hemocyanin, which transports and stores oxygen in the blood, begins to undergo structural changes at 50 °C, affecting its function. However, these changes are reversible up to 71.5 °C. At temperatures below 70 °C, there are no significant changes in the protein's structure or aggregation, but above this temperature, the protein starts to unfold and aggregate (Idakieva et al., 2012).

Another critical effect of temperature on biological processes is its influence on membrane fluidity. Cell membranes comprise a lipid bilayer that forms a barrier

between the cell and its environment. Membrane fluidity is essential for cellular processes such as membrane transport and signalling. Temperature affects membrane fluidity by altering the physical properties of the lipids that make up the membrane. Lipids become more fluid at high temperatures, leading to increased membrane permeability and altered cellular signalling (Pernet et al., 2007). Several intertidal organisms can regulate the fluidity of their membranes in response to changes in temperature, even when faced with daily temperature variations of 20-30 °C and more comprehensive seasonal temperature ranges. For example, *Mytilus californianus*, a type of mussel, is one such organism that shows seasonal changes in membrane fluidity through changes in the membrane's phospholipid head groups, fatty acid, and cholesterol composition, a process called homeoviscous adaptation (Pernet et al., 2007).

Finally, the impact of temperature on metabolism is one of the most basic and crucial effects of temperature on biological processes. Metabolism refers to the sum of all the chemical reactions that occur in an organism, and temperature plays a critical role in regulating the rate of these reactions. Metabolism affects the rate at which organisms use energy and nutrients to grow, reproduce, and survive. The rate of most chemical reactions increases with increasing temperature, following the Q10 rule, which states that for every 10 °C increase in temperature, the reaction rate doubles or triples (Ito et al., 2015).

As temperature directly affects many biological processes, from enzymatic reactions to population growth, it plays a critical role in determining the distribution and

abundance of a species. Thus, environmental temperature is a fitness-determining factor for many organisms (Killam & Clapham, 2018).

3.1.2. Heatwaves and bivalve aquaculture

Recent studies show that the effects of temperature on biological processes are becoming more pronounced due to heatwaves (Amorim et al., 2020; Dominguez et al., 2020; C. Munari, 2011; Peng et al., 2017). Atmospheric heatwaves are periods of unusual weather with thermal conditions above certain thresholds that persist for at least three consecutive days (Met Office, 2021). The proportion of the world's surface impacted by atmospheric heatwaves has risen, and several studies suggest that they are occurring more frequently and with greater intensity due to climate change (Herring et al., 2019; Meehl & Tebaldi, 2004; Schiermeier, 2018). Models project a surge in the chance of long and highly intense atmospheric heatwaves happening in the near future (Russo et al., 2014).

In recent years, there have been reports of mass mortality of oysters and other bivalves during the summer following atmospheric heatwaves (Colletta & Westfall, 2021; Hagenbuch, 2021; Timms, 2019; Troost, 2018). The harmful impacts of heatwaves on the growth, reproduction, and survival of bivalves can culminate in substantial economic detriments for the bivalve aquaculture industry.

One of the primary ways by which heat waves can impact bivalve aquaculture is by perturbing the physiological processes of bivalves. Atmospheric heatwaves increase seawater temperature through air-sea heat flux, which is the primary cause of marine heatwaves. Figure 3.1 displays subtidal water temperature recordings at Pyefleet Creek during the August 2020 heatwave in the UK. Pyefleet Creek is a production and harvest area for bivalves such as *Ostrea edulis, Crassostrea gigas, Cerastoderma edule, Mercenaria mercenaria,* and *Ruditapes philippinarum* (Cefas, 2013). The subtidal temperature peaked at 25 ± 0.5 °C. In Figure 3.2, temperature recordings during the July 2021 heatwave are shown for the high and low intertidal zones of Pyefleet Creek. The highest temperature recorded above ground in the high intertidal zone (Fig 3.2 B) was 38.95 °C, observed during low tide.

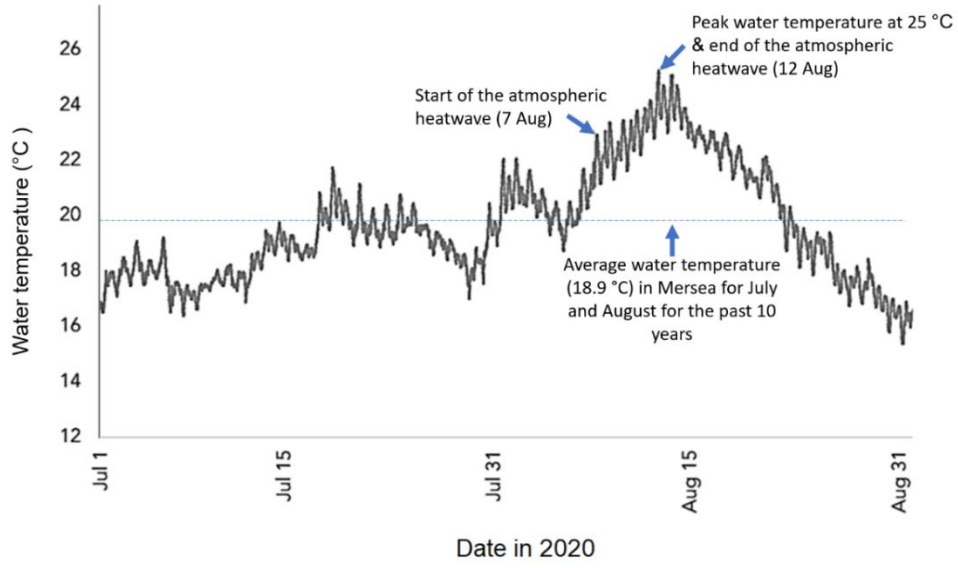


Figure 3.1 *Subtidal water temperature on 1 July to 31 August 2020 in Pyefleet Creek, Mersea Island, Essex, measured by temperature loggers attached to a floating raft. Arrows denote the start and end of an atmospheric heatwave in the UK, and the average water temperature of Mersea for July and August for the past 10 years (historical temperature taken from seatemperature.info).*

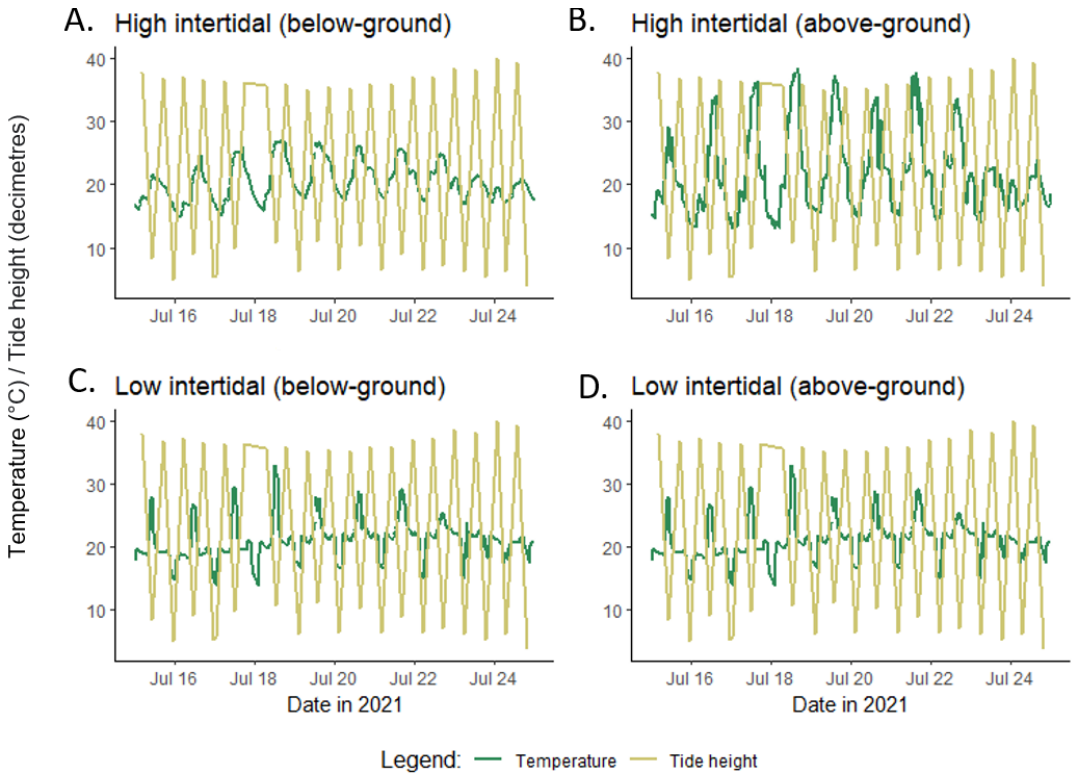


Figure 3.2 *Temperature recordings (every 10 min) (green line) and tide height (yellow line) at the high (**A and B**)and low intertidal zones (**C and D**) during an atmospheric heatwave on 15 to 25 July 2021 in Pyefleet Creek, Mersea Island, Essex. Above-ground recordings were taken from the surface of the mud, while below-ground recordings were made ~5 cm from the surface. (At some time during data collection, the low-tide below-ground logger surfaced from the mud and, hence, may incorrectly reflect the temperature below the ground). Tide height data were taken from the UK Hydrographic Office.*

Elevated water temperatures surpassing their optimal range can engender heat stress, stimulating reduced feeding and growth rates, augmented mortality, and heightened susceptibility to diseases and parasites, among other deleterious outcomes (Matozzo & Marin, 2011). Moreover, heat stress can upset the bivalves' reproductive cycle, producing lower spawning success and reduced larval survival rates (Beukema & Dekker, 2005, 2014; Philippart et al., 2003).

Another mode through which heatwaves can affect bivalve aquaculture is via perturbations in the water quality. Heightened water temperatures can reduce the dissolved oxygen levels in the water, rendering it challenging for bivalves to acquire adequate oxygen (Kennedy & Roberts, 1999; Stevens & Gobler, 2018). Further, heatwaves can spark harmful algal blooms, which can generate toxins that can harm bivalves and other aquatic organisms (Jöhnk et al., 2008). Consequently, heatwaves can provoke significant declines in the robustness and productivity of bivalve farms.

In addition to the direct impacts of heatwaves on bivalves, they can also have indirect consequences on the aquaculture industry. For instance, heatwaves can instigate disturbances in the supply chain, resulting in delays in the transportation of bivalves from farms to markets. Furthermore, if a significant number of bivalves perish due to heat stress or disease outbreaks, it can lead to financial losses for farmers and processors.

3.1.3. Ostrea edulis*: An important aquaculture species*

One of the most commercially valuable bivalve aquaculture species is the native oyster *Ostrea edulis* (Fig 3.3A). Native oysters have been cultivated for centuries, and their meat is considered a delicacy in many countries. In 2002, the production of farmed *O. edulis* was valued at £19.5 million, highlighting the continued significance of this sector in the limited regions where it is cultivated. Reefs of the European native oyster (Fig 3.3A) used to be a dominant structural and ecological component

along Europe's coasts in the 19th century and earlier, providing ecosystem services such as food to other animals, rigid substrate for attachment, protection and refuge for other invertebrates, and nursery ground for some fish species (Fig 3.3C). Native oysters have also bolstered the coastal economy for centuries. In 1864, 700 million native oysters were consumed in London, and approximately 120,000 employees were hired in Britain as dredgers (MacKenzie, 1997). In the southwest of France, shell piles from previous harvests contained one trillion shells per pile, emphasising the productivity of the native oysters and the scale of their harvest (MacKenzie, 1997).

At the end of the 19th century, however, the population of *O. edulis* began to decline, linked mainly to overexploitation. Data on *O. edulis* landings in England and Wales from 1887 to 1947 illustrates this (Fig 3.3B). Currently, native oysters are highly exhausted in the wild throughout the UK and Europe (Laing et al., 2006). *O*. *edulis* is on the list of threatened and declining species and habitats of The Oslo-Paris Commission (OSPAR; OSPAR, 2020).

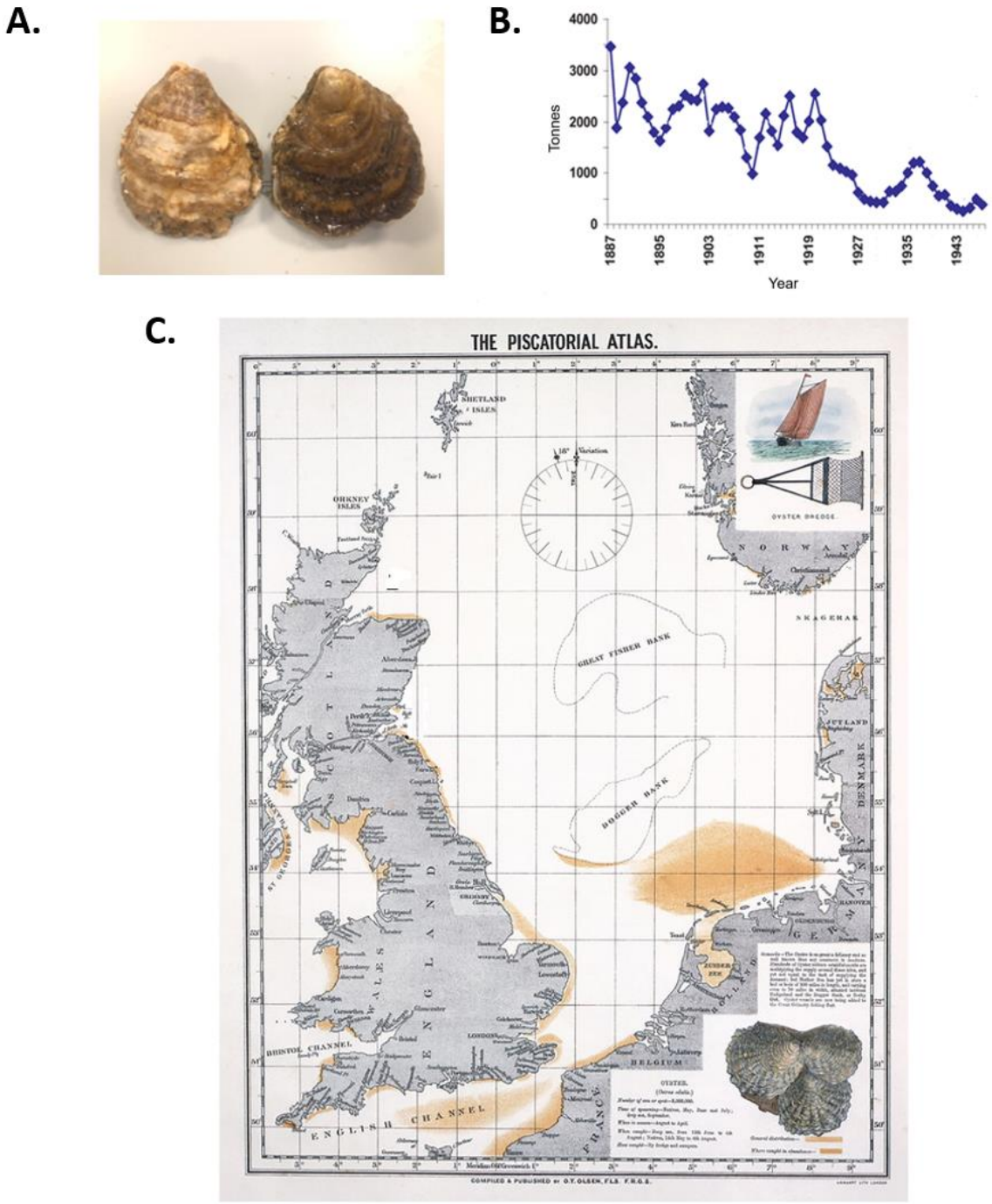


Figure 3.3 *Two adult* Ostrea edulis ***(A).*** Ostrea edulis *landings in England and Wales from 1887 to 1947* ***(B)*** *(Taken from from Laing, 2006).The Piscatorial Atlas of the North Sea entry for O. edulis (Olsen, 1883). Accounts of fishermen at that time showed abundant oyster beds (shown in orange)* (***C***). Image taken from Olsen (1883).

European marine protected areas now identify *O. edulis* beds as a priority marine habitat for protection (Fariñas-Franco et al., 2018). To coordinate the rapidly growing interest in restoring native oyster reefs, the UK and Europe established The Native Oyster Restoration Alliance (NORA) and The Native Oyster Network-UK and Ireland. Their goal is to increase awareness of the cultural and ecological value of native oysters and to encourage information-sharing between stakeholders from governments, restoration practitioners, and aquaculturists (Preston et al., 2020).

As temperature is the primary driver of climate change, it is essential to know the thermal niche of species we desire to conserve to understand the likely impacts of climate change and more frequent heatwaves on these resources. The thermal niche of organisms is often derived from studying their thermal tolerance (capacity to withstand short-term exposure to extreme temperatures) or thermal performance (examining fitness-related traits under different temperatures). Several traits, including metabolism, growth, and reproductive capacity, are used to determine thermal performance (MacLean et al., 2019). One of the traits that best represents thermal performance is the rate of metabolism, which is indirectly measured through the oxygen consumption rate (Cech & Brauner, 2011; Mueller & Diamond, 2001). The oxygen consumption rate (MO_2) is a reasonable approximation of the of metabolic rate since most organisms generate ATP aerobically using oxygen as a final electron acceptor (Clarke & Fraser, 2004).

Despite the well-documented decline in the *O. edulis* population along European coasts and the efforts to conserve their population (Fariñas-Franco et al., 2018), a

limited number of studies investigated their thermal performance and tolerance. Placing the following query on the Web of Science platform (www.webofscience.com): "*ALL=(Ostrea edulis AND (temperature OR thermal tolerance OR thermal performance OR thermal niche))"* yields 1,834 results (22 Nov. 2022). Examining each paper's abstract, only six publications investigated the thermal performance or tolerance of *O. edulis*; four focused on larvae and juveniles (Beiras et al., 1995; Child & Laing, 1998; Kamermans & Saurel, 2022; Robert et al., 2017), while there are only two studies reseraching adults (Eymann et al., 2020; Hutchinson & Hawkins, 1992).

Kamermans & Saurel (2022) investigated the growth of larval *O. edulis* at six temperatures (3, 8, 15, 20, 25 and 30 °C) at two food regimes (2 and 10 µg Chl a L^{-1}) for 6 weeks and found that optimal growth under low food concentration is at 20° C and under high food concentration is at 25 °C. Robert et al. (2017) reared *O. edulis* larvae at four different temperatures (15, 20, 25, and 30 °C) and report that *O. edulis* larvae have a relatively high-temperature tolerance (20-30 °C), with the best performances at 25 °C. The highest temperatures tested, i.e. 25 and 30 °C resulted in the best larval performances with high a survival rate (≥97%), high growth rates (≤ 21 µm day^{-1}) and high colonisation success (≥78%) (Robert et al., 2017).

Beiras et al. (1995) exposed young post-metamorphic *O. edulis* to cold (14 °C), control (20 °C), and warm treatment (26 °C) for three weeks and report a reduced scope for growth (SFG-the surplus energy available for growth, beyond the maintenance requirement) in oysters exposed to the cold treatment, while an

increase was observed in the warm treatment. Child & Laing (1998) investigated the low-temperature tolerance of juvenile *O. edulis* and *Magallana gigas* (Pacific oysters), maintaining them unfed or with low algal concentrations at 3, 6, and 9 °C for 11 weeks. Most *O. edulis* juveniles survived for 11 weeks at all temperatures, in contrast to juvenile *M. gigas* that had high mortalities (>95%) after 3–7 weeks at 3 °C. Fed *O. edulis* showed small weight loss at 3 °C, but increased in weight at 6 °C and 9 °C (Child & Laing, 1998).

Hutchinson & Hawkins (1992) assessed scope for growth on adult *O. edulis* acclimated to temperatures of 5-25 °C and a salinity of 16 to 34 and defined optimum conditions for maintaining *O. edulis* stock at temperatures between 15 and 20 °C and salinities between 28 and 34, when they are in their summer physiological state. Eymann et al. (2020) studied the response of adult *O. edulis* to a short-term warming protocol using a 2 °C increase every 48 hours from 14 to 36 °C and suggest that the optimum temperature for *O. edulis* is from 18 to 24 °C, because feeding activity was the highest at these temperatures when total energy expenditure (indicated by heart rate as a proxy for oxygen consumption rate) was moderate.

The discrepancy for optimum between the studies by Hutchinson & Hawkins (1992) and Eymann et al. (2020) can be explained by the fact that they measured different physiological parameters Suppose the goal is to assess the response of organisms to a changing environment. In that case, metabolic rate may be more appropriate as it is a more sensitive and direct measure of physiological response to environmental

stressors. On the other hand, if the goal is to evaluate the long-term growth potential and overall health of organisms, the scope for growth may be more suitable.

3.1.4. *Chapter aims*

This chapter presents the results of two experiments aimed to advance our understanding of the thermal requirements and vulnerability of adult European native oysters, *Ostrea edulis,* in the face of climate change and heatwaves. Experiment 1 builds upon the pioneering work of Eymann et al. (2020), employing a more robust and reliable approach by quantifying metabolic response through oxygen consumption rate instead of relying solely on heart rate measurements in a warming protocol of 9 to 36 °C with a 3 °C temperature increment. By focusing the investigation on oysters from the southeastern coastal region of England, which serves as a well-established stronghold for *O. edulis* (Allison et al., 2019), this study offers vital insights into their performance under both current and future environmental conditions in the said area.

Building upon the outcomes of Experiment 1, a second experiment was conducted, characterised by a narrower temperature increment (2 °C). In this subsequent investigation, both heart and oxygen consumption rate measurements were employed for a more nuanced and accurate portrayal of the species' metabolic adaptations across diverse temperature regimes. The finer resolution in this experiment was made to observe more subtle shifts in both heart rate and oxygen

consumption rate as the temperature gradually increases, facilitating a finer resolution analysis of their thermal performance. Additionally, the Arrhenius breakpoint temperature (ABT) of the heart rate was determined to provide further insights into the thermal performance of *O. edulis*.

Experiments 1 and 2 aimed to explore MO_2 and heart rate, aiming to reveal the thermal performance of *O. edulis* and identify discernible temperature thresholds beyond which *O. edulis* may experience physiological stress or reduced fitness. Experiment 1 also aimed to assess the impact of high temperatures on the overall health of oysters by comparing the condition index of oysters exposed to elevated temperatures with those exposed to control temperatures, thereby investigating the effects of temperature on their well-being.

Further, since Experiment 1 was conducted in oysters during their winter physiological state (December 2020) and Experiment 2 in oysters in their summer physiological state (June 2022), an additional analysis was conducted to determine if they exhibit similar thermal responses or if their thermal requirements differ based on their physiological state.

Based on the objectives above, this chapter was guided by the following hypotheses:

1. The MO_2 and heart rate observations in Experiments 1 and 2 will complement each other and unveil thermal performance and discernible thresholds at which the metabolic activity of *Ostrea edulis* becomes compromised.

2. High temperatures will affect the overall health of oysters, leading to a lower condition index than oysters exposed to control temperatures.
3. The oysters in winter (Experiment 1) and summer (Experiment 2) physiological states will have different thermal requirements.

These findings contribute to the body of knowledge on *O. edulis* and provide valuable insights for the bivalve aquaculture industry and conservation efforts, particularly in developing adaptive management strategies to reduce the negative impacts of heatwaves on bivalve aquaculture. A comprehensive understanding of the of the bivalve species' thermal requirements is essential for developing effective management strategies.

3.2. Materials and Methods

3.2.1. Thermal Performance of Ostrea edulis (*Experiment 1*)

3.2.1.1. Oysters and Experimental Conditions

The University of Essex Ethics Review and Management System reviewed and approved all experimental procedures in this thesis with Application number ETH2021-1358. Oysters (mean shell length: 76.7 ± sd: 7.03 mm) used in all experiments were from seabed mariculture or wild stock in the Blackwater, Crouch,

Roach and Colne Estuaries Marine Conservation Zone (BCRCE-MCZ) in Essex, UK. Sixty-three oysters were allowed to acclimate under laboratory conditions for two weeks, initially at 10 °C (field temperature during collection on 13 November 2020).

Oysters were haphazardly divided into three treatment groups: the experimental group and two control groups (Control 1 and Control 2) and placed in separate custom-built stacks of tanks (three 45.5 x 45.5 x 30 cm tanks with a combined sump). The experimental group, consisting of 21 individuals, underwent a temperature acclimation process that commenced at 9 °C and extended over three days.

Following this initial phase, I systematically increased the temperature by 3 °C every three days, gradually progressing from the starting temperature of 9 °C to a maximum of 36 °C. The decision to initiate the experiment at 9 °C was deliberate and grounded in scientific rationale. It aligned with the research findings of Eymann et al. (2020), which identified the thermal optimum for *O. edulis* as falling within the range of 18 to 24 °C. This precise starting temperature was chosen to ensure that the experiment would reach a phase where these specific temperature ranges could be accurately observed and studied. It took approximately 6.363 hours to increase the temperature to the next increment, with a ramp up of 0.4712 °C per hour. Oysters assigned to Control 1 (n=21) were exposed to a constant temperature of 10°C — the average annual temperature of the river Colne from 2011 up to the present (historical temperature taken from seatemperature.info).; and oysters assigned to Control 2 (n=21) to a constant temperature of 18°C — the average water temperature of the

Pyefleet Creek (part of BCRCE-MCZ) in the summer of 2020 (July-August, data collected from the field using HOBO data loggers).

From the second day of acclimation, the temperatures of the tanks were adjusted to reach the desired starting point temperature of the experiment, at 1 °C per day – e.g., 9, 10 or 18 °C (Fig 3.4). The MO_2 of oysters in the treatment group were measured on the third day of exposure to the target temperature before increasing the temperature to the next increment. The MO_2 of nine oysters each from Control 1 and 2, were also measured every time the MO_2 of the experimental group was quantified.

From acclimation and throughout the experiment, oysters were fed once daily with Shellfish Diet 1800® (Reed Mariculture, USA). As per the recommended feeding guidelines provided by Reed Mariculture, oysters with an average shell length of 75 mm are typically fed 4×10^{-7} mL of feed daily. In this experiment, each oyster was provided with 0.67 mL of feed daily, sufficient to meet their nutritional needs and ensure they were not underfed. Water tanks were cleaned, and 10% of the water was changed once a week. Raw local seawater was allowed to stand for sediment settlement, filtered, and UV-treated (before the arrival of oysters for three days, with UV lamps built-in with the stacked tanks) in all experiments. Where required, reverse osmosis water was used to correct for evaporation to keep salinity at 35.

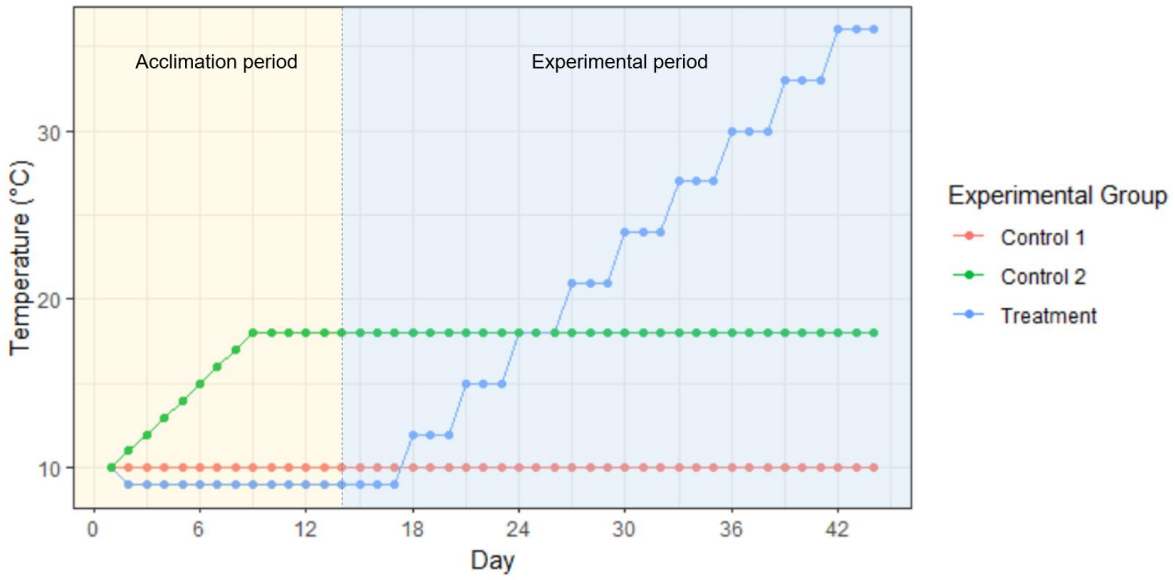


Figure 3.4 *This diagram illustrates the temperature changes experienced by each experimental group throughout the acclimation period and the entire experiment duration. The initial temperature for all groups was set at 10 °C, which corresponds to the ambient temperature at the time of oyster collection in the field. Starting from the second day of acclimation, the tank temperatures were gradually adjusted to achieve the desired starting point temperature for the experiment, at a rate of 1 °C per day. The specific starting temperatures for the experiment varied among groups and were set at 9, 10, or 18 °C. During the investigation, the treatment group (n=21) initiated at a temperature of 9 °C and underwent a 3 °C incremental temperature rise every three days, ultimately reaching a maximum temperature of 36 °C. It took approximately 6.363 hours to increase the temperature to the next increment after the thermostat was set, with a ramp up of 0.4712 °C per hour. In contrast, oysters assigned to Control 1 (n=21) were exposed to a constant temperature of 10 °C, representing the average annual temperature of the river Colne from 2011 to the present (historical temperature data obtained from seatemperature.info). Likewise, oysters assigned to Control 2 (n=21) were subjected to a constant temperature of 18 °C, reflecting the average water temperature of the river Colne in July-August 2020.*

3.2.1.2. Oxygen Consumption Rate Measurement

MO_2 measurements were done with recirculating sealed chambers maintained in a water bath at the incubation temperature using natural seawater, filtered through a polypropylene felt filter bag (pore size 25 µm; Cole-Palmer, UK) at a salinity of 35. For all experiments, circular glass food-storage containers with plastic lids (CP

Creative Products, Germany) served as respirometry chambers (see Fig 3.5). A water pump (Eheim 1048 Universal), connected using 6 mm (outside diameter) silicone tubing, circulated the water inside each chamber, which held a single oyster. The chambers were then placed inside a water bath, where temperatures were maintained using a thermostat-linked heater (Schego 300W titanium heater, D-D The Aquarium Solution Ltd, Germany). Seven oysters were assessed per water bath, including a single empty chamber that acted as a control to determine any bacterial oxygen consumption associated with the chamber over time (background oxygen consumption). Chambers were 680 mL, including all associated tubing and internal pump volume (Fig 3.5).

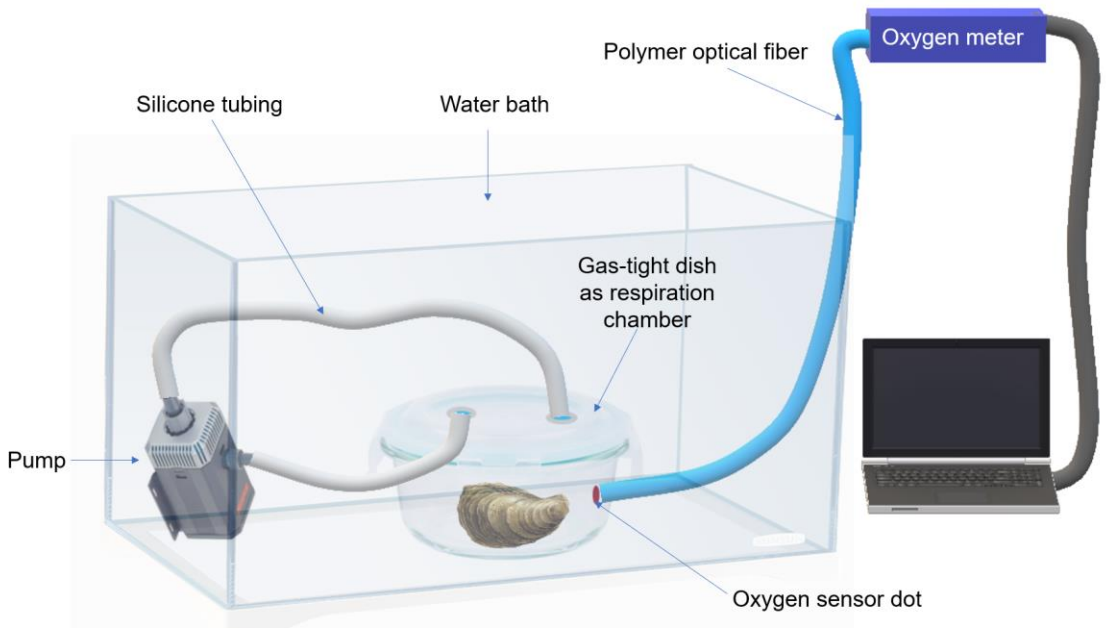


Figure 3.5 *Schematic diagram of the setup for oxygen consumption rate measurements. Individual oysters were placed in a gas-tight chamber connected to a pump, enabling water circulation during measurement. The chambers were placed in water baths set to acclimation temperatures. Each chamber had an oxygen sensor spot attached to the Multi-Channel Oxygen Meter (OXY-4 SMA (G3), PreSens, Precision Sensing GmbH, Regensburg, Germany); the percentage oxygen saturation inside the chamber was measured every 2 seconds for one minute, every hour.*

Before observation, the oysters were placed in individual chambers overnight to acclimate to handling and were not fed to reduce digestion and feeding-associated metabolic responses (Dong et al., 2011). During this time, the chambers were open at the connection point to the recirculating pump allowing water to move freely between the chambers and the external water bath. An air stone and an Eheim pump (Ecco Pro 130 external filter, Baden-Wurttemberg, Germany) aerated and continually filtered the water in the larger water bath.

Fifteen minutes before MO_2 measurements, the air stone was removed to prevent water from being supersaturated with oxygen. The recording was initiated, and the recirculating silicone tubes were attached, enclosing the oyster in its fixed volume circulatory chamber. Using a PreSens O_2 sensor dot affixed inside the glass chamber and a Polymer Optical Fibre and a Multi-channel oxygen meter (OXY-4 SMA (G3), PreSens Precision Sensing GmbH, Regensburg, Germany), the percentage oxygen saturation inside the chamber was measured every 2 seconds for one minute, every hour. O_2 saturation was measured for a maximum three hours, and when a decline of 50% saturation was observed in a chamber, the recording of oxygen consumption rates in that oyster was stopped. During measurement, temperature was automatically compensated using a temperature sensor, whilst salinity and pressure were compensated manually (Salinity: 35, Pressure: 1020 millibars). This gave rise to a series of oxygen concentration inside the chamber over a three-hour period.

The MO_2 of individual oysters was calculated using the following equation (Bayne & Widdows, 1978; Sawusdee, 2015):

$$MO_2 = \frac{(C_{oxygen})\left(\frac{V_c - V_a}{1000}\right)\left(\frac{60}{t_1 - t_0}\right)}{AFDW}$$

The variable MO_2 represents the oxygen consumption rate, measured in mL O_2 L^1 h^1. C_{oxygen} denotes the average difference in oxygen concentration, measured in mL L^{-1}, between the end time and the start time of each hour during the three-hour measurement. V_c is the chamber volume and associated tubing volume (680 mL), and V_a is the mean volume of the oyster measured by three repeated measures of water displacement in mL. The background oxygen consumption rate from control chambers without oysters was subtracted from individual oyster oxygen consumption rates to calculate an oyster-only oxygen consumption rate. The MO_2 is then divided by the ash-free dry weight of the soft tissue (AFDW) to calculate the oxygen consumption rate per g AFDW.

To obtain the dry weight of the oysters, animals that died overnight, were removed in the morning (0900) and immediately placed in a labelled bag in a freezer (-20 °C). Animals still alive at the end of the study were first opened (shucked), soft tissues and shells of oysters were separated, and then oven-dried at 80 °C on pre-dried foil dishes for 48 h. Animals from the freezer were recovered and included in this process. The dry weight of the shell and the tissue were determined using an analytical balance. To obtain the AFDW, the dried soft tissues were put into a muffle furnace for six hours at 500 °C to remove organic content and reweighed for the ash

weight. The ash weight was subtracted from the dry tissue weight to obtain the AFDW of the soft tissues.

3.2.1.3. Condition Index

A condition index (CI) is often used to determine whether a shellfish product quality is good (e.g., will it be well-received by the market) or evaluate the health of stock by measuring its physiological activity, such as growth and reproduction, under a given environmental condition (Lucas & Beninger, 1985; Wilder et al., 2016). A low value for CI often means that the organism has used a significant biological effort for maintenance under unfavourable environmental conditions or has been infected by a disease. Another reason for a low CI could be that the organisms have spent their metabolic effort producing and releasing gametes. Therefore, as CI serves as an indicator of stress or reproductive activity, it is instrumental in determining the physiological state of an organism (Lucas & Beninger, 1985; Wilder et al., 2016)**.**

The CI for all the oysters at the end of the experiment (N=63), including those that died before the investigation finished, was quantified following the methods of Noisette et al. (2014). The dry weight of the shell and the tissue were determined using the abovementioned proceduere. The CI was finally calculated with the following formula:

$$CI = \frac{Dry\ tissue\ weigh}{Dry\ shell\ weight} * 100$$

3.2.2. Thermal Performance of Ostrea edulis (*Experiment 2)*

A second experiment was performed in June 2022 to measure heart rates and respiration rates simultaneously. Eight oysters were collected and allowed to acclimatise to laboratory conditions at 18 °C for two weeks. On the third day of acclimation, heartbeat sensors (Electricblue, Porto, Portugal) were attached to the oysters' shells directly above the heart's location using superglue (Loctite® Super Glue ULTRA Gel Control™). After acclimation, oysters were placed in respirometry chambers and subjected to an incremental 2 °C increase in temperature every three days starting from 18 °C up to 32 °C in a water bath (Fig 3.6). Before the subsequent temperature increase, the oysters' MO_2 and HR were simultaneously measured. After every measurement the oysters were checked to assess mortality.

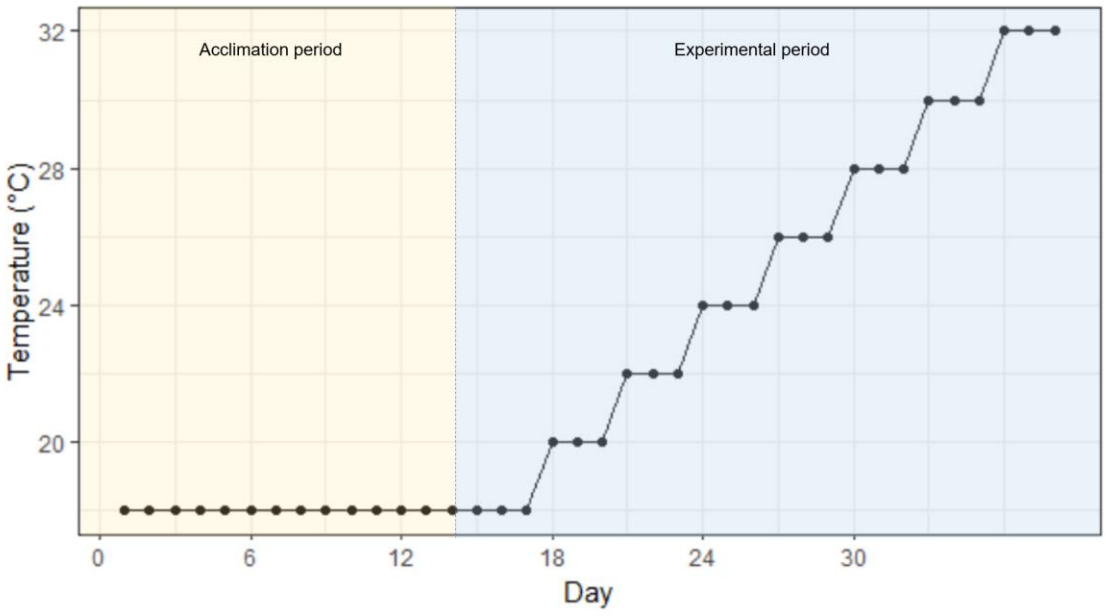


Figure 3.6 *The temperature changes experienced by the oysters in Experiment 2 throughout the acclimation period (18 °C) and the entire experiment duration. During the experiment, the oysters (n=8) initiated at a temperature of 18 °C and underwent a 2 °C incremental temperature rise every three days, ultimately reaching a maximum temperature of 32 °C. After the thermostat was set to the next increment, the ramp up rate was 0.4712 °C per hour.*

MO_2 was quantified as previously described in Section 3.2.1.2. Simultaneously, within-chamber HR were recorded using the Pulse 8 Channel Heart Frequency Logger V1.0 (Electricblue, Porto, Portugal). To do this, the respiration chamber was modified so the lid would also have a hole for the heartbeat sensor cable (See Fig 3.7). HR was measured every eight minutes for one minute (Pulse logger V1.0 cannot simultaneously log all channels, hence the eight-minute interval). This gave rise to a time series of metabolism and heart rate across a temperature gradient.

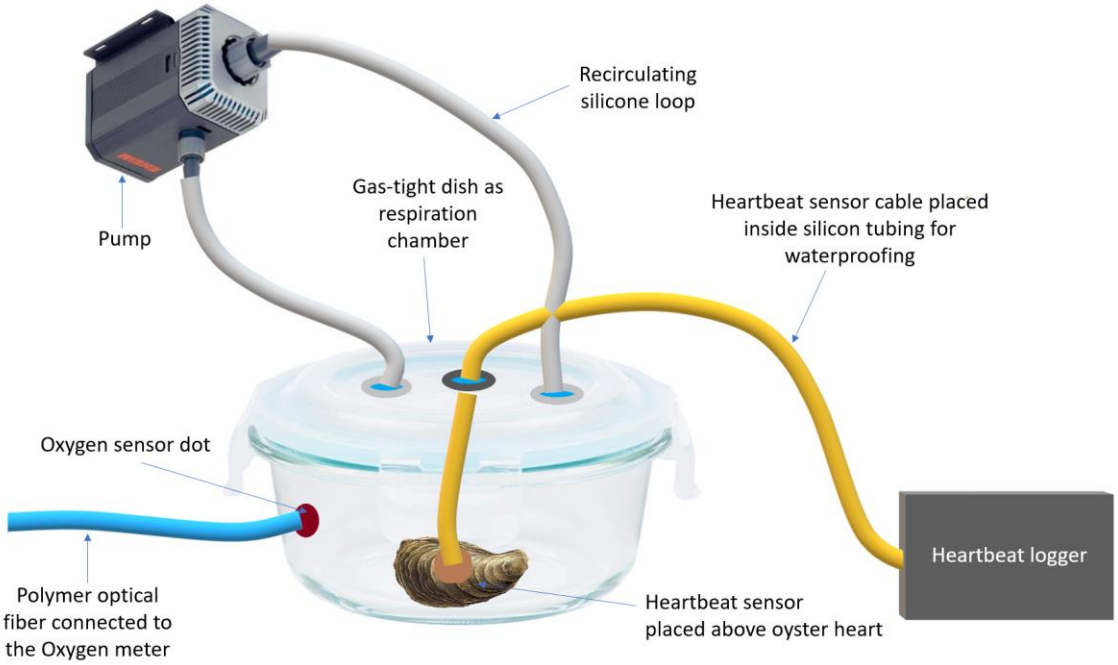


Figure 3.7 *The schematic diagram for the modified respiration chamber, already includes a hole where the heartbeat cable goes through (sealed with a silicone sealant) to allow simultaneous measurement of the oyster heartbeat and oxygen concentration inside the chamber.*

3.2.3. Data Analysis

Generalized Linear Mixed Models (GLMMs) with the Tweedie distribution were employed to analyse oxygen consumption and heart rate. This modelling approach was chosen to accommodate the positively skewed nature of the oxygen consumption rate and heart rate data, which exhibited a mixture of continuous and zero values. The GLMMs included temperature as a fixed effect and individual oyster ID as a random effect, capturing the repeated measurements within each oyster.

A Generalized Linear Model (GLM) with the Gamma family and log-link function was utilized to assess the condition index between treatment groups. The Gamma distribution was selected to account for the positive skewness observed in the condition index data.

Model fit, and goodness-of-fit were evaluated using established diagnostics. In the GLMMs for oxygen consumption and heart rate, the dispersion and model fit were assessed using the DHARMa package. Additionally, the GLM for the condition index was diagnosed by inspecting residual plots to assess assumptions of homoscedasticity and normality.

Post-hoc analyses were conducted to explore pairwise differences in oxygen consumption rates and heart rates across various temperature levels. The emmeans package was utilised, and Tukey's post-hoc test was applied to determine significant differences between the groups.

Moreover, time plots of the residuals were examined to assess autocorrelation in the model residuals. This allowed for the visual inspection of any patterns or dependencies over time, aiding in the detection of potential autocorrelation and guiding the selection of appropriate modelling strategies if needed.

The statistical analyses were performed using R version 4.1.1 (2021-08-10). The GLMMs and GLM were fitted using the glmmTMB and glm functions within the

relevant R packages, respectively. The significance of model terms and post-hoc comparisons was determined based on appropriate statistical tests and adjustments.

3.2.4. Determination of the Arrhenius Breakpoint Temperature of the Heart Rate

A well-established method of evaluating the thermal performance of an organism is to determine the Arrhenius breakpoint temperature (ABT) of the heart rate, which defines the temperature where the heart rate first deviates from its exponential increase with temperature (Ferreira et al., 2014; Stenseng et al., 2005; Stillman, 2002; Xing et al., 2016). The ABT was calculated for each oyster by plotting the natural logarithm (ln(HR)) of the heart rate against the inverse absolute Temperature in Kelvin (1/T). The breakpoint analysis was performed in R using the “segmented” package (Muggeo, 2003). The average and standard error of ABT were then determined for all oysters.

3.2.5. Winter versus summer physiological state

To determine if there was a difference in the thermal requirements between winter and summer physiological states of native oysters, the MO_2 data from Experiment 1 and 2 were analysed together. Experiment 1 represented the winter physiological

state, and Experiment 2 represented the summer physiological state. The change in MO_2 slope was calculated for each oyster in both states. Additionally, the MO_2 of oysters in the winter and summer states were compared at 18, 24, and 30°C, which common to both experiments. MO_2 were analysed for statistical difference using Welch's two-sample t-test to compare the slopes of the change in respiration rate between the winter and summer physiological states. The null hypothesis assumed no significant difference between the two states. Paired t-tests were conducted to compare respiration rates at specific temperatures.

3.3. Results

3.3.1. Thermal tolerance of Ostrea edulis *(Experiment 1)*

European native oysters were subjected to incremental increases (3 °C increments) in temperature from 9 to 36 °C. The results revealed a clear relationship between temperature and oxygen consumption in the treatment group (Fig 3.8 A). As the temperature increased, so did the rate of oxygen consumption (MO_2), from a mean ± 1 SE of 0.232 ± 0.044 at 9 °C to 0.520 ± 0.095 mL O_2 h^{-1} g $AFDW^{-1}$ at 33 °C. Notably, at the highest temperature tested (36 °C), all oysters died, and oxygen consumption rate measurements could not be done and plotted as zero (Fig 3.8 A). In contrast, the control groups (Fig 3.8 B and C) did not exhibit the same increasing trend in oxygen consumption throughout the experiment and remained stable.

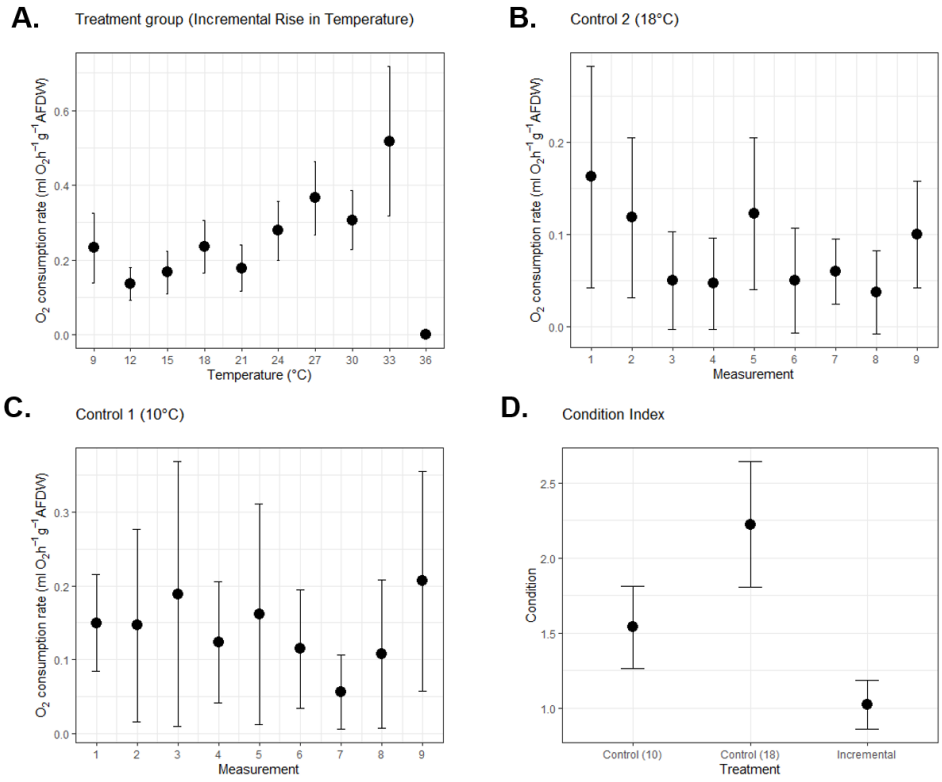


Figure 3.8 *Oxygen consumption rates of* O. edulis *exposed to 10 °C (Control 1) (**A**), 18 °C Control 2 (**B**), and incremental rise of temperature from 9 to 36 °C for 28 days (N=63) (**C**) (mean with 95% confidence intervals). Since all oysters in the treatment group died within 21 hours of exposure to 36 °C, oxygen consumption rates were measured up to 33 °C. The condition index (mean with 95% confidence intervals) of the oysters in each experimental condition are shown in the left panel (**D**).*

The statistical analysis demonstrated a significant effect of temperature on oyster respiration rate (Estimate = 0.043276, SE = 0.006463, z = 6.696, $p < 0.001$) in the treatment group. This implies that as temperature increases, the rate of oyster respiration also increases. Specifically, the estimated coefficient for temperature suggests that, on average, for each one °C rise in temperature, oyster respiration rate increased by 0.043276 mL O_2 h^{-1} g $AFDW^{-1}$.

The post hoc analysis of the data uncovered meaningful variations in the respiration rate of oysters across different temperature levels. By comparing each pair of temperature levels, statistically significant differences in respiration rate were observed between several combinations. Specifically, significant temperature differences were found between 12 and 24, 12 and 26, 15 and 27, 21 and 27, 12 and 30, 15 and 30, and 9 and 33 °C. Furthermore, there were significant differences between all lower temperatures and 33 °C, except for the comparisons involving 27 °C and 33 °C (Table 3.1) (see Appendix D for a complete list of interactions).

Table 3.1 *Post hoc comparison of respiration rates of European native oysters exposed to incremental rise in temperature from 9 to 36 °C (n=21). Mean differences are shown. Values with asterisk (*) indicate that the mean difference is significant at the 0.05 level.*

Temperature(°C)	12	15	18	21	24	27	30	33	36
9	0.51	0.30	-0.06	0.23	-0.22	-0.50	-0.36	-0.84*	1.53
12		-0.20	-0.57	-0.28	-0.73*	-1.01*	-0.87*	-1.34*	2.04
15			-0.37	-0.07	-0.53	-0.81*	-0.67*	-1.14*	1.84
18				0.30	-0.16	-0.44	-0.30	-0.77*	1.47
21					-0.45	-0.73*	-0.60	-1.07*	1.76
24						-0.28	-0.14	-0.61*	1.31
27							0.14	-0.33	1.03
30								-0.47	1.17
33									0.70

As for the controls, the GLMM analysis revealed a significant impact of the "measurement number" variable on the oxygen consumption rates of oysters exposed to a constant temperature of 18 °C (Control 2). The estimated coefficient was -2.01052 (SE = 0.31960), indicating a significant decrease in respiration rate as the measurement number increased (z = -6.291, p = 0.0388). Post hoc analysis identified a significant difference only between Measurement 1 and Measurement 8 (Estimate = 1.50362, SE = 0.440, z = 3.414, p = 0.0185).

However, for oysters in Control 1 exposed to a constant temperature of 10 °C, the coefficient for the "measurement number" variable was not statistically significant (estimate = -0.006111, SE = 0.009963, z = -0.613, p = 0.54). This suggests no significant change in the oxygen consumption rate as the experiment progressed in oysters under this temperature condition.

The analysis revealed the treatment groups' significant effects on the oysters' condition index. The pairwise comparisons indicated that the Control 1 (10 °C) group had a significantly lower condition index compared to both the Control 2 (18 °C) group (Estimate= -0.368, SE= 0.199, t.ratio = -3.195, p = 0.0081) and the Treatment group (Estimate= 0.410, SE= 0.199, t.ratio = 3.456, p = 0.0029). Furthermore, the Control 2 group exhibited a significantly higher condition index than the Treatment group (Estimate= 0.778, SE= 0.199, t.ratio = 6.561, p < 0.0001)(Fig 3.8 D).

Mortality in the treatment group was first observed at 27 °C (one oyster) and then at 33 °C (one oyster), while the remaining oysters died (19 oysters) within 21 hours after being exposed to 36 °C. No mortality was observed for either Control 1 or Control 2.

3.3.2. Thermal tolerance of Ostrea edulis *(Experiment 2)*

European native oysters were exposed to a gradual temperature increase ranging from 18 to 32 °C over 24 days. After each temperature exposure, which lasted for three days, both their heart rate (HR) and oxygen consumption rate (MO_2) were measured. Notably, none of the oysters included in this experiment experienced any mortality. In Figure 3.9 MO_2 and HR values for 36 °C were plotted as 0, based on the observation of 100% mortality in oysters exposed to this temperature in Experiment 1.

The oysters' MO_2 increased with increasing temperature, similar to observations in Experiment 1. The mean MO_2 at lower temperatures (9 to 26 °C) ranged from 0.120 to 0.166 mL O_2 h^{-1} g $AFDW^{-1}$ and started its steep increase at 28 °C, at a mean respiration rate of 0.196 ± 0.043 mL O_2 h^{-1} g $AFDW^{-1}$ (Fig 3.5A). In the analysis, the GLMM demonstrated a significant effect of temperature on MO_2 (estimate = 0.07175, SE = 0.01582, z = 4.535, $p < 0.001$). Post hoc analysis indicated significant differences in the respiration rate between various temperature pairs, including 18 and 32, 20 and 32, 22 and 32, 24 and 32, and 26 and 32 °C. (refer to *Appendix D* for detailed interactions).

The HR of the oysters followed a temperature-dependent bell curve that was the lowest at 18 °C (13.2 ± 1.0 beats per minute (bpm)) and continually increased until it reached its peak at 26 °C (18.9 ± 1.15 bpm) and started decreasing again at 28 °C (Fig 3.5B). During the HR recording, some oysters lacked distinct HR readings for the three-hour measurements, identified as periods of cardiac arrests (Fig 3.10). In two to five oysters, cardiac arrests were observed at 20, 22, and >26°C. All oysters exhibited cardiac arrests at some point, except for oysters 3 and 8 (see Fig 3.5C for specific oysters exhibiting arrests for each temperature). The mean occurrence of cardiac arrest was greater in high temperatures (>24 °C) (2.7 ± 0.96 oysters) than the mean occurrence at ≤ 24 °C (1.7 ± 1.81 oysters), however, this difference is not significant ($t_6 = 0.7845$, $p = 0.7687$). The mean ABT for heart rate was 24.88 ± 1.25 °C (Fig 3.4 D). Similarly, for the heart rate analysis, the GLMM revealed a significant effect of temperature (estimate = 0.012514, SE = 0.006032, z = 2.075, p = 0.038). Notably, post hoc analysis of the data revealed statistically significant differences in the heart rate between various pairs of temperature levels, including 18 and 24, 18 and 26, 18 and 28, 20 and 24, and 20 and 26 °C (see *Appendix E* for further details).

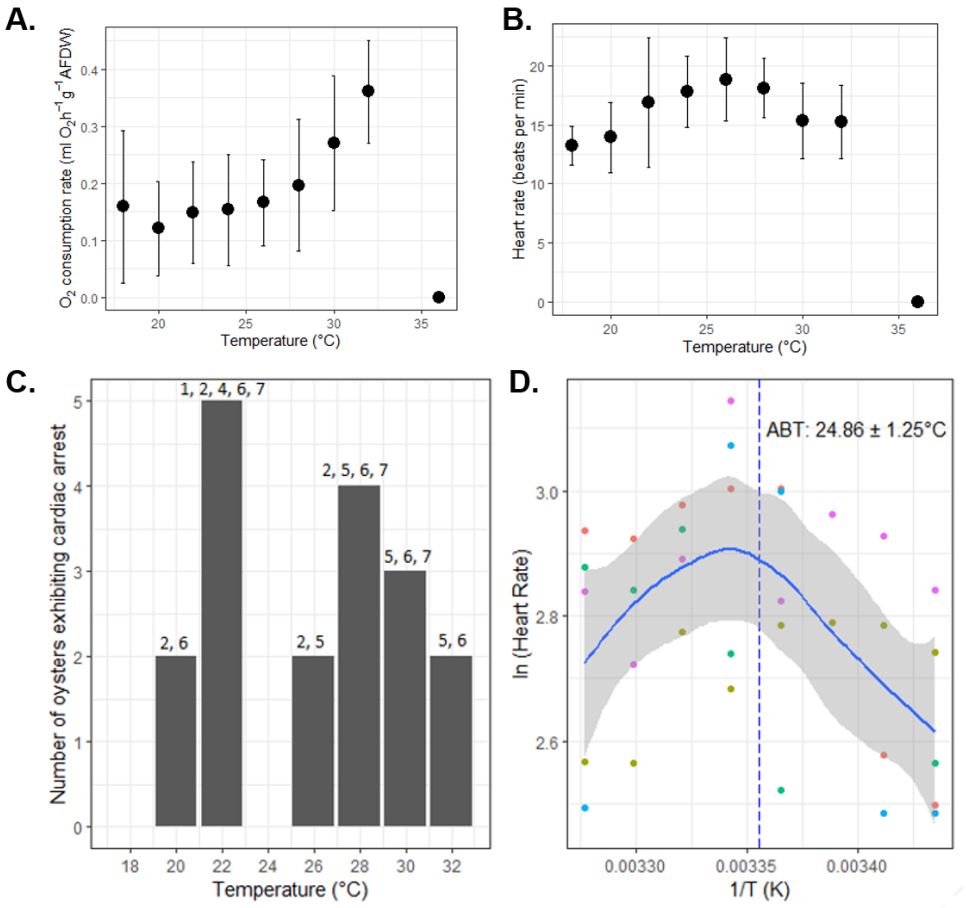


Figure 3.9 *Results of an experiment that exposed* O. edulis *to an incremental increase in temperature from 18 to 32 °C for 24 days (n=8). The panels show mean with 95% confidence interval of oxygen consumption (**A**) and heart rate (**B**), number of individuals exhibiting cardiac arrest (the numbers above the bars indicate which oysters exhibited cardiac arrests in each temperature) (**C**), and Arrhenius plot - colour of dots denote individual oysters' natural logarithm of heart rate as a factor of the inverse absolute temperature in Kelvin, solid line shows trendline with one standard error (grey shade), and the dashed blue line shows the mean Arrhenius Breakpoint Temperature for all oysters (**D**).*

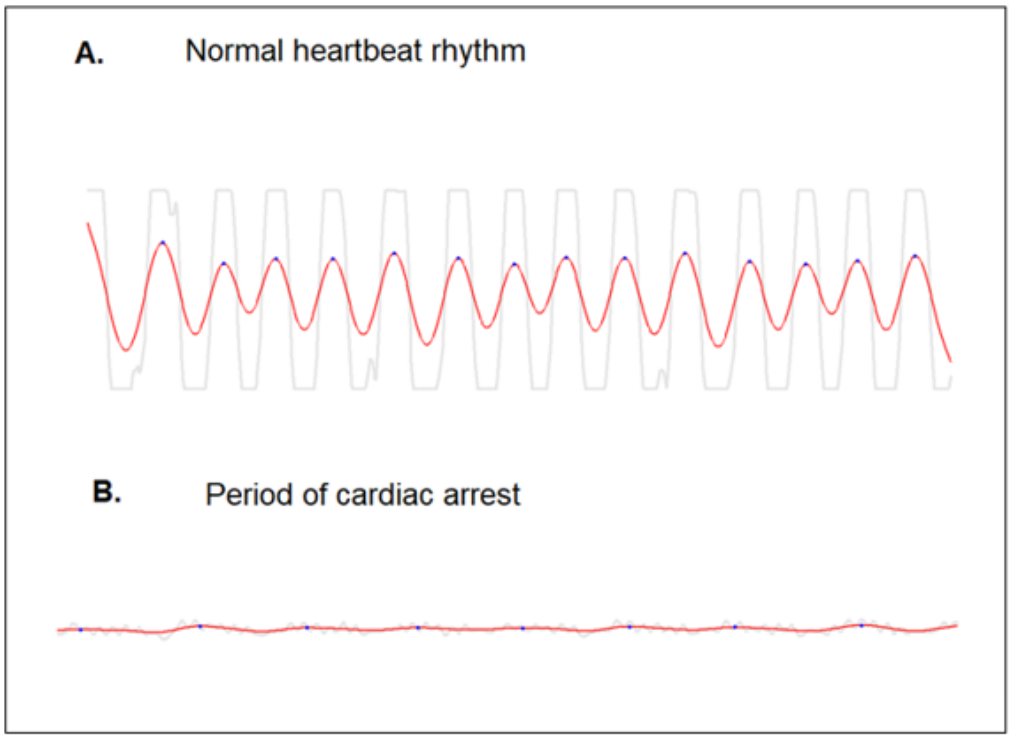


Figure 3.10. *Normal heartbeat rhythm (**A**) and periods of cardiac arrests (**B**) were observed during the data processing of Heartbeat recording in R (Screenshots).*

3.3.3. Winter vs Summer physiological state

Analyses were done to compare the thermal requirements of native oysters in their winter (Experiment 1) and summer (Experiment 2) physiological states. The change in respiration rate slope was calculated for each oyster. The average slope of the change in respiration rate with increasing temperature was 0.0119 (SE: 0.0018) for summer oysters and 0.0133 (SE: 0.0087) for winter oysters. A Welch's two-sample t-test revealed no significant difference in the slopes of the change in respiration rate between the winter and summer physiological states (t = 0.409, df = 14.565, p-value = 0.688).

Furthermore, the respiration rates of oysters in the winter and summer states were compared at temperatures of 18, 24, and 30 °C, the common temperatures between the two experiments. At 18 and 30 ° C, no significant differences were found between the two physiological states. However, at 24 °C, a significant difference was observed, with winter oysters (Mean: 0.2780561, SE: 0.03792301) exhibiting a higher respiration rate compared to summer oysters (Mean: 0.1537060, SE: 0.04112151) ($t = -2.223$, $df = 19.128$, p-value = 0.03846).

3.4. Discussion

The thermal requirements of oysters play a crucial role in the success and sustainability of bivalve aquaculture. Understanding the relationship between temperature and oyster physiology is essential for optimising growth, survival, and overall productivity. In this study, I investigated the thermal responses of European native oysters through two experiments aimed at assessing their oxygen consumption rate (MO_2), heart rate (HR), mortality, and condition index across a range of temperatures. By integrating the results of Experiment 1 and Experiment 2, I gained valuable insights into the thermal requirements of oysters and their implications for aquaculture practices. In this discussion, I will analyse and interpret the findings from both experiments, highlighting the significant outcomes and their potential impact on bivalve aquaculture management.

In Experiment 1, *O. edulis* exhibited a stable metabolic rate (MO_2) at lower temperatures (9-21°C), but a significant increase was observed from 24 °C onwards,

accompanied by initial mortality at 27 °C. Experiment 2 incorporated heart rate measurements, revealing a sharp rise in MO_2 at 28 °C and the start of the decline of HR. The Arhennius Breakpoint Temperature of 24.88 ± 1.25 °C was computed. Cardiac arrests were observed at various temperatures, with higher occurrences above 24 °C, although not significantly different from lower temperatures (≤24 °C). The comparison of spring and summer physiological states showed no differences in thermal requirements, except for significantly higher respiration rates in winter oysters at 24 °C. These findings indicate temperature-dependent physiological responses in *O. edulis*, with potential autonomous responses above 24 °C. Furthermore, the oysters exposed to increased temperatures exhibited a lower condition index, highlighting the impact of warming on their overall health.

3.4.1. Twenty-four degrees Celsius is the upper thermal optimum for Ostrea edulis

The MO_2 of native oysters increased with increasing temperature, a common phenomenon among most aquatic ectotherms, illustrated in Fig 3.11. In these organisms, MO_2 rises with increasing temperature until it reaches a critical point when the oxygen supply capacity is limited (Giomi & Poertner, 2013). A similar increasing trend in MO_2 of *O. edulis* was reported by Hutchinson and Hawkins (1992) when they exposed native oysters to 5-25 °C. Higher temperature means higher kinetic energy of biomolecules, which causes increased metabolic processes and organismal activity, explaining this positive correlation between temperature and MO_2 (Boscolo-Galazzo et al., 2018).

Furthermore, the lower ambient oxygen in the water at high temperatures is another factor that causes increased metabolism. At high temperatures, cardiovascular circulation rises to compensate for reduced oxygen concentration with increased oxygen delivery, thus increasing MO_2 and organism metabolism (Pörtner et al., 2017).

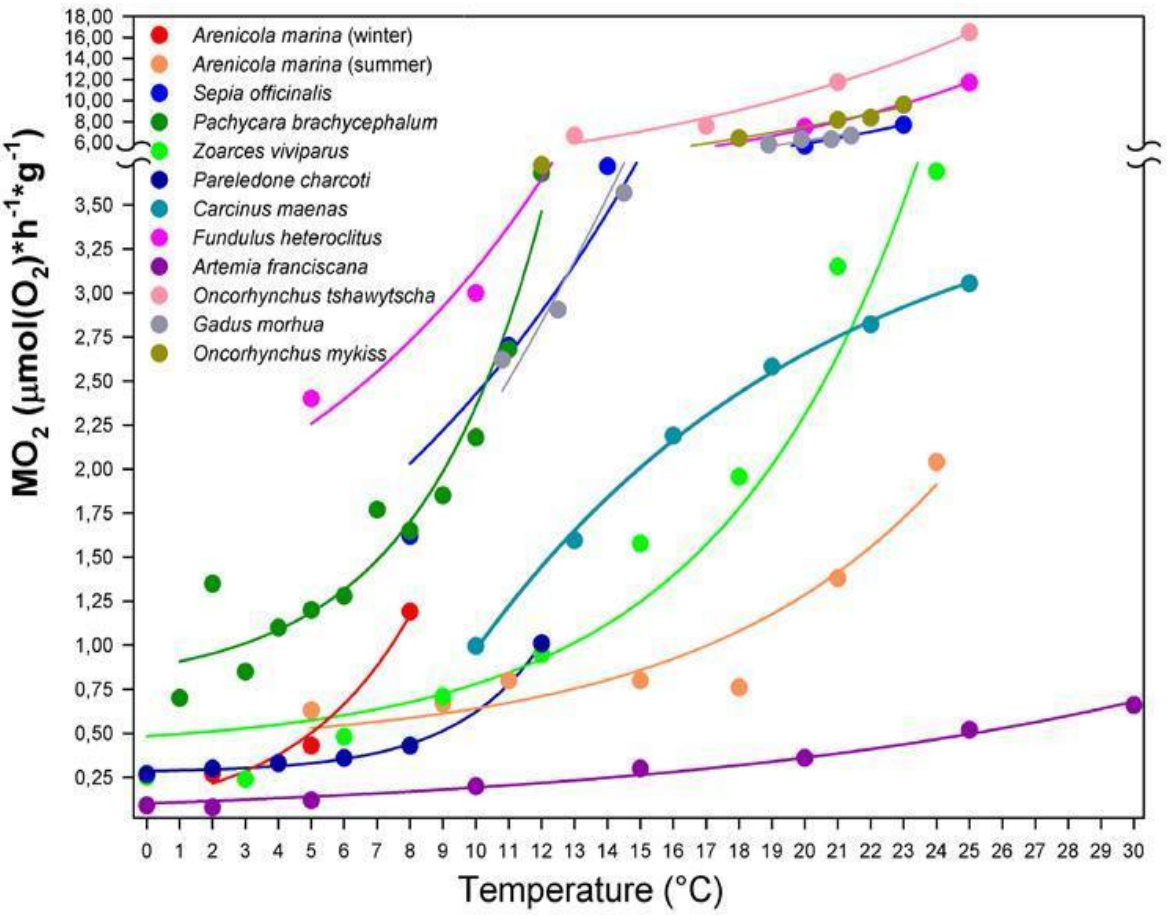


Figure 3.11 *Temperature-dependent oxygen consumption (MO_2) rate in µmol (O_2) h^{-1} g^{-1} in different marine ectotherms. Each colour denotes individual ectotherms. Image taken from Giomi and Poertner (2013).*

The observed changes in the pattern of MO_2 in oysters at 24 °C during Experiment 1, coupled with the identification of the Arrhenius Break Point temperature (ABT) at 24.88 ± 1.25 °C and the increased occurrence of cardiac arrests above 24 °C in

Experiment 2, strongly suggest that *O. edulis* exhibits an inherent physiological response to thermal stress beyond 24 °C. These findings support the notion that 24 °C may represent the upper thermal optimum for these oysters.

Furthermore, these results align with previous research by Eymann et al. (2020) that identified the thermal optimum for *O. edulis* as being between 18 and 24 °C. In their study, Eymann et al. (2020) measured heart rate as a proxy for MO_2 and found that this temperature range coincided with the highest feeding activity and moderate energy expenditure. Eymann et al. (2020) also highlighted 26 °C as a critical temperature for *O. edulis*, beyond which performance limitations were evident, including reduced filtration rates, arrhythmia, and the accumulation of anaerobic metabolites in the gills.

The present study's findings, in conjunction with the previous work by Eymann et al. (2020), support the notion that heart rate can serve as a reliable proxy for assessing oxygen consumption (MO_2) in *O. edulis*.

The expected drastic decline in metabolic activity after reaching the thermal optimum, as predicted by the theoretical thermal performance curve (Fig 3.12), was not observed in my measurement of MO_2. However, this decline was evident in the measurements of heart rate during Experiment 2 (Fig 3.8A and Fig 3.9A). Based on the results of both MO_2 and heart rate, it can be inferred that the upper critical limit (Fig 3.12) for *O. edulis* lies between 33 and 36 °C. These vital limits denote temperatures beyond which the organism can only tolerate short-term exposure.

.

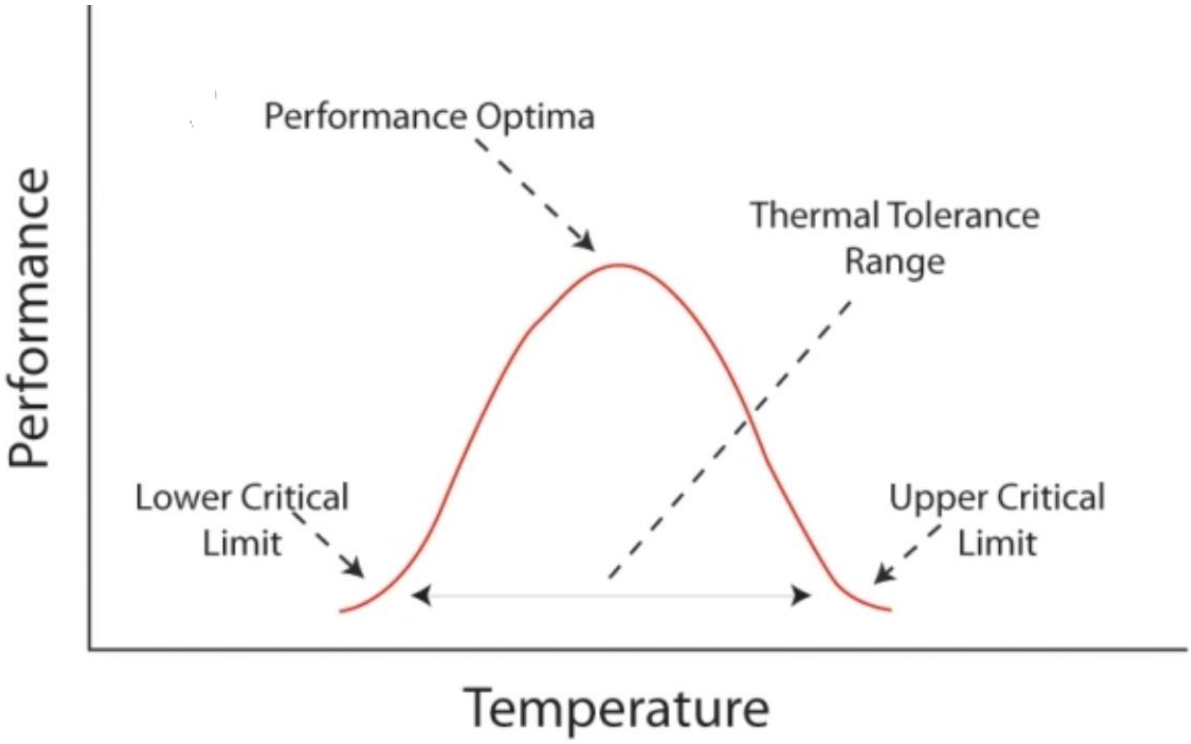


Figure 3.12. *Hypothetical physiological performance of ectothermic animals. Critical limits refer to temperatures beyond which only short-term exposure is possible. (Image taken from Miller and Stillman, 2012; Pörtner et al., 2006).*

When temperatures exceed the organism's limits, weak bonds in proteins break, leading to loss of stability and unfolding. This triggers a heat shock response characterised by the upregulation of heat shock proteins (HSPs) that aid in refolding damaged proteins. However, this process requires energy and reduces the synthesis of other proteins. While increased expression of HSPs provides protection and facilitates recovery, it comes at a cost to overall performance (Miller and Stillman, 2012). When environmental temperatures exceed the organism's capacity to cope, it cannot recover, leading to a brief survival time. This critical temperature, known as CTmax, often leads to a loss of neuromuscular coordination due to a decline in neurotransmitter function (Miller and Stillman, 2012).

Prolonged exposure to temperatures beyond 24 °C may harm *O. edulis*, necessitating temperature monitoring and management in bivalve aquaculture. Maintaining optimal water temperatures is vital for effective bivalve aquaculture management. The knowledge of *O. edulis'* thermal optima has important implications for predicting its responses to climate change and implementing effective conservation and management strategies. As global temperatures rise and heatwaves become more frequent, *O. edulis* populations may face increasing challenges.

Heatwaves contribute to surpassing the upper thermal optimum of 24 °C more frequently and for longer durations. When organisms experience frequent exposure to temperatures beyond the thermal optimum, several physiological and behavioural changes occur as they attempt to cope with the adverse conditions. From a physiological standpoint, the organism undergoes considerable stress. The normal functioning of metabolic processes is disrupted, resulting in energy production and utilization imbalances. Consequently, the organism may experience reduced performance in essential physiological functions such as growth, reproduction, and immune response. The increased metabolic demands to maintain homeostasis and repair cellular damage can divert resources away from these vital functions, compromising the overall well-being of the organism (Pörtner et al., 2017).

The reduced habitat suitability experienced by oysters may necessitate their migration to higher latitudes in search of suitable conditions. However, this shift in

range can lead to habitat fragmentation, decreased connectivity, and heightened competition for resources in the new ranges, posing additional threats to the survival of *O. edulis* populations.

The temperature range of UK waters falls within the upper thermal optimum for *O. edulis,* with average temperatures ranging from 6-10 °C in winter to 15-20 °C in summer (seatemperature.org). While native oyster larvae have shown tolerance to high temperatures (Robert et al., 2017), the impact of peak summer temperatures, particularly after spawning, on adult mortality in natural non-aquaculture environments has received less attention (González & González, 1985; Zrnčić et al., 2007).

In a mild heatwave phenomenon that was presented in the introduction of this Chapter in August 2020, subtidal temperatures of an *O. edulis* production site reached 25 °C for one day, with a total of ten days exceeding the normal summer average of around 18 °C, including three days surpassing the identified upper thermal optimum of 24 °C. Moreover, during the 2021 heatwave in the same production area, the intertidal zone experienced 7-8 consecutive days with water temperatures exceeding 24 °C during mid to peak tidal heights. Notably, during low tide, the mud surface in the high intertidal zone reached up to 38.95 °C, with three consecutive days surpassing the *O. edulis* lethal temperature of 36 °C. Although these higher intertidal areas are not the typical habitat for *O. edulis* in the southern North Sea, the species is known to naturally occur in rocky intertidal sites elsewhere in the UK (e.g., Wales, Ireland, and Northern Ireland) (Smyth et al., 2020).

While the European Native Oyster Habitat Restoration Handbook suggests that intertidal areas up to depths of 80 m are suitable for restoration sites (Preston et al., 2020), considering the results of this study and the predicted increase in heatwave frequency, lower intertidal and subtidal restoration and aquaculture sites are preferred for optimal success.

3.4.2. Warming may affect bivalve health and post-reproductive adult survival

The oysters exposed to high temperatures in Experiment 1 resulted in a significantly lower condition index (CI) for *O. edulis* than the controls. A low value for CI frequently means that the animal has spent a significant biological effort for maintenance in an unfavourable environment or has a disease. Another explanation for a reduced CI is that the animal has spent their metabolic attempt to produce and release gametes. The oysters in this study are not post-reproductive, which indicates they were in a poor physiological state due to thermal stress. These findings lead us to speculate that warming coasts and more frequent heatwaves would affect the overall health of *O. edulis* and their ability to grow and reproduce. Thermal stress may also lead to their declining ability to recover from spawning, which can reduce post-breeding adult survival.

O. edulis generally release their gametes between June and August (Maathuis et al., 2020). This suggests that *O. edulis* are most likely in a reproductive or post-reproductive stage during the summer atmospheric heatwaves. Stechele et al.' recent study on the life history traits and tolerance to changing environments shows that *O. edulis* is especially susceptible to unfavourable conditions during metamorphosis and the brooding period (Stechele et al., 2022). Stechele et al. (2022) arrived at this conclusion by estimating the dynamic energy budget (DEB) for *O. edulis* based on a comprehensive dataset. They validated it using laboratory experiments that measured growth at several temperature and food levels (Kamermans & Saurel, 2022), and with field data from two aquaculture sites in Denmark and the German Bight.

Even if an individual survives a heatwave, this exposure is found to have long-term effects. Short-term heat stress is found to significantly decrease (~26%) sperm production of American oysters (*Crassostrea virginica*) and enhance apoptosis of spermatogenetic cells (Nash & Rahman, 2019). Exposure to heat and low oxygen in the early life stages of *C. virginica* makes them more vulnerable to the same stressors, decreasing their ability for tissue and shell growth when exposed to the same stressors later (Donelan et al., 2021). Thermal tolerance of the blue mussel *Mytilus edulis* decreases when exposed to recurrent heat stress (Seuront et al., 2019). Sublethal effects of temperature in *O. edulis* have not yet been widely studied, and the current study suggests that they manifest at temperatures above 24°C.

3.4.3. Overall thermal sensitivity of respiration rate does not vary between winter and summer

The slopes of the change in respiration rate with temperature for both winter and summer oysters were analysed. Interestingly, findings revealed no significant difference in the slopes between the two physiological states. These results suggest that the overall thermal sensitivity of respiration rate does not significantly vary between winter and summer oysters. Thus, oysters in both seasons can adjust their metabolic rates similarly in response to temperature changes.

Additionally, the MO_2 at specific temperatures shared between the 18, 24, and 30 °C experiments were compared. At 18 and 30 °C, there were no significant differences in the MO_2 between the two physiological states. This implies that oysters in both winter and summer conditions exhibit similar metabolic activities at these temperatures.

However, a significant difference in respiration rates between winter and summer oysters was observed at 24°C. Winter oysters displayed a higher respiration rate than summer oysters at this temperature, indicating potential variations in the thermal requirements of oysters depending on their physiological state, particularly at intermediate temperatures like 24 °C. It is worth noting that the experimental design involved different temperature increments for winter and summer oysters, with winter oysters experiencing a 3 °C increment while summer oysters had a 2 °C increment. This discrepancy in temperature increments may have influenced the observed

differences in metabolic rates at 24 °C between the two physiological states. The gradual temperature increments might have contributed to a more stable and predictable increase in the metabolic rates of summer oysters, potentially impacting the overall findings.

This result also suggests that summer oysters might possess better physiological adaptation or acclimation to higher temperatures, enabling them to maintain more stable metabolic rates. However, further research and experimentation are required to confirm this hypothesis. Investigating oysters' specific mechanisms and physiological responses to different temperature increments would provide a more comprehensive understanding of their thermal adaptation capabilities.

3.5. Conclusion

In the investigation into the thermal requirements of European native oysters, observations that shed light on the intricate relationship between temperature and oyster physiology have been uncovered. Through careful examination and analysis, valuable insights into the adaptive responses of these bivalves have been gained, revealing their inherent ability to cope with environmental changes.

The findings suggest that *O. edulis* exhibits a preferred thermal range centred around 24°C, beyond which its physiological equilibrium is perturbed. Notably, an increase in oxygen consumption rate (MO_2) and the occurrence of cardiac arrests

were observed when these oysters were exposed to temperatures surpassing this optimal threshold. Additionally, 33°C was identified as the upper critical limit for this species.

As we face a world with increasing temperatures and more frequent heatwaves, it becomes imperative to recognise the vulnerability of *O. edulis* and implement effective conservation and management strategies. Therefore, to ensure the successful cultivation and growth of *O. edulis*, aquaculture practices must consider these thermal limits. Proper monitoring of water temperatures in farming settings is crucial, as excessively high temperatures can lead to stress, a declining condition index, and even mortality in bivalves, as shown in this study's findings. Implementing cooling systems or strategies to mitigate heat stress, such as shading or adjusting water flow, may be necessary to maintain suitable temperatures within the recommended range for *O. edulis.*

Additionally, the observations presented here show that the overall thermal sensitivity of MO_2 remains remarkably consistent between winter and summer oysters, implying a uniform capacity for metabolic adjustments in the face of temperature fluctuations. However, a notable dissimilarity emerges at 24°C, where winter oysters exhibit heightened respiration rates compared to their summer counterparts, implying potential divergences in thermal requisites influenced by physiological states. Further scientific inquiry is indispensable to unravel the intricate mechanisms underlying oyster adaptations, thereby fostering a comprehensive understanding of their response to varying temperature increments.

Moreover, a deeper understanding of the underlying mechanisms behind *O. edulis'* thermal adaptations will guide future research endeavours and inform the development of strategies to mitigate the adverse effects of warming. Further studies should investigate how changes in mean temperatures and the occurrence of heatwaves affect crucial biological processes in *O. edulis*, such as growth, reproduction, and immunity to diseases. By elucidating these responses, aquaculture practices can be refined to optimise production and minimise the negative impacts of thermal stress.

In addition to temperature changes, it is essential to examine the effects of heatwaves in conjunction with other climate change-related stressors. Factors such as hypoxia (low oxygen levels), acidification (increased seawater acidity), salinity stress, and harmful algal blooms can further compound the challenges faced by *O. edulis* in aquaculture settings. Understanding the interactive effects of multiple stressors is crucial for developing holistic management strategies that consider the complexity of the environment and aim to enhance the resilience of bivalve populations.

In conclusion, the vulnerability of *O. edulis* in the face of increasing temperatures and heatwaves necessitates proactive conservation and management efforts within aquaculture. By monitoring water temperatures, considering the species' thermal preferences and limits, and investigating the underlying mechanisms of thermal adaptations, we can improve the sustainability and resilience of *O. edulis* farming

practices. Additionally, studying the interactive effects of various stressors will enable the development of comprehensive strategies that account for the multifaceted challenges of climate change.

3.6. References

Allison, S., Hardy, M., Hayward, K., Cameron, T., & Underwood, G. (2019). Strongholds of *Ostrea edulis* populations in estuaries in Essex, SE England and their association with traditional oyster aquaculture: Evidence to support a MPA designation. Journal of the Marine Biological Association of the United Kingdom, 100, 1–10. https://doi.org/10.1017/S0025315419001048

Amorim, V. E., Gonçalves, O., Capela, R., Fernández-Boo, S., Oliveira, M., Dolbeth, M., Arenas, F., & Cardoso, P. G. (2020). Immunological and oxidative stress responses of the bivalve *Scrobicularia plana* to distinct patterns of heatwaves. Fish & Shellfish Immunology, 106, 1067–1077. https://doi.org/10.1016/j.fsi.2020.09.024

Bakhmet, I. N., & Khalaman, V. V. (2006). Heart rate variation patterns in some representatives of Bivalvia. Biology Bulletin, 33(3), 276–280. https://doi.org/10.1134/S1062359006030101

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bayne, B. L., & Widdows, J. (1978). The physiological ecology of two populations of *Mytilus edulis* L. Oecologia, 37(2), 137–162. https://doi.org/10.1007/BF00344987

Beiras, R., Camacho, A. P., & Albentosa, M. (1995). Short-term and long-term alterations in the energy budget of young oyster *Ostrea edulis* L. in response to temperature change. Journal of Experimental Marine Biology and Ecology, 186(2), 221–236. https://doi.org/10.1016/0022-0981(94)00159-B

Bennema, F. P., Engelhard, G., & Lindeboom, H. (2020). *Ostrea edulis* beds in the central North Sea: Delineation, ecology, and restoration. ICES Journal of Marine Science, 77, 2694–2705. https://doi.org/10.1093/icesjms/fsaa134

Boscolo-Galazzo, F., Crichton, K. A., Barker, S., & Pearson, P. N. (2018). Temperature dependency of metabolic rates in the upper ocean: A positive feedback to global climate change? Global and Planetary Change, 170, 201–212. https://doi.org/10.1016/j.gloplacha.2018.08.017

Child, A. R., & Laing, I. (1998). Comparative low temperature tolerance of small juvenile European, *Ostrea edulis* L., and Pacific oysters, *Magallana gigas* Thunberg. Aquaculture Research, 29(2), 103–113. https://doi.org/10.1046/j.1365-2109.1998.00934.x

Christidis, N., McCarthy, M., & Stott, P. A. (2020). The increasing likelihood of temperatures above 30 to 40°C in the United Kingdom. Nature Communications, 11(1), 3093. https://doi.org/10.1038/s41467-020-16834-0

Clarke, A., & Fraser, K. P. P. (2004). Why does metabolism scale with temperature? Functional Ecology, 18(2), 243–251. https://doi.org/10.1111/j.0269-8463.2004.00841.x

Deabes, E. A. M. (2020). The impact of thermal power stations on coastline and benthic fauna: Case study of El-Burullus power plant in Egypt. Results in Engineering, 7, 100128. https://doi.org/10.1016/j.rineng.2020.100128

Department of Energy and Climate Change. (2011). National Policy Statement for Nuclear Power Generation (EN-6). Retrieved January 11, 2022, www.official-documents.gov.uk.

Donelan, S. C., Breitburg, D., & Ogburn, M. B. (2021). Context-dependent carryover effects of hypoxia and warming in a coastal ecosystem engineer. Ecological Applications, 31(4), e02315. https://doi.org/10.1002/eap.2315

Eymann, C., Götze, S., Bock, C., Guderley, H., Knoll, A. H., Lannig, G., Sokolova, I. M., Aberhan, M., & Pörtner, H.-O. (2020). Thermal performance of the European native oyster, *Ostrea edulis* (Linnaeus, 1758)—Explaining ecological findings under climate change. Marine Biology, 167(2), 17. https://doi.org/10.1007/s00227-019-3620-3

Ferreira, E. O., Anttila, K., & Farrell, A. P. (2014). Thermal Optima and Tolerance in the Eurythermic Goldfish (*Carassius auratus*): Relationships between Whole-Animal Aerobic Capacity and Maximum Heart Rate. Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches, 87(5), 599–611. https://doi.org/10.1086/677317

Ferreira-Rodriguez, N., Fernandez, I., Leonor Cancela, M., & Pardo, I. (2018). Multibiomarker response shows how native and non-native freshwater bivalves differentially cope with heat-wave events. Aquatic Conservation-Marine and Freshwater Ecosystems, 28(4), 934–943. https://doi.org/10.1002/aqc.2884

Genner, M., Freer, J., & Rutterford, L. (2017). Future of the sea: Biological responses to ocean warming. Foresight, Government Office for Science,UK, 30.

Ghaffari, H., Wang, W., Li, A., Zhang, G., & Li, L. (2019). Thermotolerance Divergence Revealed by the Physiological and Molecular Responses in Two Oyster Subspecies of *Magallana gigas* in China. Frontiers in Physiology, 10. https://doi.org/10.3389/fphys.2019.01137

Giomi, F., & Poertner, H. (2013). A role for haemolymph oxygen capacity in heat tolerance of eurythermal crabs. Frontiers in Physiology, 4, 110. https://doi.org/10.3389/fphys.2013.00110

González, R., & González, G. (1985). Europeanf lat oyster (Ostrea edulis) cultured in rafts, in the Ría de Arosa (Galicia). Bulletin. Spanish Institute of Oceanography, 2(2), 9–16.

Halpern, B. S., Frazier, M., Potapenko, J., Casey, K. S., Koenig, K., Longo, C., Lowndes, J. S., Rockwood, R. C., Selig, E. R., Selkoe, K. A., & Walbridge, S. (2015). Spatial and temporal changes in cumulative human impacts on the world's ocean. Nature Communications, 6(1), Article 1. https://doi.org/10.1038/ncomms8615

Haure, J., Penisson, C., Bougrier, S., & Baud, J. P. (1998). Influence of temperature on clearance and oxygen consumption rates of the native oyster *Ostrea edulis*: Determination of allometric coefficients. Aquaculture, 169(3), 211–224. https://doi.org/10.1016/S0044-8486(98)00383-4

Hiebenthal, C., Philipp, E. E. R., Eisenhauer, A., & Wahl, M. (2012). Interactive effects of temperature and salinity on shell formation and general condition in

Baltic Sea *Mytilus edulis* and *Arctica islandica*. Aquatic Biology, 14(3), 289–298. https://doi.org/10.3354/ab00405

Hutchinson, S., & Hawkins, L. E. (1992). Quantification of the physiological responses of the European native oyster *Ostrea edulis* to temperature and salinity. Journal of Molluscan Studies, 58(2), 215–226. https://doi.org/10.1093/mollus/58.2.215

IUCN (2013). Guidelines for Reintroductions and Other Conservation Translocations. IUCN SSC Conservation Translocation Specialist Group. Retrieved January 11, 2022, from https://iucn-ctsg.org/project/new-rsg-re-introductions-guidelines-2013/

Kamermans, P., & Saurel, C. (2022). Interacting climate change effects on mussels (*Mytilus edulis* and *M. galloprovincialis*) and oysters (*Magallana gigas* and *Ostrea edulis*): Experiments for bivalve individual growth models. Aquatic Living Resources, 35, 1. https://doi.org/10.1051/alr/2022001

Laing, I., Walker, P., & Areal, F. (2006). Return of the native – is European oyster (*Ostrea edulis*) stock restoration in the UK feasible? Aquatic Living Resources, 19(3), 283–287. https://doi.org/10.1051/alr:2006029

Lannig, G., Cherkasov, A. S., Pörtner, H.-O., Bock, C., & Sokolova, I. M. (2008). Cadmium-dependent oxygen limitation affects temperature tolerance in eastern oysters (*Crassostrea virginica* Gmelin). American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 294(4), R1338–R1346. https://doi.org/10.1152/ajpregu.00793.2007

Lannig, G., Flores, J. F., & Sokolova, I. M. (2006). Temperature-dependent stress response in oysters, *Crassostrea virginica*: Pollution reduces temperature

tolerance in oysters. Aquatic Toxicology, 79(3), 278–287. https://doi.org/10.1016/j.aquatox.2006.06.017

Lemasson, A. J., Hall-Spencer, J. M., Kuri, V., & Knights, A. M. (2019). Changes in the biochemical and nutrient composition of seafood due to ocean acidification and warming. Marine Environmental Research, 143, 82–92. https://doi.org/10.1016/j.marenvres.2018.11.006

Lenth R (2023). emmeans: Estimated Marginal Means, aka Least-Squares Mean. R package version 1.8.6, <https://CRAN.R-project.org/package=emmeans>.

Lown, A. E., Hepburn, L. J., Dyer, R., & Cameron, T. C. (2020). From individual vital rates to population dynamics: An integral projection model for European native oysters in a marine protected area. Aquatic Conservation: Marine and Freshwater Ecosystems, 30(11), 2191–2206. https://doi.org/10.1002/aqc.3445

Lucas, A., & Beninger, P. G. (1985). The use of physiological condition indices in marine bivalve aquaculture. Aquaculture, 44(3), 187–200. https://doi.org/10.1016/0044-8486(85)90243-1

Maathuis, M. A. M., Coolen, J. W. P., van der Have, T., & Kamermans, P. (2020). Factors determining the timing of swarming of European native oyster (*Ostrea edulis* L.) larvae in the Dutch Delta area: Implications for native oyster restoration. Journal of Sea Research, 156, 101828. https://doi.org/10.1016/j.seares.2019.101828

MacKenzie, C. L. (1997). The History, Present Condition, and Future of the Molluscan Fisheries of North and Central America and Europe, Volume 3: Europe. U.S. Department of Commerce.

Mackenzie, C. L., Lynch, S. A., Culloty, S. C., & Malham, S. K. (2014). Future Oceanic Warming and Acidification Alter Immune Response and Disease Status in a Commercial Shellfish Species, *Mytilus edulis* L. Plos One, 9(6), e99712. https://doi.org/10.1371/journal.pone.0099712

Mackenzie, C. L., Lynch, S. A., Culloty, S. C., & Malham, S. K. (2014). Future Oceanic Warming and Acidification Alter Immune Response and Disease Status in a Commercial Shellfish Species, *Mytilus edulis* L. Plos One, 9(6), e99712. https://doi.org/10.1371/journal.pone.0099712

Mackenzie, C. L., Ormondroyd, G. A., Curling, S. F., Ball, R. J., Whiteley, N. M., & Malham, S. K. (2014). Ocean Warming, More than Acidification, Reduces Shell Strength in a Commercial Shellfish Species during Food Limitation. Plos One, 9(1), e86764. https://doi.org/10.1371/journal.pone.0086764

MacLean, H. J., Sørensen, J. G., Kristensen, T. N., Loeschcke, V., Beedholm, K., Kellermann, V., & Overgaard, J. (2019). Evolution and plasticity of thermal performance: An analysis of variation in thermal tolerance and fitness in 22 *Drosophila* species. Philosophical Transactions of the Royal Society B: Biological Sciences, 374(1778), 20180548. https://doi.org/10.1098/rstb.2018.0548

McCarthy, M., Armstrong, L., & Armstrong, N. (2019). A new heatwave definition for the UK. Weather, 74(11), 382–387. https://doi.org/10.1002/wea.3629

Meehl, G. A., & Tebaldi, C. (2004). More Intense, More Frequent, and Longer Lasting Heat Waves in the 21st Century. Science, 305(5686), 994–997. https://doi.org/10.1126/science.1098704

Met Office,UK (2022). A milestone in UK climate history. Met Office. Retrieved January 11, 2022, from https://www.metoffice.gov.uk/about-us/press-office/news/weather-and-climate/2022/july-heat-review

Met Office, UK (2020). Heatwave continues for parts of UK – August 2020. Met Office. Retrieved January 11, 2022, from https://www.metoffice.gov.uk/about-us/press-office/news/weather-and-climate/2020/heatwave-continues-august.

Miller, N. A. & Stillman, J. H. (2012) Physiological Optima and Critical Limits. Nature Education Knowledge 3(10):1

Miossec, L., Le Deuff, R.-M., & Goulletquer, P. (2009). Alien species alert: *Magallana gigas* (Pacific oyster). ICES Cooperative Research Report, 299. https://archimer.ifremer.fr/doc/00000/6945/

Muggeo VM (2003). “Estimating regression models with unknown break-points.” Statistics in Medicine, 22, 3055–3071

Nash, S., & Rahman, M. S. (2019). Short-term heat stress impairs testicular functions in the American oyster, *Crassostrea virginica*: Molecular mechanisms and induction of oxidative stress and apoptosis in spermatogenic cells. Molecular Reproduction and Development, 86(10), 1444–1458. https://doi.org/10.1002/mrd.23268

Olsen, O. T. (1883). The piscatorial atlas of the North Sea, English Channel, and St. George’s Channels: Illustrating the fishing ports, boats, gear, species of fish (how, where, and when caught), and other information concerning fish and fisheries. Taylor & Francis, London, UK.

OSPAR (2020). European native oyster and *Ostrea edulis* beds. https://oap.ospar.org/en/ospar-assessments/committee-assessments/biodiversity-committee/status-assesments/european-flat-oyster/

Payton, S. L., Johnson, P. D., & Jenny, M. J. (2016). Comparative physiological, biochemical and molecular thermal stress response profiles for two unionid freshwater mussel species. Journal of Experimental Biology, 219(22), 3562–3574. https://doi.org/10.1242/jeb.140129

Peperzak, L. (2005). Future increase in harmful algal blooms in the North Sea due to climate change. Water Science and Technology, 51(5), 31–36. https://doi.org/10.2166/wst.2005.0102

Peyre, M., Bernasconi, S. B., Lavaud, R., Casas, S., & La Peyre, J. (2019). Eastern oyster clearance and oxygen consumption rates in response to acute and chronic exposure to suspended sediment loads. Journal of Sea Research, 157, 101831. https://doi.org/10.1016/j.seares.2019.101831

Pörtner, H.-O., Bock, C., & Mark, F. C. (2017). Oxygen- and capacity-limited thermal tolerance: Bridging ecology and physiology. Journal of Experimental Biology, 220(15), 2685–2696. https://doi.org/10.1242/jeb.134585

Preston, J., Gamble, C., Debney, A., Helmer, L., Hancock, B., & zu Ermgassen, P. S. E. (eds). (2020). European Native oyster Habitat Restoration Handbook. The Zoological Society of London, UK., London, UK.

Quante, M., & Colijn, F. (2016). North Sea Region Climate Change Assessment. Springer International Publishing.

Robert, R., Vignier, J., & Petton, B. (2017). Influence of feeding regime and temperature on development and settlement of oyster *Ostrea edulis* (Linnaeus, 1758) larvae. Aquaculture Research, 48(9), 4756–4773. https://doi.org/10.1111/are.13297

Sawusdee, A. (2015). Restoration of the European native oyster *Ostrea edulis* using elevated broodstock reefs [Phd, University of Southampton]. https://eprints.soton.ac.uk/386063/

Sea water temperature Mersea Island | United Kingdom. (2023). SeaTemperature.info. Retrieved January 11, 2023, from https://seatemperature.info/mersea-island-water-temperature.htm

Seo, E., Sazi, T., Togawa, M., Nagata, O., Murakami, M., Kojima, S., & Seo, Y. (2016). A portable infrared photoplethysmograph: Heartbeat of *Mytilus galloprovincialis* analyzed by MRI and application to *Bathymodiolus septemdierum*. Biology Open, 5(11), 1752–1757. https://doi.org/10.1242/bio.020909

Seuront, L., Nicastro, K. R., Zardi, G. I., & Goberville, E. (2019). Decreased thermal tolerance under recurrent heat stress conditions explains summer mass mortality of the blue mussel *Mytilus edulis*. Scientific Reports, 9(1), 17498. https://doi.org/10.1038/s41598-019-53580-w

Shpigel, M., Barber, B. J., & Mann, R. (1992). Effects of elevated temperature on growth, gametogenesis, physiology, and biochemical composition in diploid and triploid Pacific oysters, *Magallana gigas* Thunberg. Journal of Experimental Marine Biology and Ecology, 161(1), 15–25. https://doi.org/10.1016/0022-0981(92)90186-E

Smyth, D. M., Horne, N. S., Ronayne, E., Millar, R. V., Joyce, P. W. S., Hayden-Hughes, M., & Kregting, L. (2020). Wild gregarious settlements of *Ostrea edulis* in a semi-enclosed sea lough: A case study for unassisted restoration. Restoration Ecology, 28(3), 645–654. https://doi.org/10.1111/rec.13124

Stechele, B., Maar, M., Wijsman, J., Van der Zande, D., Degraer, S., Bossier, P., & Nevejan, N. (2022). Comparing life history traits and tolerance to changing environments of two oyster species (*Ostrea edulis* and *Magallana gigas*) through Dynamic Energy Budget theory. Conservation Physiology, 10(1), coac034. https://doi.org/10.1093/conphys/coac034

Stenseng, E., Braby, C. E., & Somero, G. N. (2005). Evolutionary and acclimation-induced variation in the thermal limits of heart function in congeneric marine snails (genus *Tegula*): Implications for vertical zonation. The Biological Bulletin, 208(2), 138–144. https://doi.org/10.2307/3593122

Stillman, J. H. (2002). Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, genus petrolisthes. Integrative and Comparative Biology, 42(4), 790–796. https://doi.org/10.1093/icb/42.4.790

Tan, K., Zhang, H., & Zheng, H. (2020). Selective breeding of edible bivalves and its implication of global climate change. Reviews in Aquaculture, 12(4), 2559–2572. https://doi.org/10.1111/raq.12458

Taylor, A. M., Maher, W. A., & Ubrihien, R. P. (2017). Mortality, condition index and cellular responses of *Anadara trapezia* to combined salinity and temperature stress. Journal of Experimental Marine Biology and Ecology, 497, 172–179. https://doi.org/10.1016/j.jembe.2017.09.023

Trueman, E. R., & Lowe, G. A. (1971). The effect of temperature and littoral exposure on the heart rate of a bivalve mollusc, *Isognomum alatus*, in tropical conditions. Comparative Biochemistry and Physiology Part A: Physiology, 38(3), 555–564. https://doi.org/10.1016/0300-9629(71)90122-8

UK Hydrographic office (2022). ADMIRALTY EasyTide. ADMIRALTY EasyTide. Retrieved January 11, 2022, from https://easytide.admiralty.co.uk/

Wilder, S. M., Raubenheimer, D., & Simpson, S. J. (2016). Moving beyond body condition indices as an estimate of fitness in ecological and evolutionary studies. Functional Ecology, 30(1), 108–115. https://doi.org/10.1111/1365-2435.12460

Xing, Q., Li, Y., Guo, H., Yu, Q., Huang, X., Wang, S., Hu, X., Zhang, L., & Bao, Z. (2016). Cardiac performance: A thermal tolerance indicator in scallops. Marine Biology, 163(12), 244. https://doi.org/10.1007/s00227-016-3021-9

Zrnčić, S., Oraić, D., Mihaljević, Ž., & Zanella, D. (2007). Impact of varying cultivation depths on growth rate and survival of the European native oyster *Ostrea edulis*, L. Aquaculture Research, 38(12), 1305–1310. https://doi.org/10.1111/j.1365-2109.2007.01804.x

CHAPTER FOUR

Combined effects of heatwave and *Prorocentrum lima* bloom on the toxin accumulation, metabolism, immune response, and survival of the Pacific rock oysters *Magallana gigas*

Abstract

The increasing sea-surface temperature facilitates the intensification of toxic algal blooms, two important stressors for benthic marine invertebrates. Corroborated by ongoing climate change, these two stressors can co-occur more frequently in the future. Despite this, few studies have investigated the effects of both stressors on the marine environment, specifically in sedentary bivalves, which cannot relocate in response to increasing water temperatures and are exposed to toxic algae while they filter-feed. This study investigated the immediate and delayed effects of a heatwave and a bloom (3×10^6 cells L^{-1}) of the diarrhetic shellfish-toxin-producing algal dinoflagellate *Prorocentrum lima* on the survival, physiology (oxygen consumption rate, condition index, immune parameters), and toxin accumulation in the Pacific rock oyster *Magallana* (*Crassostrea*) *gigas*. Oysters exposed to both stressors contained higher mean diarrhetic shellfish-toxin concentrations (mean: 173.26 ± 1 SE:19.78 µg kg^{-1} soft tissue) than those exposed to *P. lima* bloom alone (120.38 ± 20.90 µg kg^{-1} soft tissue) and exceeded maximum permitted levels for human consumption, which is 160 µg kg^{-1} soft tissue. Furthermore, exposure to individual stressors and their combination modified the physiology of *M. gigas.* Oysters exposed to heatwave alone had significantly higher oxygen consumption rate than the control (0.73 ± 0.06 mg O_2 h^{-1} g^{-1} ash-free dry weight); however, this was not observed in oysters exposed to both heatwave and *P. lima* (0.49 ± 0.06 mg O_2 h^{-1} g^{-1}). This alteration of the metabolic response in the presence of *P. lima* may affect the ability of rock oysters to survive an environment that requires an immediate response. Immunomodulation, through changes in total haemocyte count, was observed in oysters exposed to *P. lima* and combined stressors 21 days after

stressor exposure. The findings of this study highlight the vulnerability of rock oysters to foreseen increased frequency of heatwaves and toxic algal blooms and the likelihood of more toxic shellfish events with warming coasts. To ensure the production of safe bivalves for consumption, constant and more efficient monitoring of toxic algal cells and toxins in the coasts and shellfish meat should be implemented, especially during warmer months when they can accumulate more toxins.

4.1. Introduction

4.1.1. Heatwaves can promote harmful algal bloom

The growing global demand for seafood to meet the nutritional needs of a rapidly expanding population has placed significant pressure on aquaculture as a sustainable food production method. However, the viability and reliability of aquaculture face substantial challenges in the face of environmental change.

Climate change-induced heatwaves are becoming more frequent and intense, posing increased risks and economic losses for bivalve aquaculture operations. As discussed in the previous chapter of this thesis, higher seawater temperatures during heatwaves disrupt the optimal conditions necessary for successful cultivation. They can result in the mortality of farmed bivalves. The susceptibility of oysters and other bivalves to mass mortality events following heatwaves directly impacts bivalve

aquaculture. Heat stress hampers growth rates, impairs reproduction, and compromises immune function in bivalves, rendering them more vulnerable to diseases and infections (Colletta & Westfall, 2021; Hagenbuch, 2021; Timms, 2019; Troost, 2018). The loss of mature oysters significantly impacts production levels and disrupts the supply chain, affecting local economies and global seafood markets (Herring et al., 2019; Meehl & Tebaldi, 2004; Schiermeier, 2018).

Additionally, heatwaves pose a considerable challenge to the aquaculture industry by promoting the emergence of harmful algal blooms (HABs), which have gained global attention for their profound impact on marine ecosystems and human health (Wang & Wu, 2009). Planktonic microalgae, serving as a food source for primary consumers like suspension-feeding bivalves, can occasionally proliferate, forming ‘algal blooms’ characterised by millions of cells per litre (Lloyd et al., 2013). While algal blooms can offer benefits to aquaculture and wild fisheries, they also have the potential to become harmful algal blooms (HABs), causing significant economic damage to aquaculture, fisheries, and tourism industries, as well as adverse effects on the environment, animal health, and human well-being (Turner et al., 2021).

Heatwaves directly impact the physiology of microalgae and promote their growth and reproduction. Warmer waters stimulate increased metabolic activity and accelerated algae growth rates, creating favourable conditions for the development of algal blooms. Furthermore, heatwaves exacerbate the effects of other environmental factors that contribute to HAB formation, such as nutrient availability and water layer stratification (Visser et al., 2016; Brandenburg et al., 2021).

4.1.2. Harmful algal blooms are a challenge in bivalve aquaculture

The intensification and frequency of HABs are influenced by a range of climate change-associated factors, extending beyond just warming and heatwaves. Coastal zones, which often experience the co-occurrence of HABs and elevated CO_2 levels, present unique challenges for aquaculture. The changing chemistry of seawater, caused by increased concentrations of CO_2, can have profound effects on the physiology of toxic algae, potentially promoting their growth and toxin production. CO_2 is crucial in algal photosynthesis, making it a vital resource for metabolic processes. Studies indicate that certain harmful algae species exhibit accelerated growth rates under acidified conditions resulting from CO_2 absorption. This phenomenon contributes to the development and persistence of toxic algal blooms, further complicating matters for aquaculturists (Brandenburg et al., 2019; Gao et al., 2021; Gobler et al., 2017; Hallegraeff, 2010; Moore et al., 2008).

There are 5,000 extant species of phytoplankton, and 300 species are known to occasionally occur in such high abundance that they visibly discolour the surface of the sea (e.g., red tides formed by dinoflagellates) (Hallegraeff et al., 2003). This excessive algal growth leads to a decrease in light and oxygen in the water, which other aquatic organisms need to survive. Additionally, 80 phytoplankton species produce harmful toxins that can bioaccumulate finfish and shellfish and cause

disease and physical harm to other animals, including humans. It is worth noting that these toxin-producing phytoplankton can sometimes form low biomass ‘blooms’ that may not be visually apparent or dominate the phytoplankton community but still pose a significant risk to shellfish, leading to shellfish-associated illnesses (Berdalet et al., 2015; Hallegraeff et al., 2003).

During HABs, the accumulation of phycotoxins in the flesh of filter-feeding bivalves can reach levels dangerous for human health and result in the closure of shellfish-production sites. Different categories of human shellfish poisoning have been identified, including paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and azaspiracid shellfish poisoning (AZP) (Butzke et al., 2013). In the European Atlantic Arc, closures of shellfish production sites are primarily caused by the regular occurrence of diarrhetic shellfish toxins (DST), specifically the OA group of toxins, during the summer period (Fernandes-Salvador et al., 2021).

Among the toxin-producing phytoplankton, *Prorocentrum* is a genus of dinoflagellates distributed globally. In temperate regions, they frequently thrive and bloom in eutrophic marine environments during the summer when water temperatures exceed 24°C (Heil et al., 2005; Türkoğlu, 2010; Türkoğlu & Erdoğan, 2010), but blooms are also observed in late winter and early spring (Hu et al., 2022; Li et al., 2021; Tas & Okuş, 2011). *Prorocentrum lima*, a species within this genus, is found worldwide in tropical, subtropical, and temperate regions, inhabiting marine and brackish waters (Türkoglu, 2016). Commonly found in the UK waters, *P. lima* is

an epiphytic-benthic dinoflagellate that exhibits weak motility and can disperse over long distances by "rafting" on floating detritus (Coates et al., 2014, 2015; Harrison et al., 2016, 2017; Leftley & Hannah, 2009; Parks et al., 2018, 2019, Tarazona-Janampa, 2020). It has been observed in association with macroalgae and macrophytes in Fleet Lagoon, Dorset, where shellfish production sites occasionally face closure due to elevated levels of Diarrhetic Shellfish Toxins (DST) in shellfish (Foden et al., 2005).

HAB events can lead to the closure of aquaculture operations as a precautionary measure to protect human health. The presence of toxic algae in the surrounding waters can contaminate bivalves, making them unsuitable for consumption and resulting in significant economic losses for aquaculturists (Anderson et al., 2012; Brandenburg et al., 2019; Gao et al., 2021; Gobler et al., 2017; Hallegraeff, 2010; Moore et al., 2008). The closure of aquaculture sites during HAB events disrupts the production and supply of bivalves, affecting both local markets and global seafood trade. Aquaculturists may experience financial hardships due to the loss of income and investment in affected stocks. The long-term reputation of aquaculture operations can also be affected, as consumers may lose confidence in the safety and quality of bivalve products following HAB-related closures.

Moreover, the negative impacts of HABs on bivalve health and growth can further challenge aquaculture operations. Bivalves may experience reduced growth rates and impaired physiological functions due to the stress caused by exposure to toxic algae. This can result in diminished yields and prolonged production cycles,

impacting the profitability and efficiency of aquaculture ventures (Lassudrie et al., 2020; Li et al., 2002; Tan et al., 2020).

As our planet's climate continues to evolve, HAB occurrences are expected to shift, with higher latitudes becoming more suitable for algal growth. In comparison, lower latitudes become excessively warm, limiting algal bloom formation (Brandenburg et al., 2019).

4.1.3. Combined effects of climate drivers and harmful algal blooms

Despite the frequent co-occurrence of heatwaves and HAB in coastal zones, the combined effects of these stressors on aquatic organisms remain poorly understood. Limited knowledge exists regarding their combined impact. One relevant study by Turner et al. (2019) focused on understanding the influence of multiple climate drivers on the physiological health and toxin load of a tropical filter-feeding clam called *Meretrix meretrix*. They manipulated climate drivers such as warming, freshening, and acidification, along with the presence of toxic microalgae, to simulate projected marine climates. Using structural equation modelling, they observed direct adverse effects on metabolic and immunological function in the clams exposed to these climate conditions. The results were more pronounced when clams were simultaneously exposed to multiple climate drivers. Interestingly, when the clams were fed a PST-producing dinoflagellate (*Alexandrium* sp.), their physiological responses and toxin load exhibited different patterns than clams exposed to climate

drivers alone. Exposure to the toxic dinoflagellate caused the clams to show no response to increased temperature or combined treatments of increased temperature, acidification, and decreased salinity. Furthermore, the study found that warming had a decreasing effect on the toxin load, and this reduction was more noticeable when the clams were exposed to a combination of warming, freshening, and acidification. However, it is essential to note that this result on toxin load did not reach statistical significance.

In another study by Farrel (2015), the accumulation of PSTs in oysters (*Saccostrea glomerata* and diploid and triploid *Crassostrea gigas*) acclimated to different temperatures was examined. The researchers observed that warm-acclimated oysters had lower toxin concentrations after *consuming Alexandrium minutum*, indicating reduced toxin accumulation. However, these warm-acclimated oysters also exhibited lower detoxification rates, suggesting a potential trade-off.

Similarly, Tang (2021) investigated the effects of seawater surface temperature (SST) and toxic algal abundance (TAA) on PSTs in mussels (*Mytilus coruscus*). The mussels were exposed to *A. catenella* under various environmental conditions. The study revealed that increasing SST resulted in lower PST levels in mussels due to rapid toxin elimination, while higher TAA led to increased PST concentrations.

Braba (2018) focused on the impact of seawater temperature increase and acidification on PST in shellfish, specifically mussels (*Mytilus galloprovincialis*). The mussels were acclimated to different climate change scenarios and exposed to

Gymnodinium catenatum. Under current conditions, high toxicity levels were observed. However, mussels adjusted to warming or acidification exhibited significantly lower toxin levels, and the combined effect of warming and acidification led to slightly higher toxicity but still lower than the control.

The studies above offer significant insights into the combined effects of climate drivers and PST-associated algae on the physiology and toxin accumulation of different shellfish species. They contribute valuable knowledge to understanding the potential implications for shellfish production and food safety in various regions. However, it is important to acknowledge a gap in the research concerning diarrhetic shellfish toxin-associated algae, which is more commonly occurring (Fernandes-Salvador et al., 2021). Further investigation and studies are needed to bridge this gap and provide a comprehensive understanding of the combined effects of climate drivers on the physiology and toxin dynamics associated with diarrhetic shellfish toxin in shellfish species. Such research would significantly ensure shellfish safety and production in different regions.

4.1.4. Chapter aims

This study was initiated to address the knowledge gap discussed in the previous section and assist the aquaculture industry. The study focused on investigating the combined effects of increased temperature (through a simulated heatwave) and the presence of the diarrhetic shellfish toxin (DST)-producing dinoflagellate *P. lima* on the widely cultured and commercially important Pacific rock oyster *Magallana gigas.*

This study aimed to identify and evaluate the potential immediate and delayed effects of these stressors on the physiological response of *M. gigas*. Various parameters include toxin accumulation, metabolic activity (through the measurement of oxygen consumption rate), body condition, immune function (total haemocyte count, haemocyte neutral red uptake, haemolymph protein concentration), and survival.

Based on the literature and the previously stated aims, I hypothesise that the co-occurrence of a heatwave and *P. lima* bloom will lead to the following immediate and delayed effects in *M. gigas:*

1. Lower Toxin Accumulation: The simultaneous exposure to a heatwave and *P. lima* bloom is expected to lead to reduced toxin accumulation in *M. gigas* compared to the group exposed solely to *P. lima* bloom.

2. No Response to Warming: *M. gigas* exposed to the combined treatment of heatwave and *P. lima* bloom will exhibit no significant physiological response to the warming conditions. This could be due to the potential interactive effects of *P. lima* toxins and elevated temperature, which may hinder the normal physiological responses of the oysters to warming.

3. Reduced Body Condition, Immune Function, and Increased Mortality: *M. gigas* exposed to the combined stressors of heatwave and *P. lima* bloom will experience

reduced body condition, compromised immune function, and increased mortality rates. The simultaneous heatwave and *P. lima* bloom may impose additional stress on the oysters, impairing physiological health and increasing susceptibility to diseases or other adverse effects.

The findings of this study hold significant implications for coastal managers and stakeholders involved in bivalve aquaculture. Understanding the interactions between heatwaves, DST-associated algal blooms, and their impacts on key aquaculture species like *M. gigas* will provide crucial baseline information for assessing the viability and dependability of bivalve aquaculture in coastal seas affected by anthropogenic stressors. Additionally, the insights gained from this research can contribute to developing effective management strategies to mitigate the adverse effects of HAB and environmental change on aquaculture systems.

4.2. Methods

4.2.1. Animal Collection and Husbandry

Adult Pacific rock oysters (*M. gigas*) (mean: 91.96 ± SD: 17.24 mm) were obtained from Mersea Island, Essex, UK on 2 October 2021 and acclimated in the laboratory for 14 days in locally collected seawater, filtered through a polypropylene felt filter bag (pore size 25 µm; Cole-Palmer, UK), set to a controlled temperature of 14°C,

matching the field water temperature during collection. During acclimation and the entire duration of the experiment, oysters were exposed to a consistent 12:12 day-night cycle. This acclimation procedure was designed to minimise any potential effects of seasonal variations and entrain the oysters to a stable diurnal rhythm, ensuring that the observed responses in the experiment were primarily driven by the experimental treatments rather than external factors related to oyster collection timing.

Starting from the second day of the acclimation period, the water temperature in the acclimation tank was raised by one °C every day until it reached 20 °C, the average water temperature of Pyefleet Creek, Mersea, in August 2020, and the control temperature in this experiment. Salinity was measured using the Practical Salinity Scale and adjusted to a salinity of 35 daily by adding reverse osmosis water when necessary. Nitrate, nitrite, and ammonia concentrations were monitored every other day using a test kit (Red Sea Marine Care Test Kit, Tel Aviv, Israel). During the acclimation period, the oysters were fed a commercial feed containing a mix of five marine microalgae including *Isochrysis* sp., *Pavlova* sp., *Tetraselmis* sp., *Thalassiosira weissflogii and Thalassiosira pseudonana* (Shellfish Diet 1800®; Reed Mariculture, California, USA).

4.2.2. Microalgae

The *P. lima* used in this study was isolated from Vigo, Spain, and purchased from the Centre for Culture of Algae and Protozoa (CCAP) in 2018 (Oban, Scotland, UK). The dinoflagellate cultures were maintained at the University of Essex in flasks with

1 L K medium (Keller, 1987) made with filtered natural seawater (0.2 µm), at 15°C and under a 12:12 light-dark cycle at mean light intensity (± standard error) of 221 ± 12 µmol m^{-2} s^{-1}, measured using a light meter (Li-Cor Li-250A, California, USA). In these standard culture conditions, the total toxin concentration of the algae was 2.26 pg/cell (OA: 1.84 pg/cell; DTX: 0.42 pg/cell). The microalgae *Thalassiosira pseudonana*, maintained in the same culture conditions as *P. lima,* was used as a non-toxic diet in the control treatment. Cell counts for both microalgal cultures were determined using a Neubauer haemocytometer and a compound microscope (Olympus BH-s BHT; Shinjuku, Japan) at 100 × magnification. Cells were harvested by centrifugation (800 × g for 10 min) and adjusted to the required concentrations with filtered seawater (FSW).

4.2.3. Experimental design

The experiment consisted of five days exposure of oysters to each of the four treatment combinations of two conditions: with or without the simulated heatwave; and with or without *P. lima* (Fig 4.1). The work was conducted in a static system, with constant aeration, in 6L plastic tanks (Exo Terra Faunarium; Montreal, Canada) with 5L seawater. Each condition included three replicate tanks, and each containing six oysters (3 replicates each to quantify short-term and long-term effects, N=72). The temperature in the tanks was maintained using independent thermostats (Ink bird

Temperature Controller, Guangdong, China).

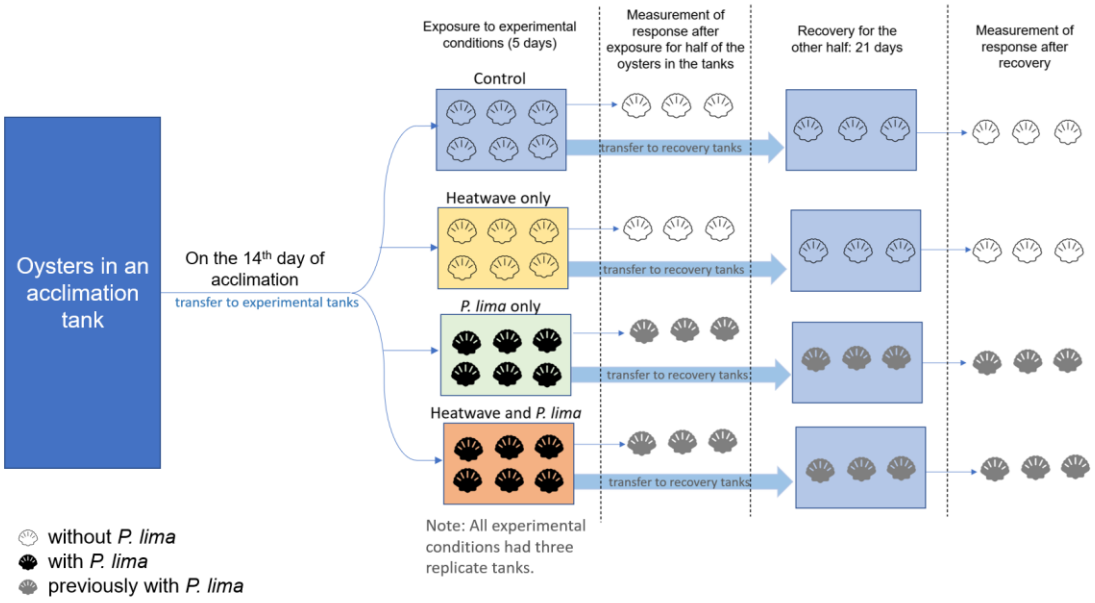


Figure 4.1 *Schematic diagram of the experimental design with four treatment combinations of two conditions: with or without the simulated heatwave; and with or without* P. lima*. Each 6 L tank contained six oysters (*M. gigas*) exposed to experimental conditions for five days. All four conditions had three replicate tanks. Three oysters from each tank were evaluated for response immediately after exposure and the rest were allowed to recover for 21 days to investigate possible delayed responses.*

To simulate the local heatwave conditions observed in Pyelfeet Creek, Essex in 2020, the water temperature of the tanks assigned to the heatwave treatment was raised from 20 to 22°C on day 1, then increased to 24°C on day two, and then on day three raised to 25 °C, the peak of the heatwave. The tanks assigned to heatwave conditions were maintained at this temperature for the remainder of the experiment (2 days). The temperature ramp up rate was at 0.02 °C minute $^{-1}$, calculated using recordings of the water temperature in the tanks using HOBO data loggers (Onset Computer Corp, MA, USA). The control temperature of 20 °C was based on the mean water temperature of Pyefleet Creek in August 2020, which is

also representative of local mean summer seawater temperatures for the period 2000-2010 (Centre for Environment, Fisheries and Aquaculture Science (CEFAS) Bradwell Station data). Figure 4.2 shows a schematic diagram of the of heatwave and control temperature conditions from acclimation to recovery period).

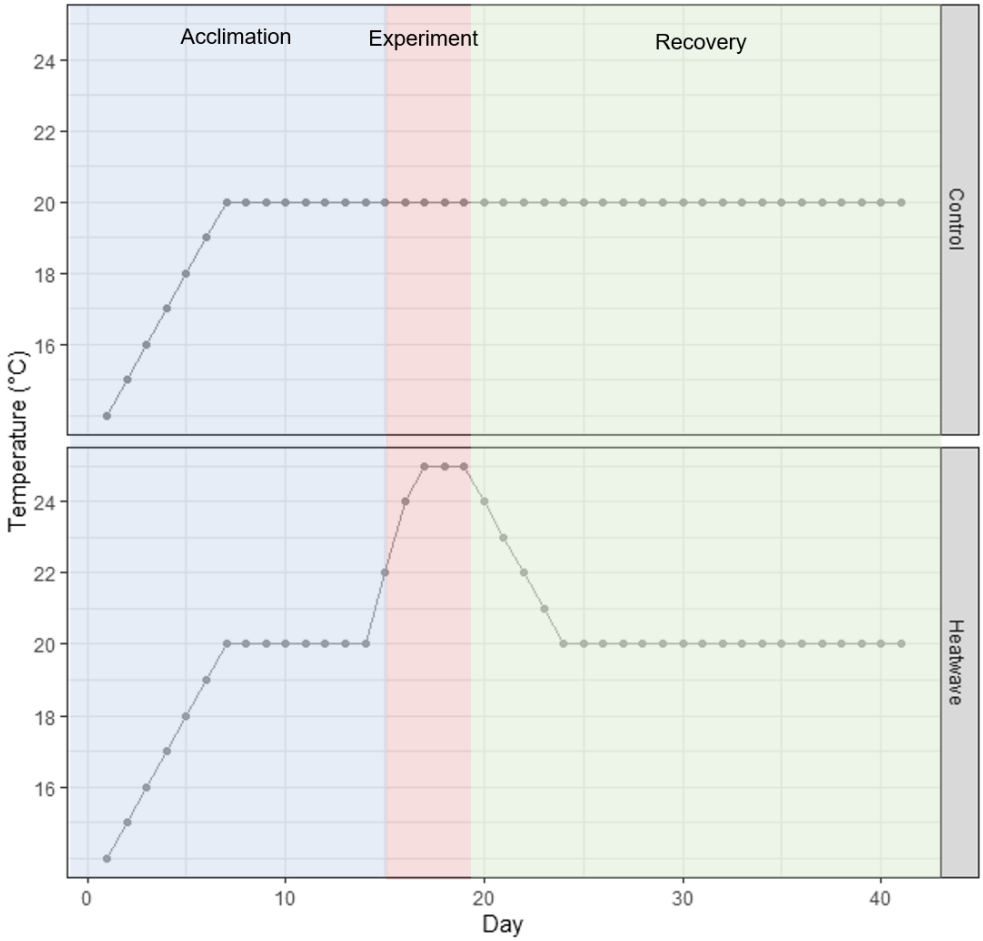


Figure 4.2 *Schematic diagram showing the temperatures oysters were exposed to in the control and heatwave conditions from the 14-day acclimation, 5-day experimental, and 21-day recovery period. The temperature ramp up rate was at 0.02 °C minute* $^{-1}$

Oysters in the toxic algal bloom treatment were exposed to a daily suspension of *P. lima* (Equivalent Spherical Diameter (ESD): 35 µm; 3×10^6 cells L^{-1}), simulating a natural bloom (Türkoglu, 2016). The control group was exposed to an equivalent biovolume of *T. pseudonana* (ESD: 3.9 µm; 2.70×10^7 cells L^{-1}). In addition, a fixed

amount of *T. pseudonana* was added to all tanks (1×10^6 cells L^{-1}) to stimulate feeding in the treatment group. Before the microalgae were provided as a single dose once every 24 hours, 100% water changes (water adjusted to appropriate temperatures) were performed in all the tanks. Other environmental parameters such as light cycles and salinity were maintained the same as during the acclimation period.

After the conclusion of the exposure period, half of the oysters from each tank (i.e., three oysters) were selected for immediate response measurements under experimental conditions. In contrast, the remaining half were transferred to fresh seawater (see Fig 4.2). They underwent a 21-day recovery period, fed daily with commercial feed, following the same regimen as the acclimation period. Subsequently, their delayed response to the experimental conditions was assessed.

4.2.4. Metabolic response - oxygen consumption rate

O_2 consumption rate (MO_2) measurements were conducted after the five-day exposure to stressors and after twenty-one days. The methodology for MO_2 measurements followed the experiments described in Chapter 3 section 3.2.1.

4.2.5. Measurement of Immune Response

Haemolymph Collection Methods for measuring the immune response were based on the work of Matozzo et al. (2012). By prying the oysters open, approximately 700 µL of haemolymph per individual was collected from the adductor muscle of the bivalves using a 19-gauge needle with a one mL disposable syringe and stored on ice. Total haemocyte count (THC) was determined using 5 µL of the haemolymph, and 500 µL was used for the neutral red (NR) uptake assay. As for the total protein concentration in the cell-free haemolymph (CFH), 190 µL of the pooled haemolymph was used. CFH is the fluid component of the bivalve circulatory system that lacks haemocytes or other cellular elements. It consists of a transparent or pale yellowish fluid containing dissolved proteins, hormones, nutrients, and ions, and it plays a role in nutrient transport, waste removal, immune defence, and intercellular communication.

4.2.5.1. Total haemocyte count

To dilute samples and prevent clogging, 5 µl of the haemolymph was added to 195 µl of 0.38% sodium citrate in FSW (pore size: 0.45 µm) with a pH of 7.5. Twenty microlitres of the haemolymph and sodium citrate mixture were placed on a haemocytometer. The number of haemocytes was quantified using a compound microscope (Olympus BH-s BHT; Shinjuku, Japan) at 100× magnification.

4.2.5.2. Neutral Red (NR) uptake

NR uptake assays indicate damage to membranes, including lysosomes. Lysosomes are important in receiving and degrading macromolecules, be they secretory, endocytic, autophagic, or phagocytic membrane trafficking pathways (Zhao et al., 2011). They are responsible for detoxification and defence against pathogens in marine organisms (Zhao et al., 2011).

In vitro studies have used NR uptake widely to determine the effect of stressors on the lysosomal membrane stability of haemocytes. The dye is uptaken by viable haemocytes through pinocytosis or passive diffusion, while non-viable cells do not take it up. The differences in the amount of retained dye can indicate damage to membranes, including that of the lysosomes, and/or reduction of the pinocytotic capacity of the haemocytes (Matozzo et al., 2012).

The haemolymph used for NR uptake was centrifuged at 780 × g for 10 min. Haemocytes were then resuspended in an equal volume of 8 mg L^{-1} NR dye (Merck) solution in FSW and incubated at room temperature for 30 min. After it was then centrifuged at 780 × g for 10 min, resuspended in distilled water, and lysed using Precelly's ® Evolution homogeniser (Bertin Technologies Montigny-le-Bretonneux, France) at 6.5 m s^{-1} for 2 minutes, and centrifuged

again at 780 × g for 10 mins. The supernatant, corresponding to haemocyte lysate (HL), was then collected for the NR uptake assay. Absorbance at 550 nm was recorded on a microplate reader. The results were then expressed as optical density per ml haemolymph (OD mL^{-1} haemolymph).

4.2.5.3. Haemolymph protein concentration

Most proteins found in the haemolymph play a role in immune defence against microbes, fungi, protozoans, and viruses. Gianazza et al. (2021) extensively reviewed these functions in molluscs and crustaceans and describe haemolymph proteins as “marine drugs.” There are also proteins in the haemolymph that perform multiple functions, including coordination of cellular activities and switching between pathways to respond to changes inside and outside the organism (Gianazza et al., 2021). The main pathways in which haemolymph proteins are involved are those related to immunity and metabolism (Gianazza et al., 2021). Thus, haemolymph protein concentration (HPC) can serve as a useful biomarker when evaluating changes in the immune and physiological functions of bivalves.

The haemolymph used to determine protein concentration in CFH was first centrifuged at 780 × g for 10 min. The supernatant corresponding to CFH was then collected for protein quantification. A BCA protein assay kit (Pierce™, Pennsylvania, USA) was used to measure protein concentrations in CFH

using bovine serum albumin as standard. Five microliters of CFH diluted with 20 µl deionised water were incubated at 37 °C for 30 minutes with 200 µl of the BCA kit working reagent. The absorbance was measured at 562 nm, and the results were expressed as mg protein mL^{-1} haemolymph.

4.2.6. Condition index and ash-free dry weight determination

Every morning (0900), the tanks were inspected for dead oysters, and those that died were immediately placed in a labelled bag in a freezer (-20 °C). Animals that were alive at the end of the study were first opened (shucked) and soft tissues and shells of oysters were separated. The wet weight of the soft tissue (WW_{Tissue}) was determined using an analytical balance. After weighing, the soft tissue was homogenised with mortar and pestle, and two grams of the soft tissue for each oyster were taken for toxin accumulation determination described in section 4.2.7.

The remaining soft tissue and shells were oven-dried at 80°C on pre-dried foil dishes for 48 h. Animals from the freezer were recovered and included in this process. The dry weight of the shell (DW_{Shell}) and the tissue (DW-2) were determined using an analytical balance. DW_{shell} and DW-2 measurements were used to obtain the condition index of each oyster, described below. Accounting for the 2 g reduction in soft tissue used for toxin quantification, the ratio of the wet weight after 2 g reduction (WW-2), the quantified dry weight of soft tissue (DW-2), and the true wet weight of

the soft tissue (WW_{Tissue}) were used to obtain the true dry weight of soft tissue (DW_{Tissue}), following Equation 2:

$$DW_{Tissue} = \frac{\mathrm{DW}-2}{WW-2}\ x\ WW1 \qquad \text{(Equation 2)}$$

The CI was calculated using equation 3:

$$CI = \frac{DW_{Tissue}}{DW_{Shell}}\ x\ 100 \qquad \text{(Equation 3)}$$

CI is used in aquaculture work in adult bivalves due to its ease of measurement and practicality (Lucas & Beninger, 1985). A low value for this index means that the organism has spent a major biological effort either for maintenance under a poor environment or to produce and release gametes. Thus, this index can indicate stress or sexual maturity (Lucas & Beninger, 1985).

To obtain the ash-free dry weight (AFDW), the dried soft tissues were placed in a muffle furnace for six hours at 500 °C to remove organic content and reweighed for the ash weight. The ash weight was subtracted from the DW_{Tissue} to obtain the AFDW of the soft tissues.

4.2.7. *Diarrhetic shellfish toxin determination*

A three-step extraction based on the EU Reference Laboratory for Monitoring Marine Biotoxins protocol (EURL, 2015) was used. For this, 2.0 ± 0.01 g aliquots of each homogenised tissue were extracted in 9.0 mL methanol. After centrifugation at 2900 × g (10 mins; room temperature) the supernatants were removed and collected. The remaining solid pellets were subjected to a second and third extraction step involving the addition of a further 6.0 mL aliquot of methanol, before re-homogenization with a multi-tube vortex mixer. The supernatants from the three extraction steps were combined and diluted to 20.0 mL with methanol before filtering using 0.2 µm nylon syringe filters (Phenomenex, Manchester, UK). For Internal Quality Control purposes samples were again extracted in batches alongside certified calibrants. Methanolic extracts from all samples and controls were hydrolysed to allow the determination of acyl esters of OA-group toxins as per EURL (2015).

Both unhydrolysed and hydrolysed extracts were analysed from each sample using an Acquity I-class UHPLC system, which was coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters Ltd., Manchester, UK). Chromatography of toxins was conducted using an Acquity BEH C18 column (1.7µm x 2.1mm x 50 mm) with a Waters BEH C18 guard cartridge. UHPLC and MS/MS conditions utilised are described by Dhanji-Rapkova et al. (2018, 2019). This method allowed the quantitation of the lipophilic toxins (LT) okadaic acid (OA), Dinophysistoxins 1 and 2 together with associated acyl esters (DTX1-3), pectentoxins (PTX1,2,11), yessotoxin

analogues (YTX, h. YTX, 45 OH YTX, 45 OH h. YTX) and aspiracids (AZA1-3) using a 6-point calibration curve prepared from dilutions of certified calibrants (NRC, Canada). Results were reported as µg OA and DTX kg^{-1} soft tissue.

4.2.8. Calculation of Survival-odds ratio and confidence intervals

The calculations of the survival-odds ratio (OR) and OR confidence intervals were based on a method described by Szumilas (2010), following the equations 4.3, 4.4, and 4.5. OR values equal to 1 meant that treatment did not affect odds of mortality, OR>1 indicated that the treatment was associated with higher odds of mortality. In contrast, OR<1 meant that exposure to treatment was associated with lower odds of mortality. Since no death was observed in some groups, a Haldane-Anscombe correction was employed. Haldane correction adds 0.5 to all values in the OR equation if any of the values is equal to zero.

$$OR = \frac{a/c}{b/d} \qquad \text{(Equation 4.3)}$$

$$Upper\ 95\%\ CI = e^{\wedge}\ [\ln(OR) + 1.96\sqrt{(\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d})} \qquad \text{(Equation 4.4)}$$

$$Lower\ 95\%\ CI = e^{\wedge}\ [\ln(OR) - 1.96\sqrt{(\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d})} \qquad \text{(Equation 4.5)}$$

Where :

a = Number of oysters exposed to the treatment that died

b = Number of oysters exposed to the treatment that did not die
c = Number of oysters in the control that died
d = Number of oysters in the control that did not die

4.2.9. Data analysis

The data analysis involved investigating the effects of different treatment conditions on oysters by subjecting them to four distinct groups: heatwave only, algae only, heatwave and algae combined, and a control group. The objective was to determine if the treatment conditions significantly influenced the response variable (toxin accumulation, MO_2, CI, THC, HPC).

To address this question, linear mixed-effects models were employed using the "lme4" and "lmerTest" packages in R. Initially, a model was constructed with the following specifications:

response ~ treatment + (1|tank).

The model incorporated the response variable, denoted as "response," the treatment conditions, represented by the factor "treatment," and a random effect factor (1|tank) to account for potential random variability across individual tanks. Visual examination of the residual plots did not indicate any apparent deviations from the assumptions of

homoscedasticity and normality. The "anova" function was utilised to perform an analysis of variance (ANOVA) to assess the overall significance of the treatment conditions on the response variable.

Subsequently, the potential interaction between heatwave and toxic algae in the combined treatment was evaluated by comparing two linear mixed-effects models to assess the significance of an interaction term. The primary model, referred to as `modA`, was constructed with the following specifications:

response ~ heatwave + algae + heatwave:algae + (1|tank).

The model included the response variable denoted as "response" and the two stressors represented by the predictors `heatwave` and 'algae`. Additionally, an interaction term `heatwave:algae` was included to examine the potential interaction between the treatments. The random effect `(1|tank)` was incorporated to account for potential random variability across individual tanks.

A second model, denoted as `modB`, was also constructed without the interaction term:

response ~ heatwave + algae + (1|tank).

To assess the significance of the interaction term and evaluate its contribution to the model, an analysis of variance (ANOVA) was performed using the `anova` function in R. This allowed for a direct comparison of the nested models `modA` and `modB`, providing insights into the significance of the interaction of the two stressors.

These analytical procedures were deemed suitable for addressing the research question as they allowed for assessing the treatment conditions' effects on the response variable while considering potential random effects associated with individual tanks. Incorporating random effects enabled the control of potential confounding factors and enhanced the precision of the results. Moreover, by directly testing the significance of the interaction term and comparing the nested models, I could evaluate the significance of the interaction between heatwave and toxic algae. The likelihood ratio test (LRT) implemented in the ANOVA provided a statistically rigorous comparison.

The results of the LME analysis comparing toxin accumulation between oysters exposed to a heatwave and those not, did not yield statistical significance. However, it is important to note that the small sample size might have hindered the detection of a substantial difference between groups. To assess the magnitude of the difference in toxin accumulation, the "lsr" R Package was utilised and Cohen's d was computed using the "CohensD" function.

For Neutral Red uptake data analysis, I employed a Generalized Linear Mixed Effects Model (GLMM) with a Tweedie distribution due to violated assumptions in a

linear mixed effects model (LME). The Tweedie distribution was chosen to accommodate the non-normality and skewness observed in the response variable, and to model the non-constant variance indicated by heteroscedasticity in the residuals. Additionally, the GLMM with a Tweedie distribution provided flexibility in the choice of link function, allowing for capturing potential nonlinear relationships. To evaluate the significance of the treatment term in the GLMM, a likelihood ratio test was conducted using the "drop1" function with the "test" argument set to "Chisq." This test allowed me to assess the statistical significance of the treatment effect on the Neutral Red uptake. Considering these reasons, the GLMM with a Tweedie distribution proved to be a suitable alternative, overcoming the limitations of the LME model for the Neutral Red uptake data.

4.3. Results

4.3.1. DST accumulation and elimination

Soft tissues of *M. gigas* were evaluated for DST content one day and twenty-one days after exposure to *P. lima,* under control temperature and a heatwave (Fig 4.3). Most of the DST quantified was OA, but low levels of DTX1 were also detected. One day after exposure, oysters exposed to heatwave and *P. lima* reached an OA concentration (mean ± 1 SE) of 173.3 ± 19.78 µg kg^{-1} soft tissue, higher than the maximum permitted levels of DST in shellfish for safe consumption in the UK, which is at 160 µg OA eq. kg^{-1} soft tissue. Oysters exposed to *P. lima* alone had lower OA,

with a mean of 120.3 ± 20.90 µg kg^{-1} soft tissue, but this difference was not statistically significant ($F_{1,5}$ = 2.79, p = 0.17). The effect size, as measured by Cohen's d, was d = 0.84, indicating a large effect. After 21 days, the OA in the group exposed to *P. lima* alone and those exposed to both stressors (50.6 ± 14.26 and 57.7 µg ± 9.23 OA kg^{-1} soft tissue, respectively) did not differ significantly ($F_{1,4}$ =0.31, p=0.73). The effect size, measured by Cohen's d was 0.20, indicating a small effect.

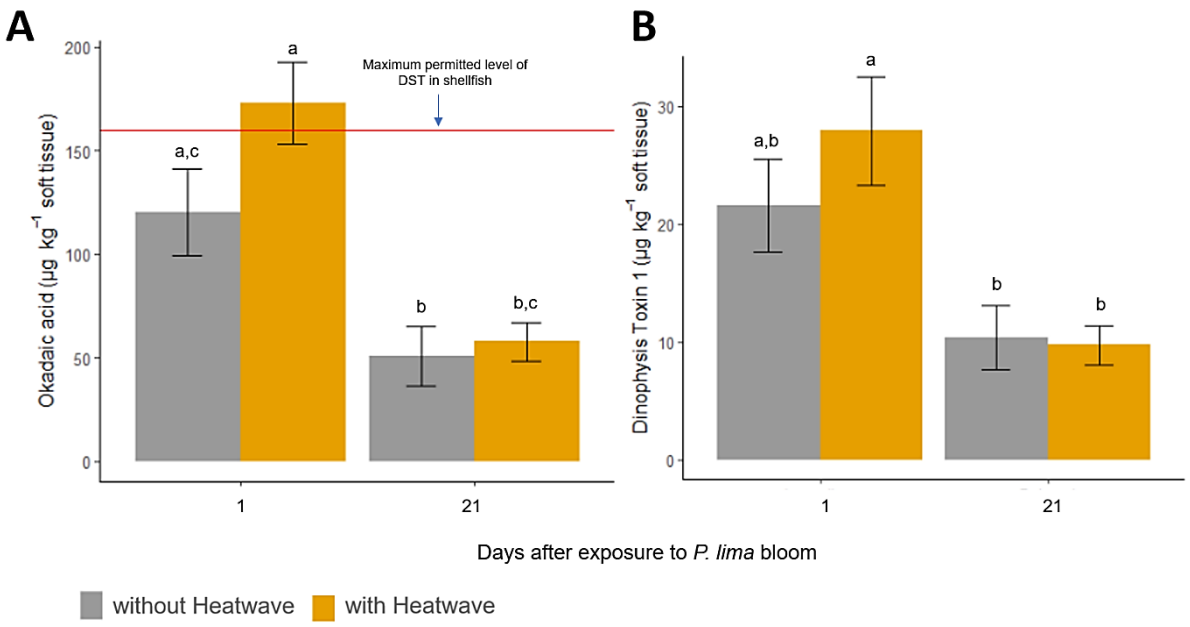


Figure 4.3 *Okadaic acid (OA) content (**A**) and Dinophysis Toxin 1 (DTX1) content (**B**) in the soft tissue of* Magallana gigas*. Oysters were exposed to two temperature conditions: control temperature (20°C) and a heatwave condition (peak: 25°C). Samples were collected one and twenty-one days after exposure* to Prorocentrum lima *at a concentration of 3 million cells L^{-1}. Data are presented as means with error bars representing ± 1 standard error (n = 9). Small letters above the bars indicate statistical differences (p≤0.05). Note that both OA and DTX1 content decreased over time.*

One day after exposure, the concentration of DTX1 in the group exposed to *P. lima* alone was lower than those exposed to both stressors (21.6 ± 3.94 and 27.9 ± 4.61 µg DTX1 kg^{-1} soft tissue, respectively). Still, this difference was not significant ($F_{1,4}$ = 0.91, p = 0.39). The effect size, as measured by Cohen's d, was d = 0.37, indicating a medium effect. Twenty-one days after exposure, the DTX1 in oysters exposed to *P. lima* bloom alone and both stressors had very similar values (10.3 ± 2.73 and 9.7 ± 1.65 µg DTX1 kg^{-1} soft tissue, respectively). They did not differ significantly ($F_{1,4}$ = 0.02, p = 0.87). The effect size, as measured by Cohen's d, was d = 0.09, indicating a small effect.

4.3.2. Physiological responses

One day after exposure, the oysters not exposed to heatwave nor *P. lima* (henceforth the control group) had the lowest oxygen consumption rate (0.2 ± 0.05 mL O_2 h^{-1} g^{-1} AFDW) among the four treatment groups (Fig 4.4A). The treatment groups had a significant main effect on O_2 consumption rates (MO_2) one day after stressor exposure ($F_{3,20}$ =8.52, p<0.001) but not 21 days after exposure ($F_{3,8}$ = 0.27, p = 0.85) (Fig 4.4A & 4.5A). Only the group exposed to heatwave alone had a significantly higher MO_2 than the control, with a mean of 0.5 ± 0.07 mL O_2 h^{-1} g^{-1} AFDW (t_8 = -4.90, p = 0.005). Specifically, *P. lima* alone and heatwave alone increased MO_2 rate a day after exposure by 0.1 and 0.5 mg O_2 h^{-1} g^{-1} AFDW, respectively, suggesting a 0.6 mg O_2 h^{-1} g $AFDW^{-1}$ increase when applied in combination if they did not interact. Instead, putting the two stressors together had

an antagonistic effect, with the mean MO_2 in the combined stressors treatment showing only a 0.2 mg O_2 h^{-1} g $AFDW^{-1}$ increase. The analysis revealed an interactive effect of stressors ($F_{1,19}$ =0 8.756, p = 0.008), in which combined treatment had a higher oxygen consumption rate than the control but less than the expected additive effect.

Results revealed that the treatment groups did not have a significant main effect on the Condition Index (Immediate: $F_{3,33}$ = 0.40, p = 0.75; Delayed: $F_{3,31}$ = 2.39, p = 0.09) and the total protein concentration in CFH (Immediate: $F_{3,32}$ = 0.18, p = 0.90; Delayed: $F_{3,31}$ = 1.22, p = 0.32) of *M. gigas* (Fig 4.4B & E; Fig 4.5B & E). One day after exposure, the Neutral red uptake of oysters exhibits a notable degree of variability (Fig 4.4D and 4.5D). However, it is important to note that these differences in mean uptake were not found to be statistically significant both one day after exposure and after twenty days (Immediate: $\chi 2_{3,}$ = 6.57, p = 0.09; Delayed: $\chi 2_3$ =1.03, p = 0.79).

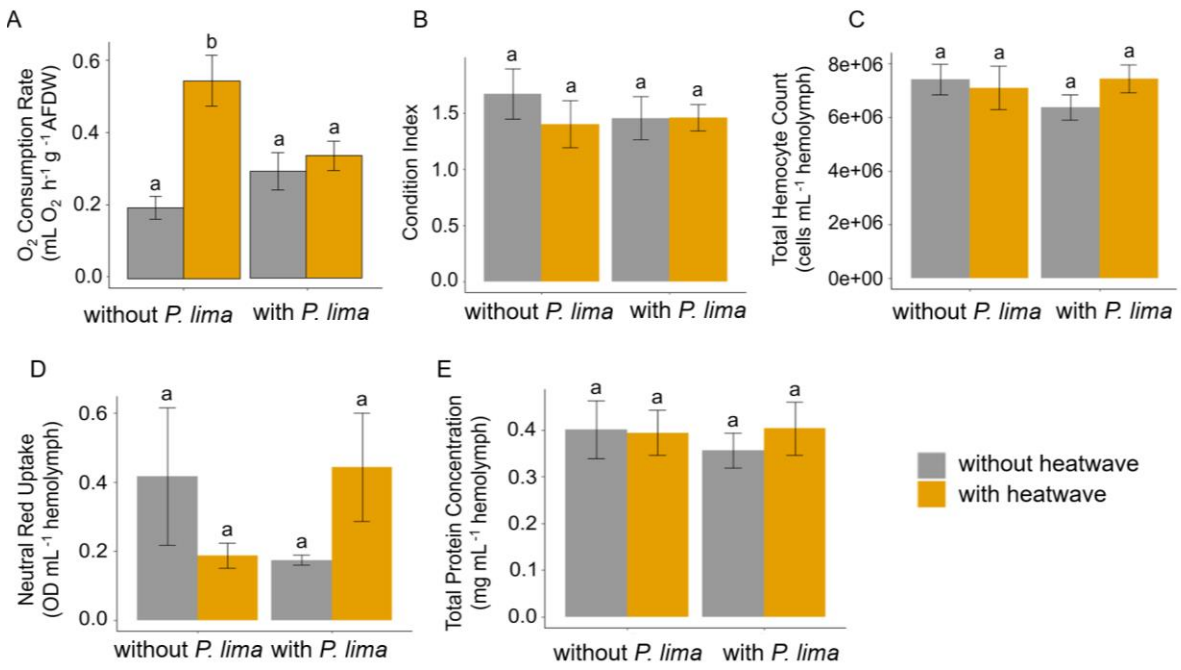


Figure 4.4 *Immediate effects of a heatwave and* Prorocentrum lima *on the oxygen consumption rate (**A**), Condition Index (**B**), Total Haemocyte Count (**C**), Haemolymph Neutral Red Uptake (**D**), and Haemolymph total protein concentration (**E**) of Pacific rock oysters (*Magallana gigas*). Data means + 1 SE (n=9). Small letters above the bars indicate statistical differences (p≤0.05).*

No effect of treatment groups was found for THC a day after exposure ($F_{3,32} = 0.68$, $p = 0.57$), but a significant main effect was detected after 21 days ($F_{3,31} = 4.80$, $p = 0.007$) (Fig 4.4E & 4.5E). The THC of the group exposed to *P. lima alone* was significantly lower than those exposed to heatwave alone and both stressors, 21 days after exposure ($t_{31} = -2.69$, $p = 0.05$; and $t_{31} = -3.59$, $p = 0.006$, respectively). If there was no interaction, the expected additive effect of the combined treatment 21 days after exposure is a THC decrease of 2.23×10^6 cells/mL relative to the control. Instead, the combined treatment had higher THC than control, suggesting an interactive effect between the stressors. This interaction is confirmed by the analysis performed ($F_{1,31} = 6.24$, $p = 0.018$).

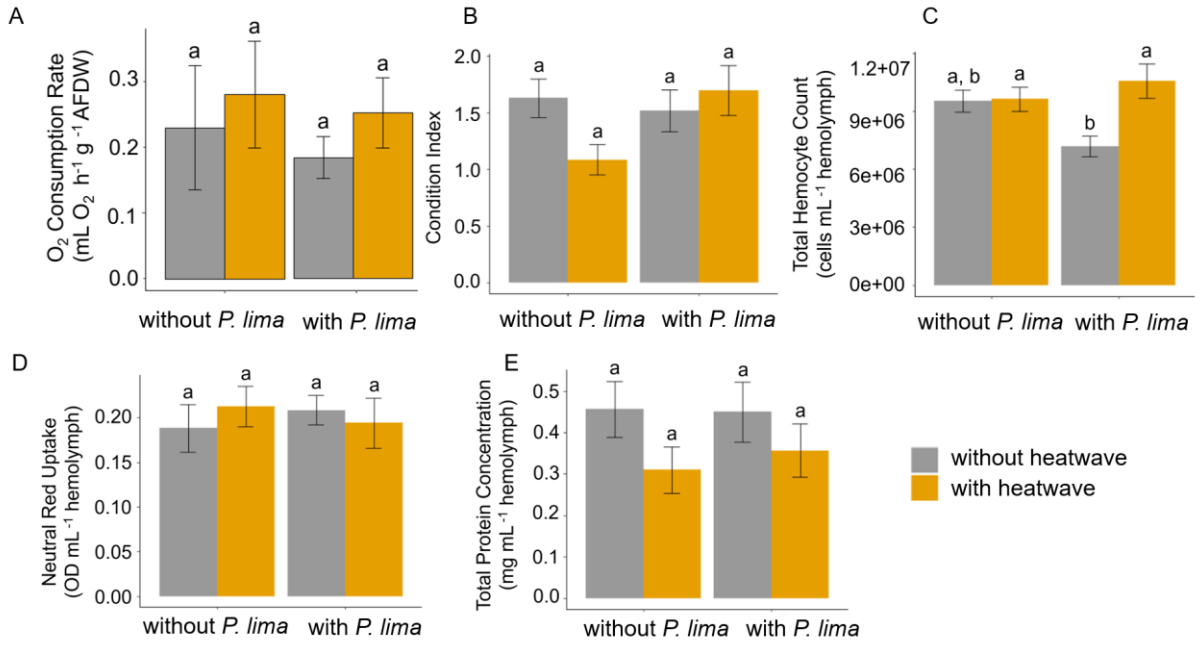


Figure 4.5 *Delayed effects of a heatwave and* Prorocentrum lima *on the oxygen consumption rate (**A**), Condition Index (**B**), Total Haemocyte Count (**C**), Haemolymph Neutral Red Uptake (**D**), and Haemolymph total protein concentration (**E**) of Pacific rock oysters (*Magallana gigas*). Data means + 1 SE (n=9). Small letters above the bars indicate statistical differences (p≤0.05).*

4.3.3. Mortality

On the fifth day of stressor exposure, one mortality was observed from the 18 oysters assigned to the heatwave and *P. lima* bloom. All the other oysters in the experiment survived the stressor exposure and recovery period. Calculating the survival-odds ratio in oysters exposed to both stressors gave a value of 3.2 [95% Confidence Interval: 0.12 - 83.17]. Although the calculated OR was positive, the 95% Confidence interval includes 1.0, indicating exposure to both stressors may not lead to a higher chance of death in oysters.

4.3. Discussion

Environmental change impacts coastal environments and contributes to trends of increasing and more variable temperatures, decreased seawater pH, changes in dissolved oxygen levels, and salinity, together with evidence of increased likelihood of harmful algal blooms. These changes are predicted to co-occur more frequently and thus challenge marine organisms as co-stressors. While multiple studies have been done about the individual effects of these climate change stressors on aquatic organisms, there is still limited knowledge on how they interact. The present study investigated how a heatwave and a bloom of DST-producing algae affect bivalve toxin accumulation, physiology, and survival.

4.4.1. Magallana gigas *exposed to* Prorocentrum lima *and heatwave show high levels of toxins above the safe consumption limit*

This study showed that exposure of Pacific oysters to *P. lima*, with or without a concurrent heatwave, resulted in the accumulation of diarrhetic shellfish toxins (DSTs) in their soft tissues. Most of the DSTs quantified were okadaic acid (OA), with low dinophysis 1 (DTX 1) level.

Although this study did not find a statistically significant difference in the DST content of *P. lima*-exposed oysters with or without a heatwave, Cohen's d analysis revealed a large effect size. Notably, the mean DST accumulation was higher in oysters exposed to high temperatures. Of particular concern, one day after exposure, the concentration of total OA in oysters exposed to both *P. lima* and the heatwave was higher than the maximum permitted levels of DST in shellfish for safe consumption in Europe and other countries. This finding highlights the potential risk to public health, as even brief exposure to toxic blooms, especially under elevated temperature conditions, could result in DST accumulation above regulatory limits in Pacific oysters.

Remarkably, the warming conditions did not appear to affect the ability of rock oysters to eliminate DST, as evidenced by the negligible difference in total DST levels between oysters exposed to only *P. lima* and those exposed to both *P. lima* and a heatwave, 21 days after exposure. This suggests that the rock oysters maintained their capacity to process harmful toxins despite the challenging environmental conditions. This phenomenon has practical implications for aquaculturists. To minimise potential risks associated with OA or DST contamination in shellfish, aquaculture management practices are urged to consider the timing of harvesting. Waiting for an appropriate period following exposure to harmful algal blooms, such as *P. lima*, before harvesting shellfish like *M. gigas* can help reduce the accumulation of OA and DST in their soft tissues. This precautionary measure aligns with food safety standards and ensures that the shellfish remains safe for consumption.

Depuration or purification, which uses the bivalve's natural ability to eliminate toxins, is a technique used in many parts of the world to remove microbial contaminants from bivalves by placing them in tanks of clean seawater for several hours to a few days. However, this technique is not presently considered an effective means of lowering biotoxin contamination to safe levels due to the variation of depuration rate between the toxin and the species of bivalves, inconsistency, and individual variation (Lee et al., 2008). The Food and Agriculture Organization (FAO) suggests that toxin elimination in the natural environment may be quicker than in tanks due to the presence of natural food (Lee et al., 2008).

As depuration cannot reliably remove biotoxins, and with the possibility of toxin accumulation exceeding safe levels at high temperatures, more frequent and constant monitoring of shellfish meat and toxic algal bloom in the waters should be employed. For some European countries, monitoring of toxic algae concentration in the water is done weekly in the summer and less frequently in other seasons. If algal cells are detected higher than warning levels, shellfish meat is tested for toxins. However, there are cases when toxins can accumulate in bivalve meat in less than a week (Fernandes-Salvador et al., 2021; Whyte et al., 2014). One of the possible methods for constantly monitoring toxic algal blooms in the water is using biosensors.

To further investigate DST accumulation and elimination in oysters exposed to warming, future work should involve exposing the animals to different concentrations

of *P. lima*, e.g., 100 cells $L^{-1,}$ which is the trigger level for *P. lima* in European waters, to establish whether oysters can still accumulate high toxin levels at lower *P. lima* concentration.

4.4.2. Prorocentrum lima *silence oyster's metabolic response to the heatwave*

This study demonstrated that exposure to heatwave and *P. lima* bloom negatively affected the metabolic response of *M. gigas* to heat stress. The substantial upregulation of metabolic activity (demonstrated by an increase in oxygen consumption) of oysters exposed to a heatwave alone is a typical response of aquatic ectotherms to higher temperatures (Rubalcaba et al., 2020), with a 133% increase in oxygen consumption observed in our study. However, this expected upregulation of metabolic activity under heat stress was not observed in the oysters exposed to heatwave and *P. lima*. Instead, the combined stressors had an antagonistic effect, resulting in only a 67% increase in oxygen consumption in oysters exposed to both stressors, much lower than the 133% increase observed in oysters exposed to heatwave alone.

This finding suggests that exposure to DST-producing dinoflagellates changes the metabolic response of oysters to heat stress and may have decreased their metabolic capacity. The toxic algae appear to silence the physiological response of the oysters to heat stress, thus may influence the ability of the oysters to survive in a

stressful environment that requires a fast metabolic response (e.g., an abrupt temperature increase during emersion while in a heatwave). The lack of increased metabolic rate in oysters exposed to *P. lima* suggests that the toxin produced by this dinoflagellate may be inhibiting the normal metabolic response of oysters to heat stress. Similar silencing of physiological response was also observed in the clam *Meretrix meretrix* exposed to the toxic PST-producer *Alexandrium* sp., where the clams do not respond to increased temperature or combined treatments of increased temperature, acidification, and decreased salinity (Turner et al., 2019).

Notably, the metabolic response to the combined stressors is not simply the sum of the individual responses to each stressor. Instead, the statistical analysis revealed an interactive effect of stressors, in which the combined treatment had a higher oxygen consumption rate than the control but less than the expected additive effect. This result suggests that the combination of a heatwave and *P. lima* has a unique impact on the metabolic response of the oysters that cannot be predicted based on the individual effects of each stressor.

The observed lack of increase in metabolic rate in oysters exposed to both stressors can be explained by the interaction between the two stressors. *P. lima* produces potent toxins, including okadaic acid and dinophysistoxins, that can disrupt cellular signalling pathways and impair physiological processes such as feeding, respiration, and reproduction (He et al., 2019; Lv et al., 2021). It is possible that exposure to these toxins, even in the absence of a heatwave, may have already decreased the metabolic capacity of the oysters, making them less able to respond to an additional

stressor such as a heatwave. This is supported by previous studies that have shown that exposure to harmful algal blooms can lead to reduced metabolic rates (Basti et al., 2016; Bricelj & Kuenstner, 1989; Colin & Dam, 2003; Haque et al., 2023). Therefore, the combined exposure to *P. lima* and heatwave may have resulted in a synergistic effect, with the toxic effects of the algae exacerbating the negative impact of heat stress on the oysters' metabolism.

Finally, it is essential to note that no detectable difference in oxygen consumption rate was found between treatments in the delayed response measurement, suggesting that exposure of the oyster to heat stress, toxic algal bloom, and their combination do not have a long-term effect on metabolic activity. Whether this result is maintained with longer heatwaves or different toxins remains to be tested.

4.4.3. Condition Index is not an effective tool in assessing heatwave and/or Prorocentrum lima *effect on bivalve health*

Condition is the ‘ability of an animal to withstand adverse environmental stress, be this physiological, chemical, or biological’ (Mann, 1978). The body condition index (CI) measures the physiological state of the bivalve and the amount of energy they store. This ecological and physiological concept is widely used in other animals,

including humans, to determine the organism's health, growth, meat yield, sexual maturity and effects of environmental stressors (Bricelj et al., 2011; Zeng & Yang, 2021). CI is generally based on the changing relationship between body masses and body sizes. In humans, the equivalent of CI is our body mass index (BMI), a widely used index based on the relationship between body mass and height. BMI is a universal method of assessing human and other vertebrate mammals' health status and development (Zeng & Yang, 2021). In this study, the CI calculations were based on the relationship between the dry weight of soft tissues and the dry weight of the oyster shells. This method is recognised and recommended for CI evaluation for regular aquaculture work in adult bivalves due to its ease of measurement and practicality (Lucas & Beninger, 1985). A low value for this index means that the organism has spent a major biological effort either for maintenance under a poor environment or the production and release of gametes. Thus, this index can indicate stress or sexual maturity (Lucas & Beninger, 1985).

The treatments did not affect the CI immediately after exposure and after recovery. There are no published studies investigating the combined effects of a heatwave and toxic algal bloom on the CI of bivalves. Yet, some studies involve heat stress alone and toxic algae alone or combined with other stressors.

Contrary to the findings presented in Chapter 3 of this thesis , those oysters exposed to heat stress in the 'heatwave only' and "heatwave and *P. lima* " did not differ in CI compared to the control. The initial analysis would lead us to infer that this is because of the difference in the duration of stressor exposure and temperature. The

experiment on *O. edulis* in Chapter 3 exposed native oysters to an incremental temperature increase for over a month at higher temperatures. In contrast, the current study exposed Pacific oysters to the stressors for five days and at a lower temperature. Nevertheless, this lack of effect on bivalve CI is recorded in experiments that involved both short (Amorim et al., 2020; Payton et al., 2016) and long (Lannig et al., 2006; Lemasson et al., 2018) duration of exposure to higher temperatures that induce heat stress. However, several studies report a decrease in CI after heat stress (Hiebenthal et al., 2012; Lemasson et al., 2018; Mackenzie et al., 2014; Shpigel et al., 1992; Taylor et al., 2017), and one study reports an increase (Payton et al., 2016).

Most studies investigating the effects of toxic algae observed no impact on bivalve CI, similar to my findings (Borcier et al., 2017; Haberkorn et al., 2010; Hégaret et al., 2009; Lassudrie et al., 2014, 2015). One study investigated three different strains of *A. minutum* including strains that contain: (1) Paralytic shellfish toxin (PST) only, (2) uncharacterised bioactive extracellular compounds with cytotoxic properties (BEC) only, and (3) both PST and BEC, reports lower CI for Manila clams that were exposed to the BEC strain only (Castrec et al., 2018).

This study demonstrates that CI may not be an effective tool for monitoring the physiological effects of heat stress and DST-producing algae in bivalves. This result agrees with Payton et al. (2016) who observe an increase in CI after heat stress in freshwater mussels, surprisingly not accompanied by increases in growth rate or glycogen stores (critical energy reserve in bivalves). Instead, there was a rise in triglyceride stores which they attribute to the physiological and behavioural

modification that initially led to higher metabolic rates close to the upper thermal limits (Payton et al., 2016).

However, it is important to consider the timing of the experiment in relation to the reproductive cycle of the oysters, as it may contribute to the observed lack of effect on the body condition index (CI). The experiment was conducted in October, a period when the oysters had already undergone spawning (post-reproduction). It is well known that during the spawning phase, bivalves allocate a significant energy towards gamete production and release. Consequently, post-reproductive individuals often exhibit lower CI values due to the substantial biological effort in reproduction. This natural physiological state of the oysters at the time of the experiment might have influenced the lack of observable effects on CI in response to the treatment conditions. Future studies conducted during different phases of the reproductive cycle would be beneficial for further understanding the potential influences on CI in bivalves subjected to similar stressors.

4.4.4. Total haemocyte count is modulated by Prorocentrum lima *and combined heatwave and* P. lima *exposure*

4.4.4.1. Total haemocyte count

Haemocyte functionality is essential in investigating the potential impact of environmental stressors. They indicate changes in physiological performance, which

increase the vulnerability to diseases and decrease survival capacity (Munari et al., 2020). The combined effects of a heatwave and toxic algal bloom on immune parameters were evaluated in this study. Matozzo & Marin (2011) and Lassudrie et al. (2020) have given excellent reviews on the individual effects of temperature and HAB, respectively, on the immune response of oysters.

THC did not vary among experimental conditions one day after exposure. Twenty-one days after exposure, oysters exposed to *P. lima* bloom alone had lower THC than those exposed to heatwave alone and both stressors. An interaction of stressors was detected after recovery, showing an antagonistic response, wherein the combined treatment had the highest mean THC among the group, higher than the expected additive effect. This finding suggests a modulation mechanism of circulating haemocyte numbers in the recovery period after exposure to the combined stressors.

In bivalves, an increase in THC is commonly thought to result from the production or movement of cells from tissues to haemolymph. At the same time, a decrease is likely because of cell lysis or the movement of cells from haemolymph to tissues (Matozzo et al., 2012). Previous research has shown no effect on THC for bivalves exposed to increased temperature or HAB (Castrec et al., 2018; Haberkorn et al., 2010; Hégaret et al., 2007, 2010; Lassudrie et al., 2014, 2015; Malagoli et al., 2007). Other studies, however, report both increases in THC (Bricelj et al., 2011; Carballal et al., 1997; Chen et al., 2007; Malagoli et al., 2007; Paillard et al., 2004; Perrigault et al., 2011) as well as decrease (Fisher et al., 1996; Galimany et al., 2008).

4.4.4.2. Neutral Red Uptake

Low uptake of Neutral Red (NR) suggests damage to lysosomal membranes, which are essential for the detoxification process and act as a defence mechanism against pathogens in marine organisms (Zhao et al., 2011). Impairment of these organelles indicates compromised immune defence. One day after exposure, the mean NR uptake in oysters exhibited variability between treatment groups. However, it is important to note that the observed differences in this study were not statistically significant. The control group displayed higher NR uptake compared to those exposed to a heatwave only or *P. lima* only, which aligns with previous studies. Elevated temperatures have been known to decrease lysosomal membrane stability in certain bivalves. For example, the freshwater bivalves *Corbicula fluminea* and *Unio delphinus* showed reduced stability when exposed to a water temperature of 24°C for eight days (Ferreira-Rodriguez et al., 2018). Similar results were observed in *M. galloprovincialis* after exposure to low pH and high temperatures of up to 28°C (Matozzo et al., 2012). Bivalves exposed to toxic dinoflagellates also demonstrated lower lysosomal membrane stability. This trend was evident in *M. edulis* exposed to toxic dinoflagellate blooms of *A. catenella* and *A. tamarense* (PST-producers) for seven days, as well as in *M. galloprovincialis* exposed to a bloom of *Ostreopsis* cf. ovata (palytoxin-producer) for 7-14 days (Bianchi et al., 2019; Gorbi et al., 2013).

Interestingly, the mean NR uptake in the combined treatment was higher than that in the control group. This suggests that the interaction between the stressors may have antagonistic effects, leading to the mitigation of the negative effects caused by the heatwave and toxic algae. Additional research with a larger sample size is necessary

to investigate these effects further. Furthermore, the high variability in NR uptake observed 21 days after exposure implies that the stressors did not have long-term effects on lysosomal membrane stability.

4.4.4.3. Haemolymph protein concentration

In the present study, there was no change in the HPC of the oysters after exposure to individual stressors and their combination. The only published study involving toxic algae and HPC measurement had similar findings. Mello et al. (2010) investigated the effects of *D. acuminata* bloom on the mussel *P. perna*, the clam *Anomalocardia brasiliana* and the oyster *M. gigas*. They found a significant increase in HPC in the mussels and the clam (22% and 13%, respectively), while the Pacific oyster's HPC remained unchanged compared to reference individuals (Mello et al., 2010). In their study, Mello et al. (2010) conclude that of the three bivalve species, *M. gigas* is the least affected species in the bloom, as they exhibit low tissue toxin accumulation and little immunological changes. The present study's findings reaffirm that DST-associated algal bloom brings little changes to immune parameters in *M. gigas*.

As for the effects of temperature on bivalve HPC in previous research, *R. philipinarum* HPC was not affected by the combined effects of various temperatures (5, 15, 30 °C) and salinity values (salinities of 18, 28 and 38) (Munari et al., 2011). In the same species exposed to 8, 14 and 21°C, no temperature effect on HPC is found (Paillard et al., 2004). Similarly, in a study investigating the effects

of seawater acidification and increased temperature, *M. galloprovincialis* HPC did not differ between 22 and 28 °C at normal salinity and pH (Matozzo et al., 2012). In contrast, when oysters *C. virginica* were exposed to 12, 20 and 28 °C and the toxic metal cadmium (Cd, 50 µg L−1), high temperatures resulted in higher HPC and Cd levels in oyster haemolymph plasma and haemocytes (Cherkasov et al., 2006).

4.5. Conclusion

In conclusion, this study on the effects of a toxic algal bloom (*P. lima*) and a heatwave on Pacific oysters (*M. gigas*) has provided valuable insights into the multiple stressors these bivalves face and their implications for aquaculture and public health.

The study findings revealed that the exposure of Pacific oysters to the toxic algal bloom and heatwave resulted in high levels of diarrhetic shellfish toxins (DSTs), particularly okadaic acid (OA). Importantly, the levels of DSTs exceeded safe consumption limits, highlighting potential risks to public health. Continuous monitoring of shellfish meat and toxic algal blooms is therefore crucial to safeguard consumers and ensure adherence to safety regulations. To mitigate potential hazards related to OA or DST contamination in shellfish, aquaculture management practices should take into account the timing of harvest. Delaying the harvest of shellfish like *M. gigas* for a suitable period after exposure to harmful algal blooms, can aid in lowering the levels of OA and DST in their soft tissues. This precautionary

step adheres to food safety guidelines and ensures the safety of shellfish for consumption.

Furthermore, the study demonstrated that the combined stressors had a negative impact on the metabolic response of the oysters to heat stress. Specifically, *P. lima* appeared to silence the oysters' normal metabolic response to heat stress, resulting in a reduced increase in oxygen consumption rate compared to oysters exposed to a heatwave alone. This highlights the unique and interactive effect of multiple stressors on oyster metabolism, emphasising the challenges faced by oysters in coping with simultaneous environmental stressors.

The examination of the body condition index (CI), which measures the physiological state and energy storage of bivalves, in relation to the combined effects of the heatwave and toxic algal bloom showed no immediate and delayed impact on the CI of the oysters. Previous studies have reported mixed results regarding the impact of heat stress and toxic algae on CI, with some observing decreases and others finding no significant changes. The timing of the experiment in relation to the oysters' reproductive cycle may have influenced the lack of observable effects on CI. Consequently, CI may not be an effective tool for monitoring the physiological effects of heat stress and toxic algae in bivalves. Further studies conducted during different phases of the reproductive cycle are recommended for a better understanding of CI in response to similar stressors.

Additionally, oysters exposed to the toxic algal bloom alone had significantly lower THC than the control, while the combined treatment showed an antagonistic

response with higher THC during recovery. These findings suggest immune modulation in oysters exposed to these stressors.

The implications of these findings for aquaculture are significant. Aquaculture operations that cultivate Pacific oysters should prioritise the monitoring of water quality, temperature fluctuations, and the presence of toxic algal blooms. Regular testing of shellfish meat for DSTs is essential to ensure compliance with safe consumption limits and protect public health. Furthermore, strategies should be developed and implemented in aquaculture practices to mitigate the negative impacts of combined stressors, such as heatwaves and toxic algal blooms, on oyster metabolism and immune function. Continued research in this field will contribute to developing targeted management approaches that minimise the risks associated with multiple stressors in aquaculture systems, promoting the sustainability and resilience of oyster populations and the industry as a whole.

In addition, an essential consideration for aquaculture operations is the potential use of biosensors for continuous monitoring of toxic algae cells in the water. Biosensors are analytical devices that detect and quantify specific substances or biological entities. They offer a rapid and sensitive method for monitoring the presence of toxic algae, such as *P. lima*, in real time. The forthcoming chapter of this thesis will delve into the exploration of oysters as biosensors and their potential applications in the continuous monitoring of toxic algae cells in aquaculture water.

4.6. References

Amorim, V. E., Gonçalves, O., Capela, R., Fernández-Boo, S., Oliveira, M., Dolbeth, M., Arenas, F., & Cardoso, P. G. (2020). Immunological and oxidative stress responses of the bivalve *Scrobicularia plana* to distinct patterns of heatwaves. Fish & Shellfish Immunology, 106, 1067–1077. https://doi.org/10.1016/j.fsi.2020.09.024

Anderson, D. M., Cembella, A. D., & Hallegraeff, G. M. (2012). Progress in understanding harmful algal blooms (HABs): Paradigm shifts and new technologies for research, monitoring and management. Annual Review of Marine Science, 4, 143–176. https://doi.org/10.1146/annurev-marine-120308-081121

Basti, L., Nagai, S., Watanabe, S., Oda, T., & Tanaka, Y. (2016). Neuroenzymatic activity and physiological energetics in Manila clam, *Ruditapes philippinarum*, during short-term sublethal exposure to harmful alga, *Heterocapsa circularisquama*. Aquatic Toxicology, 176, 76–87. https://doi.org/10.1016/j.aquatox.2016.04.011

Berdalet, E., Fleming, L. E., Gowen, R., Davidson, K., Hess, P., Backer, L. C., Moore, S. K., Hoagland, P., & Enevoldsen, H. (2015). Marine harmful algal blooms, human health and wellbeing: Challenges and opportunities in the 21st century. Journal of the Marine Biological Association of the United Kingdom. Marine Biological Association of the United Kingdom, 2015. https://doi.org/10.1017/S0025315415001733

Bianchi, V. A., Langeloh, H., Tillmann, U., Krock, B., Müller, A., Bickmeyer, U., & Abele, D. (2019). Separate and combined effects of neurotoxic and lytic

compounds of *Alexandrium* strains on *Mytilus edulis* feeding activity and hemocyte function. Fish & Shellfish Immunology, 84, 414–422. https://doi.org/10.1016/j.fsi.2018.10.024

Borcier, E., Morvezen, R., Boudry, P., Miner, P., Charrier, G., Laroche, J., & Hegaret, H. (2017). Effects of bioactive extracellular compounds and paralytic shellfish toxins produced by *Alexandrium minutum* on growth and behaviour of juvenile great scallops *Pecten maximus*. Aquatic Toxicology, 184, 142–154. https://doi.org/10.1016/j.aquatox.2017.01.009

Braga, A. C., Camacho, C., Marques, A., Gago-Martínez, A., Pacheco, M., & Costa, P. R. (2018). Combined effects of warming and acidification on accumulation and elimination dynamics of paralytic shellfish toxins in *mussels Mytilus galloprovincialis*. Environmental Research, 164, 647–654. https://doi.org/10.1016/j.envres.2018.03.045

Brandenburg, K. M., Velthuis, M., & Van de Waal, D. B. (2019). Meta-analysis reveals enhanced growth of marine harmful algae from temperate regions with warming and elevated CO2 levels. Global Change Biology, 25(8), 2607–2618. https://doi.org/10.1111/gcb.14678

Bricelj, V. M., Ford, S. E., Lambert, C., Barbou, A., & Paillard, C. (2011). Effects of toxic *Alexandrium tamarense* on behavior, hemocyte responses and development of brown ring disease in Manila clams. Marine Ecology Progress Series, 430, 35–48. https://doi.org/10.3354/meps09111

Bricelj, V. M., & Kuenstner, S. H. (1989). Effects of the “Brown Tide” on the Feeding Physiology and Growth of Bay Scallops and Mussels. In E. M. Cosper, V. M. Bricelj, & E. J. Carpenter (Eds.), Novel Phytoplankton Blooms (pp. 491–509). Springer. https://doi.org/10.1007/978-3-642-75280-3_28

Carballal, M. J., López, C., Azevedo, C., & Villalba, A. (1997). In vitrostudy of phagocytic ability of *Mytilus galloprovincialis* Lmk. Haemocytes. Fish & Shellfish Immunology, 7(6), 403–416.

Castrec, J., Soudant, P., Payton, L., Tran, D., Miner, P., Lambert, C., Le Goïc, N., Huvet, A., Quillien, V., Boullot, F., Amzil, Z., Hégaret, H., & Fabioux, C. (2018). Bioactive extracellular compounds produced by the dinoflagellate *Alexandrium minutum* are highly detrimental for oysters. Aquatic Toxicology, 199, 188–198. https://doi.org/10.1016/j.aquatox.2018.03.034

Chen, M., Yang, H., Delaporte, M., & Zhao, S. (2007). Immune condition of *Chlamys farreri* in response to acute temperature challenge. Aquaculture. http://dx.doi.org/10.1016/j.aquaculture.2007.04.051

Cherkasov, A. S., Ringwood, A. H., & Sokolova, I. M. (2006). Combined effects of temperature acclimation and cadmium exposure on mitochondrial function in eastern oysters *Crassostrea virginica* gmelin (Bivalvia: Ostreidae). Environmental Toxicology and Chemistry, 25(9), 2461–2469. https://doi.org/10.1897/05-584R.1

Coates, L., Stubbs, B., Turner, A. D., Williams, O., Milligan, S., & Algoet, M. (2014). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England & Wales—2014. 146.

Coates, L., Wilkinson, T., Stubbs, B., Turner, A. D., Milligan, S., & Algoet, M. (2015). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England and Wales—2015. 150.

Colin, S. P., & Dam, H. G. (2003). Effects of the toxic dinoflagellate *Alexandrium fundyense* on the copepod *Acartia hudsonica*: A test of the mechanisms that

reduce ingestion rates. Marine Ecology Progress Series, 248, 55–65. https://doi.org/10.3354/meps248055

Colletta, A., & Westfall, S. (2021, July 8). Crushing heat wave in Pacific Northwest and Canada cooked shellfish alive by the millions. The Seattle Times. https://www.seattletimes.com/seattle-news/environment/crushing-heat-wave-in-pacific-northwest-and-canada-cooked-shellfish-alive-by-the-millions/

Dhanji-Rapkova, M., O'Neill, A., Maskrey, B. H., Coates, L., Swan, S. C., Teixeira Alves, M., Kelly, R. J., Hatfield, R. G., Rowland-Pilgrim, S. J., Lewis, A. M., & Turner, A. D. (2019). Variability and profiles of lipophilic toxins in bivalves from Great Britain during five and a half years of monitoring: Azaspiracids and yessotoxins. Harmful Algae, 87, 101629. https://doi.org/10.1016/j.hal.2019.101629

Dhanji-Rapkova, M., O'Neill, A., Maskrey, B. H., Coates, L., Teixeira Alves, M., Kelly, R. J., Hatfield, R. G., Rowland-Pilgrim, S. J., Lewis, A. M., Algoet, M., & Turner, A. D. (2018). Variability and profiles of lipophilic toxins in bivalves from Great Britain during five and a half years of monitoring: Okadaic acid, dinophysis toxins and pectenotoxins. Harmful Algae, 77, 66–80. https://doi.org/10.1016/j.hal.2018.05.011

EURL. (2015). EU Reference Laboratory for Monitoring Marine Biotoxins. https://www.aesan.gob.es/en/CRLMB/web/public_documents/seccion/crlmb_standard_operating_procedures.htm

Farrell, H., Seebacher, F., O'Connor, W., Zammit, A., Harwood, D. T., & Murray, S. (2015). Warm temperature acclimation impacts metabolism of paralytic shellfish toxins from *Alexandrium minutum* in commercial oysters. Global Change Biology, 21(9), 3402–3413. https://doi.org/10.1111/gcb.12952

Fernandes-Salvador, J. A., Davidson, K., Sourisseau, M., Revilla, M., Schmidt, W., Clarke, D., Miller, P. I., Arce, P., Fernández, R., Maman, L., Silva, A., Whyte, C., Mateo, M., Neira, P., Mateus, M., Ruiz-Villarreal, M., Ferrer, L., & Silke, J. (2021). Current Status of Forecasting Toxic Harmful Algae for the North-East Atlantic Shellfish Aquaculture Industry. Frontiers in Marine Science, 8. https://www.frontiersin.org/article/10.3389/fmars.2021.666583

Ferreira-Rodriguez, N. (2019). Spatial aggregation of native with non-native freshwater bivalves and activity depletion under summer heat waves: "dangerous liaisons' in a climate change context. Hydrobiologia, 834(1), 75–85. https://doi.org/10.1007/s10750-019-3910-2

Fisher, W., Oliver, L., & Edwards, P. (1996). Hematologic and serologic variability of eastern oysters from Apalachicola Bay, Florida. 15, 664–564.

Foden, J., Purdie, D. A., Morris, S., & Nascimento, S. (2005). Epiphytic abundance and toxicity of *Prorocentrum lima* populations in the Fleet Lagoon, UK. Harmful Algae, 4(6), 1063–1074. https://doi.org/10.1016/j.hal.2005.03.004

Galimany, E., Sunila, I., Hégaret, H., Ramón, M., & Wikfors, G. H. (2008). Experimental exposure of the blue mussel (*Mytilus edulis*, L.) to the toxic dinoflagellate *Alexandrium fundyense*: Histopathology, immune responses, and recovery. Harmful Algae, 5(7), 702–711. https://doi.org/10.1016/j.hal.2008.02.006

Gao, G., Zhao, X., Jiang, M., & Gao, L. (2021). Impacts of Marine Heatwaves on Algal Structure and Carbon Sequestration in Conjunction with Ocean Warming and Acidification. Frontiers in Marine Science, 8. https://www.frontiersin.org/articles/10.3389/fmars.2021.758651

Gianazza, E., Eberini, I., Palazzolo, L., & Miller, I. (2021). Hemolymph proteins: An overview across marine arthropods and molluscs. Journal of Proteomics, 245, 104294. https://doi.org/10.1016/j.jprot.2021.104294

Gobler, C. J., Clark, H. R., Griffith, A. W., & Lusty, M. W. (2017). Diurnal Fluctuations in Acidification and Hypoxia Reduce Growth and Survival of Larval and Juvenile Bay Scallops (*Argopecten irradians*) and Hard Clams (*Mercenaria mercenaria*). Frontiers in Marine Science, 3. https://doi.org/10.3389/fmars.2016.00282

Gorbi, S., Avio, G. C., Benedetti, M., Totti, C., Accoroni, S., Pichierri, S., Bacchiocchi, S., Orletti, R., Graziosi, T., & Regoli, F. (2013). Effects of harmful dinoflagellate *Ostreopsis* cf. Ovata exposure on immunological, histological and oxidative responses of mussels *Mytilus galloprovincialis*. Fish & Shellfish Immunology, 35(3), 941–950. https://doi.org/10.1016/j.fsi.2013.07.003

Haberkorn, H., Lambert, C., Le Goïc, N., Moal, J., Suquet, M., Guéguen, M., Sunila, I., & Soudant, P. (2010). Effects of *Alexandrium minutum* exposure on nutrition-related processes and reproductive output in oysters *Crassostrea gigas*. Harmful Algae, 9(5), 427–439. https://doi.org/10.1016/j.hal.2010.01.003

Hagenbuch, B. (2021, February 8). Pacific Northwest heat wave causes vibrio bacteria outbreak in oysters | SeafoodSource. https://www.seafoodsource.com/news/food-safety-health/pacific-northwest-heatwave-causes-vibrio-bacteria-outbreak-in-oysters

Hallegraeff, G., Andersson, D., & Cembella, A. (2003). Manual on Harmful Marine Microalgae (2nd ed.). UNESCO. https://www.nhbs.com/manual-on-harmful-marine-microalgae-book

Hallegraeff, G. M. (2010). Ocean Climate Change, Phytoplankton Community Responses, and Harmful Algal Blooms: A Formidable Predictive Challenge1.

Journal of Phycology, 46(2), 220–235. https://doi.org/10.1111/j.1529-8817.2010.00815.x

Haque, Md. N., Nam, S.-E., Lee, M., Kim, H.-W., Gil, H.-W., Park, H. S., & Rhee, J.-S. (2023). Chronic exposure to environmental concentrations of harmful algal bloom-forming dinoflagellates induces oxidative stress and reduces immune and hepatic functions in red seabream. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 266, 109573. https://doi.org/10.1016/j.cbpc.2023.109573

Harrison, K., Walton, A., Coates, L., Turner, A., & Algoet, M. (2017). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England & Wales – 2017. 32.

Harrison, K., Wilkinson, T., Coates, L., Turner, A. D., Milligan, S., & Algoet, M. (2016). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England & Wales—2016. 43.

He, Z.-B., Duan, G.-F., Liang, C.-Y., Li, H.-Y., Liu, J.-S., & Yang, W.-D. (2019). Up-regulation of Nrf2-dependent antioxidant defenses in *Perna viridis* after exposed to *Prorocentrum lima*. Fish & Shellfish Immunology, 90, 173–179. https://doi.org/10.1016/j.fsi.2019.05.003

Hégaret, H., da Silva, P. M., Sunila, I., Shumway, S. E., Dixon, M. S., Alix, J., Wikfors, G. H., & Soudant, P. (2009). Perkinsosis in the Manila clam *Ruditapes philippinarum* affects responses to the harmful-alga, *Prorocentrum minimum*. Journal of Experimental Marine Biology and Ecology, 371(2), 112–120. https://doi.org/10.1016/j.jembe.2009.01.016

Hégaret, H., Wikfors, G. H., & Shumway, S. E. (2007). Diverse feeding responses of five species of bivalve mollusc when exposed to three species of harmful algae.

Journal of Shellfish Research, 26(2), 549–559. https://doi.org/10.2983/0730-8000(2007)26[549:DFROFS]2.0.CO;2

Heil, C. A., Glibert, P. M., & Fan, C. (2005). *Prorocentrum minimum* (Pavillard) Schiller: A review of a harmful algal bloom species of growing worldwide importance. Harmful Algae, 4(3), 449–470. https://doi.org/10.1016/j.hal.2004.08.003

Herring, S. C., Hoell, A., Hoerling, M., Christidis, N., & Stott, P. A. (2019). Introduction to Explaining Extreme Events of 2017 from a Climate Perspective. https://doi.org/10.1175/BAMS-D-18-0307.1

Hiebenthal, C., Philipp, E. E. R., Eisenhauer, A., & Wahl, M. (2012). Interactive effects of temperature and salinity on shell formation and general condition in Baltic Sea Mytilus edulis and Arctica islandica. Aquatic Biology, 14(3), 289–298. https://doi.org/10.3354/ab00405

Hu, Z., Liu, Y., Deng, Y., & Tang, Y. Z. (2022). The Notorious Harmful Algal Blooms-Forming Dinoflagellate *Prorocentrum donghaiense* Produces Sexual Resting Cysts, Which Widely Distribute Along the Coastal Marine Sediment of China. Frontiers in Marine Science, 9. https://www.frontiersin.org/articles/10.3389/fmars.2022.826736

Keller, M. D., Selvin, R. C., Claus, W., & Guillard, R. R. L. (1987). Media for the Culture of Oceanic Ultraphytoplankton1,2. Journal of Phycology, 23(4), 633–638. https://doi.org/10.1111/j.1529-8817.1987.tb04217.x

Lannig, G., Flores, J. F., & Sokolova, I. M. (2006). Temperature-dependent stress response in oysters, Crassostrea virginica: Pollution reduces temperature tolerance in oysters. Aquatic Toxicology, 79(3), 278–287. https://doi.org/10.1016/j.aquatox.2006.06.017

Lassudrie, M., Hégaret, H., Wikfors, G. H., & Mirella da Silva, P. (2020). Effects of marine harmful algal blooms on bivalve cellular immunity and infectious diseases: A review. Developmental and Comparative Immunology, 103660. https://doi.org/10.1016/j.dci.2020.103660

Lassudrie, M., Soudant, P., Richard, G., Henry, N., Medhioub, W., da Silva, P. M., Donval, A., Bunel, M., Le Goïc, N., Lambert, C., de Montaudouin, X., Fabioux, C., & Hégaret, H. (2014). Physiological responses of Manila clams *Venerupis* (=*Ruditapes*) *philippinarum* with varying parasite *Perkinsus olseni* burden to toxic algal *Alexandrium ostenfeldii* exposure. Aquatic Toxicology, 154, 27–38. https://doi.org/10.1016/j.aquatox.2014.05.002

Lassudrie, M., Wikfors, G. H., Sunila, I., Alix, J. H., Dixon, M. S., Combot, D., Soudant, P., Fabioux, C., & Hégaret, H. (2015). Physiological and pathological changes in the eastern oyster Crassostrea virginica infested with the trematode *Bucephalus sp*. And exposed to the toxic dinoflagellate *Alexandrium fundyense*. Journal of Invertebrate Pathology, 126, 51–63. https://doi.org/10.1016/j.jip.2015.01.011

Lee, R., Lovatelli, A., & Ababouch, L. (2008). Bivalve depuration: Fundamental and Practical Aspects [Technical Paper]. FAO Fisheries.

Leftley, J. W., & Hannah, F. (2009). Evidence, A Literature review of the potential health effects of marine microalgae and macroalgae. Environment Agency. https://www.gov.uk/government/organisations/environment-agency

Lemasson, A. J., Hall-Spencer, J. M., Fletcher, S., Provstgaard-Morys, S., & Knights, A. M. (2018). Indications of future performance of native and non-native adult oysters under acidification and warming. Marine Environmental Research, 142, 178–189. https://doi.org/10.1016/j.marenvres.2018.10.003

Li, M., Zhang, F., & Glibert, P. M. (2021). Seasonal life strategy of Prorocentrum minimum in Chesapeake Bay, USA: Validation of the role of physical transport using a coupled physical–biogeochemical–harmful algal bloom model. Limnology and Oceanography, 66(11), 3873–3886. https://doi.org/10.1002/lno.11925

Li, S.-C., Wang, W.-X., & Hsieh, D. P. H. (2002). Effects of toxic dinoflagellate *Alexandrium tamarense* on the energy budgets and growth of two marine bivalves. Marine Environmental Research, 53(2), 145–160. https://doi.org/10.1016/S0141-1136(01)00117-9

Lloyd, J. K., Duchin, J. S., Borchert, J., Quintana, H. F., & Robertson, A. (2013). Diarrhetic Shellfish Poisoning, Washington, USA, 2011. Emerging Infectious Diseases, 19(8), 1314–1316. https://doi.org/10.3201/eid1908.121824

Lucas, A., & Beninger, P. G. (1985). The use of physiological condition indices in marine bivalve aquaculture. Aquaculture, 44(3), 187–200. https://doi.org/10.1016/0044-8486(85)90243-1

Lv, J., Yuan, K., Lu, M., He, Z., Li, H., & Yang, W. (2021). Responses of JNK signaling pathway to the toxic dinoflagellate *Prorocentrum lima* in the mussel *Perna viridis.* Ecotoxicology and Environmental Safety, 227, 112905. https://doi.org/10.1016/j.ecoenv.2021.112905

Mackenzie, C. L., Ormondroyd, G. A., Curling, S. F., Ball, R. J., Whiteley, N. M., & Malham, S. K. (2014). Ocean Warming, More than Acidification, Reduces Shell Strength in a Commercial Shellfish Species during Food Limitation. Plos One, 9(1), e86764. https://doi.org/10.1371/journal.pone.0086764

Malagoli, D., Casarini, L., Sacchi, S., & Ottaviani, E. (2007). Stress and immune response in the mussel *Mytilus galloprovincialis*. Fish & Shellfish Immunology, 23(1), 171–177. https://doi.org/10.1016/j.fsi.2006.10.004

Mann, R. (1978). A comparison of morphometric, biochemical, and physiological indexes of condition in marine bivalve molluscs. In: J.H. Thorp and I.W. Gibbons (Editors), Energy and Environmental Stress In Aquatic Systems. DOE Symp. Ser. No. 48, pp.484-497.

Matozzo, V., Chinellato, A., Munari, M., Finos, L., Bressan, M., & Marin, M. G. (2012). First Evidence of Immunomodulation in Bivalves under Seawater Acidification and Increased Temperature. PLoS ONE, 7(3). https://doi.org/10.1371/journal.pone.0033820

Matozzo, V., & Marin, M. (2011). Bivalve immune responses and climate changes: Is there a relationship? Invertebrate survival journal, 8, 70–77.

Meehl, G. A., & Tebaldi, C. (2004). More Intense, More Frequent, and Longer Lasting Heat Waves in the 21st Century. Science, 305(5686), 994–997. https://doi.org/10.1126/science.1098704

Mello, D. F., Proença, L. A. de O., & Barracco, M. A. (2010). Comparative Study of Various Immune Parameters in Three Bivalve Species during a Natural Bloom of *Dinophysis acuminata* in Santa Catarina Island, Brazil. Toxins, 2(5), 1166–1178. https://doi.org/10.3390/toxins2051166

Moore, S. K., Trainer, V. L., Mantua, N. J., Parker, M. S., Laws, E. A., Backer, L. C., & Fleming, L. E. (2008). Impacts of climate variability and future climate change on harmful algal blooms and human health. Environmental Health, 7(2), S4. https://doi.org/10.1186/1476-069X-7-S2-S4

Munari, C. (2011). Effects of the 2003 European heatwave on the benthic community of a severe transitional ecosystem (Comacchio Saltworks, Italy). Marine Pollution Bulletin, 62(12), 2761–2770. https://doi.org/10.1016/j.marpolbul.2011.09.011

Paillard, C., Allam, B., & Oubella, R. (2004). Effect of temperature on defense parameters in manila clam *Ruditapes philippinarum* challenged with *Vibrio tapetis*. Diseases of Aquatic Organisms, 59(3), 249–262. https://doi.org/10.3354/dao059249

Parks, R., Bear, E., Coates, L., & Maskrey, B. (2019). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England & Wales – 2019. 39.

Parks, R., Walton, A., Coates, L., & Maskrey, B. (2018). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England & Wales – 2018. 37.

Payton, S. L., Johnson, P. D., & Jenny, M. J. (2016). Comparative physiological, biochemical and molecular thermal stress response profiles for two unionid freshwater mussel species. Journal of Experimental Biology, 219(22), 3562–3574. https://doi.org/10.1242/jeb.140129

Perrigault, M., Dahl, S. F., Espinosa, E. P., Gambino, L., & Allam, B. (2011). Effects of temperature on hard clam (*Mercenaria mercenaria)* immunity and QPX (Quahog Parasite Unknown) disease development: II. Defense parameters. Journal of Invertebrate Pathology, 106(2), 322–332. https://doi.org/10.1016/j.jip.2010.11.004

Rubalcaba, J. G., Verberk, W. C. E. P., Hendriks, A. J., Saris, B., & Woods, H. A. (2020). Oxygen limitation may affect the temperature and size dependence of

metabolism in aquatic ectotherms. Proceedings of the National Academy of Sciences, 117(50), 31963–31968. https://doi.org/10.1073/pnas.2003292117

Schiermeier, Q. (2018). Droughts, heatwaves and floods: How to tell when climate change is to blame. Nature, 560(7717), 20–23.

Shpigel, M., Barber, B. J., & Mann, R. (1992). Effects of elevated temperature on growth, gametogenesis, physiology, and biochemical composition in diploid and triploid Pacific oysters, *Crassostrea gigas* Thunberg. Journal of Experimental Marine Biology and Ecology, 161(1), 15–25. https://doi.org/10.1016/0022-0981(92)90186-E

Szumilas, M. (2010). Explaining Odds Ratios. Journal of the Canadian Academy of Child and Adolescent Psychiatry, 19(3), 227–229.

Tan, K., Zhang, H., & Zheng, H. (2020). Selective breeding of edible bivalves and its implication of global climate change. Reviews in Aquaculture, 12(4), 2559–2572. https://doi.org/10.1111/raq.12458

Tas, S., & Okuş, E. (2011). A review on the Bloom Dynamics of a Harmful Dinoflagellate *Prorocentrum minimum* in the Golden Horn Estuary Introduction. Turkish Journal of Fisheries and Aquatic Sciences, 11, 673–681. https://doi.org/10.4194/1303-2712-v11_4_03

Taylor, A. M., Maher, W. A., & Ubrihien, R. P. (2017). Mortality, condition index and cellular responses of *Anadara trapezia* to combined salinity and temperature stress. Journal of Experimental Marine Biology and Ecology, 497, 172–179. https://doi.org/10.1016/j.jembe.2017.09.023

Timms, P. (2019, November 24). “Wiped out overnight”: The marine heatwaves threatening our oyster industry. ABC News. https://www.abc.net.au/news/2019-11-25/marine-heatwaves-threaten-oyster-industry-great-barrier-reef/11726630

Troost, K. (2018, August 22). Extreme cockle mortality on tidal flats of Dutch coastal waters. WUR. https://www.wur.nl/en/newsarticle/Extreme-cockle-mortality-on-tidal-flats-of-Dutch-coastal-waters.htm

Türkoğlu, M. (2010). Temporal variations of surface phytoplankton, nutrients and chlorophyll a in the Dardanelles (Turkish Straits System): A coastal station sample in weekly time intervals. Turkish Journal of Biology, 34, 319–333. https://doi.org/10.3906/biy-0810-17

Türkoğlu, M., & Erdoğan, Y. (2010). Türkoğlu, M., Erdogan, Y., 2010. Diurnal variations of summer phytoplankton and interactions with some physicochemical characteristics under eutrophication of surface water in the Dardanelles (Çanakkale Strait, Turkey). Turk J Bio, 34 (2): 211-225. Turkish Journal of Biology, 34, 211–225. https://doi.org/10.3906/biy-0807-7

Turner, L. M., Havenhand, J. N., Alsterberg, C., Turner, A. D., Girisha, S. K., Rai, A., Venugopal, M. N., Karunasagar, I., & Godhe, A. (2019). Toxic Algae Silence Physiological Responses to Multiple Climate Drivers in a Tropical Marine Food Chain. Frontiers in Physiology, 10, 373. https://doi.org/10.3389/fphys.2019.00373

Visser, P. M., Verspagen, J. M. H., Sandrini, G., Stal, L. J., Matthijs, H. C. P., Davis, T. W., Paerl, H. W., & Huisman, J. (2016). How rising CO_2 and global warming may stimulate harmful cyanobacterial blooms. Harmful Algae, 54, 145–159. https://doi.org/10.1016/j.hal.2015.12.006

Wang, J., & Wu, J. (2009). Occurrence and potential risks of harmful algal blooms in the East China Sea. Science of The Total Environment, 407(13), 4012–4021. https://doi.org/10.1016/j.scitotenv.2009.02.040

Whyte, C., Swan, S., & Davidson, K. (2014). Changing wind patterns linked to unusually high Dinophysis blooms around the Shetland Islands, Scotland. Harmful Algae, 39, 365–373. https://doi.org/10.1016/j.hal.2014.09.006

Zeng, Y., & Yang, H. (2021). Review of molluscan bivalve condition index calculations and application in Northern Quahogs *Mercenaria mercenaria*. Aquaculture Research, 52(1), 23–36. https://doi.org/10.1111/are.14866

Zhao, C., Li, X., Lou, S., & Chang, Y. (2011). Assessments of lysosomal membrane responses to stresses with neutral red retention assay and its potential application in the improvement of bivalve aquaculture. African Journal of Biotechnology, 10(64), 13968–13973.

CHAPTER FIVE

Unveiling the Potential of *Magallana gigas* Valve Behaviour as an Early Detection Tool for Diarrhetic Shellfish Toxin-Producing Dinoflagellate *Prorocentrum lima* Blooms

Abstract

Toxic algal blooms threaten aquatic systems, human health, and the economy. Given the escalating occurrence of harmful algal blooms due to environmental changes, early detection techniques for the onset of diarrhetic shellfish toxin (DST)-associated algal blooms are urgently needed. This study investigated the valve behaviour of the rock *oysters Magallana* (*Crassostrea*) *gigas* in response to regulatory levels (10^2 cells L^{-1}) and bloom concentrations (10^6 cells L^{-1}) of the DST producer *Prorocentrum lima*. The study aimed to determine if oysters could potentially serve as biosensors for DST-associated algal blooms. *M. gigas* exposed to 10^6 cells L^{-1} of *P. lima* was found to spend significantly less time widely open on days they were exposed to *P. lima* compared to the days they were exposed to the same concentration of the non-toxic algal control ($\chi2=4.61$, df=1, p=0.03). This effect was particularly pronounced during the first three hours (control day: 34.5 ± 5.89%; treatment day: 21.2 ± 5.61) of observation and remained significant throughout the entire twenty-four-hour experimental days (control day: 10.9 ± 2.12% ; treatment day: 7.6 ± 2.01%). Additionally, oysters exposed to 10^6 cells L^{-1} of *P. lima* spent significantly less time widely open (5.7 ± 1.78%) than those exposed to 10^2 cells L^{-1} of *P. lima* (24.9 ± 4.80%), specifically 11 to 13 hours after exposure ($\chi2=1.46$, df=3, p=0.01). These findings highlight the significant potential of *M. gigas* as a valuable biomonitoring tool for detecting DST-associated blooms, especially concerning the duration of time they spend with widely open valves. This advancement offers promise in enhancing the production of safe bivalves and effectively mitigating the risks associated with toxic algal blooms. Continued research in this direction could

lead to more efficient and timely monitoring systems to protect ecosystems and human health from the harmful impacts of algal blooms.

5.1. Introduction

The increased occurrence of toxic algal blooms linked to climate change is becoming a significant concern worldwide because of its effects on aquatic ecosystems, human health, and the economy. The accumulation of phycotoxins in the flesh of filter-feeding bivalves during a harmful algal bloom (HAB) can reach levels dangerous for human health and lead to the closure of shellfish production sites. Currently, five different categories of human shellfish poisoning have been identified: paralytic shellfish poisoning (PSP) caused by saxitoxins, neurotoxic shellfish poisoning (NSP) caused by brevetoxins, diarrhetic shellfish poisoning (DSP) caused by the OA group of toxins (okadaic acid and dinophysis toxins), amnesic shellfish poisoning (ASP) caused by domoic acid, and azaspiracid shellfish poisoning (AZP) caused by azaspiracids (Butzke et al., 2013).

Across the European Atlantic Arc (Scotland, Ireland, England, France, Spain, and Portugal), closures of shellfish production sites are primarily caused by the regular occurrence of Diarrhetic Shellfish Toxins (DST) over the summer period (Fernandes-Salvador et al., 2021). Data obtained from the UK Centre for Environment, Fisheries, and Aquaculture Science (CEFAS) reports for England and Wales from 2014-2019

suggest that periods with DSP-inducing toxins above the maximum permitted levels in shellfish are more frequent than the presence of any other toxins (Figure 5.1) (Coates et al., 2014, 2015; Harrison et al., 2016, 2017; Parks et al., 2018, 2019) (See *Appendix F* for specific shellfish-production sites and months). DSP symptoms include diarrhoea, vomiting, and abdominal pain that appears 30 minutes to 40 hours after shellfish consumption (Chen et al., 2013). OA's primary effect in humans is the specific inhibition of serine and threonine phosphatases 1 (PP1) and 2A (PP2A), causing the hyperphosphorylation of many cellular proteins. Phosphatases are associated with many physiological processes in the body and are crucial for cell development.

The proteins that are affected by OA are primary components of signal transduction pathways inside eukaryotic cells, which control gene expression, cell cycle, neurotransmission, ion balance, and metabolism (Romero-Geraldo et al., 2016). Okadaic acid and its derivatives are potential tumour promoters in the human digestive system (Fujiki & Suganuma, 1993; Suganuma et al., 1988). Reports show that the residual levels of DSP toxins ingested through shellfish consumption could increase colorectal cancer incidence (Lopez-Rodas et al., 2006). OA is also found to initiate apoptosis, development of DNA adducts, chromosome loss, cytotoxicity, changes in neuropeptide Y (peptide in the brain that controls appetite), DNA breakage, and cell cycle arrest (Lee et al., 2016).

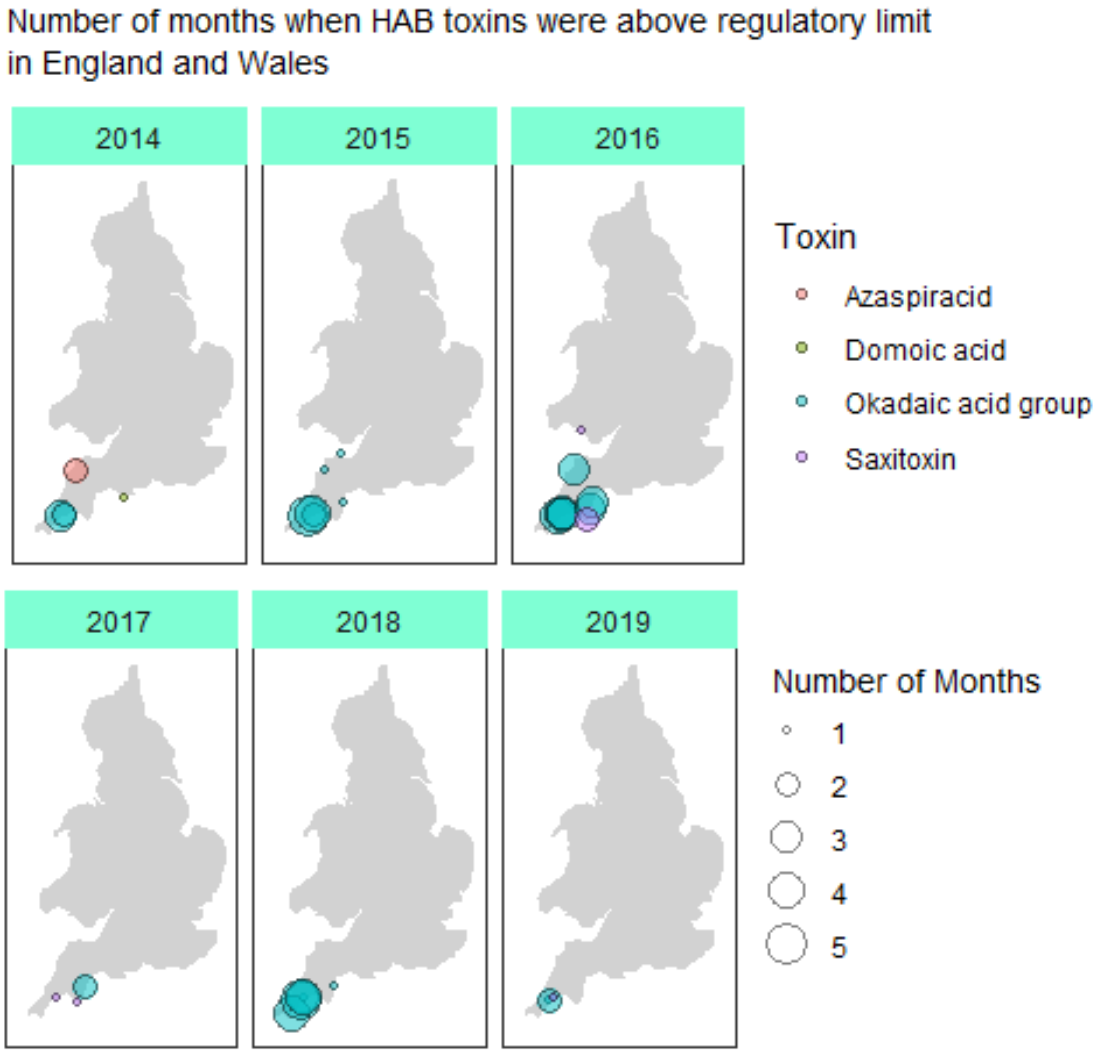


Figure 5.1 *Number of months when either Azaspiracids, Domoic acid, Okadaic acid group and Saxitons were detected above the maximum permitted levels in shellfish from 2014 to 2019, based on data gathered from CEFAS (Centre for Environment, Fisheries, and Aquaculture Science) reports for England and Wales, clearly showing that Okadaic acid group occurrence (blue bubble) is more frequent than other HAB toxins. Okadaic acid group of toxins is known to cause diarrhetic shellfish poisoning in humans (Coates et al., 2014, 2015; Harrison et al., 2016, 2017; Parks et al., 2018, 2019).*

To warn about elevated toxin levels in bivalves, countries within the European Atlantic Arc monitor the concentration of toxins in shellfish and the number of toxin-producing algal cells in the water. When the toxicity is above the regulatory limit, harvesting is suspended. For DST, a regulatory level of 160 µg per kg shellfish tissue

is in place for oysters to be sold to the public in Europe (Regulation EC No.853/2004, 29) and other countries (FAO/WHO, 2016). DST symptoms in a 60-kg human adult can manifest at a dose of 48 µg OA or 32 µg DTX-1 (Leite et al., 2020), which suggests that at the regulatory level, at least 200 g or around 40 oysters have to be consumed. The regulations may successfully protect human health; however, they do not give an early warning to the aquaculture industry to conduct business planning and manage harvesting (Fernandes-Salvador et al., 2021).

For most countries, the regulatory water sampling for toxic algal cells is carried out weekly in the summer months and less frequently in other seasons. However, there are instances when the harmful cells can accumulate rapidly in less than a week which happened during a bloom in Scotland in 2013. This HAB was caused by a change in the prevalent wind pattern that brought with it the DST-producing phytoplankton *Dinophysis*, which proliferated rapidly. This phenomenon led to shellfish toxin content reaching more than the regulatory levels within 2 3 days after the bloom's arrival. The intoxicated shellfish was sold on the market as the duration for the toxin accumulation was shorter than the weekly frequency of the water-monitoring program. Hence, seventy people were reported ill after the event, suffering symptoms associated with diarrhetic shellfish poisoning (Fernandes-Salvador et al., 2021; Whyte et al., 2014).

Biosensors can serve as an early warning system in areas where toxic algae may present a risk to the health of humans or marine organisms (Guterres et al., 2020; Harris, 2020). This system can be possible by recording the changes in the gaping

reaction of shellfish to harmful algae to alert when conventional water sampling is needed. This bivalve gaping response has been observed in several studies that mimic the bloom of PSP-producing algae (Comeau et al., 2019; Coquereau et al., 2016; Durier et al., 2022; Haberkorn et al., 2011; Lavaud et al., 2021, 2021; May et al., 2010; Tran et al., 2010). With this approach, the costs of routine algal bloom monitoring schemes can be decreased, and conventional sampling is restricted to periods when biosensors indicate the onset of a bloom event (Andrade et al., 2016).

Of the studies that looked into PSP-associated blooms, the most common response observed is a decrease in mean opening amplitude (Basti et al., 2019; Coquereau et al., 2016; Lavaud et al., 2021; May et al., 2010; Tran et al., 2010), but some of the studies report no effect in opening amplitude (Comeau et al., 2019; Durier et al., 2022; May et al., 2010) (Table 5.1). Of the previous studies that looked into the number of microclosures, four report increased occurrence (Basti et al., 2019; Comeau et al., 2019; Haberkorn et al., 2011; Tran et al., 2010) and one reports no effect (Durier et al., 2022) (Table 5.1). Microclosures are interpreted as a reaction of bivalves to irritating substances entering the pallial cavity (Comeau et al., 2019).

Table 5.1 *Summary of studies investigating how valve activity of bivalves is affected by toxic algae exposure.*

Reference	Bivalve	Toxic algae	Responses observed	Result*	DOI
1. Basti et al., 2009	*Ruditapes philipinarum*	*Heterocapsa circularisquama*	opening duration	=	https://doi.org/10.1016/j.aquaculture.2009.02.029
			opening amplitude	↓	
			microclosure	↑	
2. May et al., 2010	*Crassostrea virginica*	*Alexandrium monilatum*	opening amplitude	↓	https://doi.org/10.1016/j.hal.2009.11.005
	Mercenaria mercenaria	*Alexandrium monilatum*	opening amplitude	↓	
	Perna viridis	*Alexandrium monilatum*	opening amplitude	=	
3. Tran et al., 2010	*Magallana gigas*	*Alexandrium minutum*	opening amplitude	↓	https://doi.org/10.1016/j.aquaculture.2009.10.030
			opening duration	↑	
			microclosure	↑	
4. Haberkorn et al., 2011	*Magallana gigas*	*Alexandrium minutum*	opening duration	↑	https://doi.org/10.1016/j.marpolbul.2011.03.034
			microclosure	↑	
5. Coquereau et al., 2016	*Pecten maximus*	*Alexandrium minutum*	opening amplitude	↓	https://doi.org/10.1371/journal.pone.0160935
			number of expulsions	↑	
			number of closures	↑	
6. Comeau et al., 2019	*Mytilus galloprovincialis*	*Alexandrium minutum*	opening duration	=	https://doi.org/10.1016/j.aquaculture.2018.10.025
			opening amplitude	=	
			microclosure	↑	
7. Lavaud et al., 2021	*Mytilus edulis*	*Alexandrium catanella*	opening amplitude	↓	https://doi.org/10.1016/j.hal.2021.102097
			total number of closures	=	
			Mean number closures	=	
			The total duration of the closure	=	
8. Durier et al., 2022	*Mytilus edulis*	*Alexandrium catanella*	opening amplitude	=	https://doi.org/10.3389/fmars.2022.98787
			total number of closures	=	
			Mean number closures	↓	
			The total duration of the closure	↓	
			microclosure	=	

* = not affected; ↑ significantly increased; ↓ significantly decreased

To this date, to the author's knowledge, only one study investigates the effect of DST-algae on the valve activity of bivalves. Neves et al. (2019) measured the reaction time and the number of stimuli necessary for shell valve closure response after a predator approach (simulated by pricking the mantle with a needle, three times for each assay) in the brown mussel *Perna perna*. They found a significant increase in reaction time and the number of stimuli required for valve closure 72h after *P. lima* exposure. The authors attribute this to the observed ability of OA to disorganise the actin cytoskeleton, rounding and detaching cultured fibroblastic heart cells of clams *Ruditapes decussatus* (Hanana et al., 2012), and upregulating calcium current leading to the activation of phosphorylation kinase in cultured heart cells of *M. gigas* (Talarmin et al., 2008). However, the authors claim that the methodology they used may not be the most adequate since more than half of the mussels from the *P. lima* treatment (58%) and the control (61%) did not react to the stimuli even after three needle pricks (Neves et al., 2019). One study by Romero-Geraldo et al. (2016) reports a visual observation of adult *M. gigas* staying narrowly open (in their study, gaping amplitude was not specified or quantified) during the first three hours after *P. lima* exposure which they did not observe in oysters exposed to the control algae *I. galbana* (Romero-Geraldo et al., 2016).

Despite the risks brought by DST and it being the number one cause of closures in shellfish production sites in the European Atlantic Arc — affecting the livelihood of many people, there are no studies that evaluate whether bivalves themselves can be used as a remote biosensor to provide an early warning system for the occurrence of DST–associated algal blooms.

This research aims to assess the potential of bivalves, specifically *M. gigas*, as a reliable monitoring tool for detecting regulatory levels (10^2 cells L^{-1}) and bloom concentrations (10^6 cells L^{-1}) of DST-producing algae levels in the water column. A non-invasive oyster sensor (NOSy) was employed to record the valve behaviour of the bivalves to achieve this. Previous findings have indicated that *M. gigas* exhibits increased opening duration and microclosures, along with decreased opening amplitude, following exposure to *Alexandrium minutum*, a PSP producer (Haberkorn et al., 2011; Tran et al., 2010). Additionally, observations made by Romero et al. discussed above further contribute to this line of investigation. Building upon these findings, the study seeks to test the following hypotheses:

1. *M. gigas* exposed to *P. lima* will spend significantly less time in a widely open state than when exposed to the control algae.
2. *M. gigas* exposed to *P. lima* will spend significantly more time in a closed state than when exposed to the control algae.
3. The opening amplitude of *M. gigas* exposed to *P. lima* will show a significant decrease than when exposed to the control algae.
4. *M. gigas* exposed to *P. lima* will exhibit more microclosures than when exposed to the control algae.

As the results of Chapter 4 of this thesis showed that *P. lima* affects the metabolic response of *M. gigas,* this chapter provides a wholistic view of how these bivalves deal with the presence of DST-producing algae in the water and informs whether they can be an effective monitoring tool to detect its presence in shellfish production areas or the natural environment.

5.2. Methods

5.2.1. Animal Collection and Management

Adult Pacific rock oysters (*M. gigas*) were obtained from Colchester Oyster Fishery, West Mersea, Essex, in June 2021 and acclimated in the laboratory in custom-built stacks of tanks (three 45.5 × 45.5 × 30 cm tanks with a combined sump) for 14 days in natural seawater, filtered through a polypropylene felt filter bag (pore size 25 µm; Cole-Palmer, UK), at ambient laboratory temperatures of 17.9 - 18.5 °C. Salinity was measured using the Practical Salinity Scale and adjusted to a daily salinity of 35. Nitrate, nitrite, and ammonia concentrations were monitored every other day using a multi-test kit (Red Sea Marine Care Multi Test Kit, Tel Aviv, Israel) . During the acclimation, the oysters were fed 6mL (2.4×10^7 cells) per stack, with a commercial diet that is a mix of five marine microalgae - *Isochrysis, Pavlova, Tetraselmis, Thalassiosira weissflogii and Thalassiosira pseudonana* (Shellfish Diet 1800®; Reed Mariculture, California, USA).

5.2.2. Microalgae

This study used the benthic dinoflagellate *Prorocentrum lima*, one of the most common DST-producing algal species (David et al., 2018). It is an epiphytic-benthic dinoflagellate of cosmopolitan distribution found in tropical and temperate coastal

waters. The *P. lima* (CCAP 1136/11) used in this study was isolated from Vigo, Spain, in 2002; and purchased from the Centre for Culture of Algae and Protozoan in 2018 (Oban, Scotland, UK). Cultures were maintained at the University of Essex in 1 L flasks with K medium in filtered natural seawater (0.2 µm) at 18°C, at a mean light intensity (± standard error) of 221 ± 12 µmol m^{-2} s^{-1}, measured using a light meter (Li-Cor Li-250A, California, USA). In these standard culture conditions, the total toxin quantity of the algae was found to be 2.26 pg/cell (OA: 1.85 pg/cell; DTX: 0.42 pg/cell). To match the size of *P. lima* (32-50 µm in length), the diatom *Surirella* sp. (21-54 µm) was used as a non-toxic diet in the control and was kept in the same culture conditions as *P. lima.* Cell counts for both microalgal cultures were determined using a Neubauer chamber and a light microscope. Cells were harvested by centrifuge (800 × g for 10 min) and adjusted to the required concentrations with filtered seawater.

5.2.3. Valvometry

The University of Essex has developed a non-invasive oyster sensor (NOSy) (Shakspeare et al., 2003, using a Hall Effect (HE) magnetic sensor as described and reviewed by Clements & Comeau (2019). One week before the start of the experiment, Hall sensors (Honeywell, North Carolina, USA) were attached to the posterior edge of one valve of the oysters using epoxy putty (Milliput, Wales, UK). A neodymium magnet (6 mm diameter) (Eclipse, South Yorkshire, UK) was then attached to the other valve so it was directly on the opposite side of the Hall sensor (Fig 5.2). The magnetic field (flux density) between the sensor and the magnet corresponded to the gap between the two valves and thus measured gaping

amplitude and frequency. Data recorded as output voltage was stored in a microcontroller board equipped with a memory card (Arduino Uno, Massachusetts, USA). Recordings were retrieved from NOSy at the end of the experimentsm and recordings were subsequently processed using Phyton version 3.10.8. The daily voltage recording was converted into relative opening amplitudes every second ranging from 0 (minimum opening amplitude) to 1 (maximum opening amplitude). The voltage recording captured the electrical impulses generated by the oysters' movements, providing valuable insights into their opening and closing patterns. A conversion process was employed to establish relative opening amplitudes for each second of the recording to translate this electrical data into meaningful measurements.

The minimum and maximum voltage recordings for each oyster were identified during this conversion process. By pinpointing the lowest and highest voltage values specific to each oyster, the minimum and maximum opening amplitudes were established as reference points.

With these reference values in place, the relative opening amplitudes were determined on a scale from 0 to 1. This scale represented the range of oyster opening amplitudes, where 0 denotes the minimum opening amplitude, indicating the oysters were closed, and 1 represents the maximum opening amplitude, signifying the oysters were opened entirely. A comprehensive profile of the oysters' gaping behaviour was constructed by associating each second of the voltage recording with a corresponding opening amplitude value within this range (Appendix G).

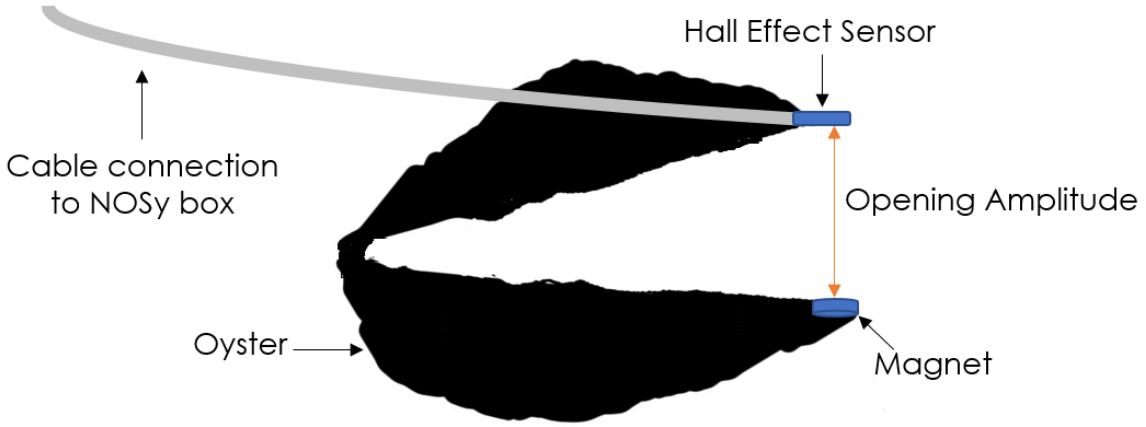


Figure 5.2 *Illustration of the attachment location of the neodymium magnet and Hall effect sensor for the opening amplitude measurement using the non-invasive oyster sensor. The magnet and the Hall effect sensor were attached to each valves' posterior edges opposite each other.*

5.2.4. Experimental design

The NOSy set-up simultaneously measured gaping for 16 bivalves at a rate of 10 Hz. Hence, to increase the sample size to 32, this study consisted of two identical experiments - block 1 and block 2. The procedures described in this section are for one experiment, with the second experiment starting one week after finishing the first.

The laboratory was accessed for essential maintenance only to prevent any stressful stimulation or disturbance of oysters from human activities. Three days before the start of the valve-gaping experiment, oysters were transferred to 16 identical 6 L tanks filled with filtered seawater with light aeration at the bottom to allow algae

suspension and mixing of water at ambient room temperature. Each tank contained one oyster (Fig 5.3).

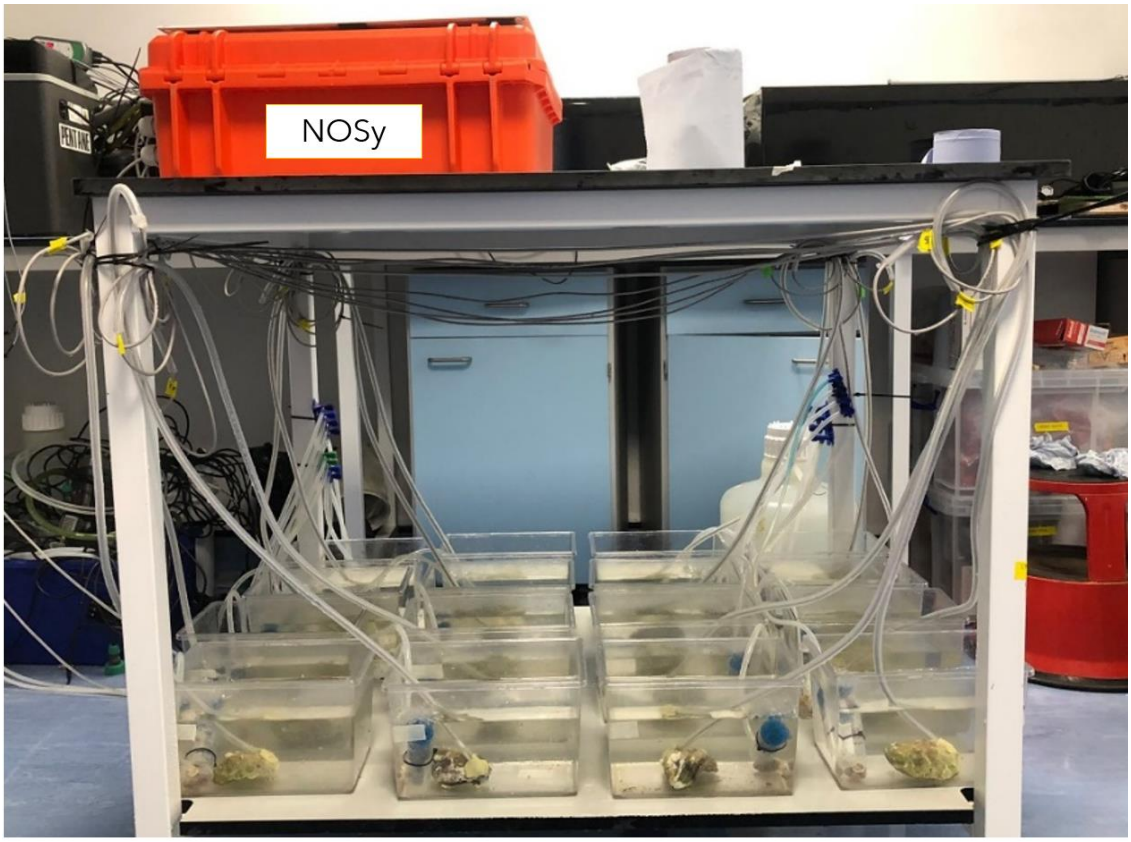


Figure 5.3 *Photograph of the experimental setup showing 6L tanks with one Pacific rock oyster each attached to the non-invasive oyster sensor (NOSy) used to measure gaping behaviour.*

The experiment lasted for six days — three control days (CD) and three treatment days (TD). It consisted of four experimental groups that had four replicates each (Fig 5.4). Two of these experimental groups were the treatment groups, alternately fed with the non-toxic algae on control days and with *P. lima* on the treatment days. One treatment group is fed 10^2 cells L^{-1} (T100) of the algae corresponding to the threshold levels for *P. lima* in the UK (Coates et al., 2019). The other treatment

group was fed with 10^6 cells L^{-1} (T1M), corresponding to a high concentration of *P. lima* recorded in a bloom (Türkoglu, 2016). The other two experimental groups were the control groups, fed with the control algae throughout the study, one with 10^2 cells L^{-1} (C100) and the other with 10^6 cells L^{-1} of the algae (C1M). All algal doses were mixed with a fixed amount of *Thalasssiosira pseudonana* (10^6 cells L^{-1}) to stimulate feeding. Algal exposure was started at the same time each day.

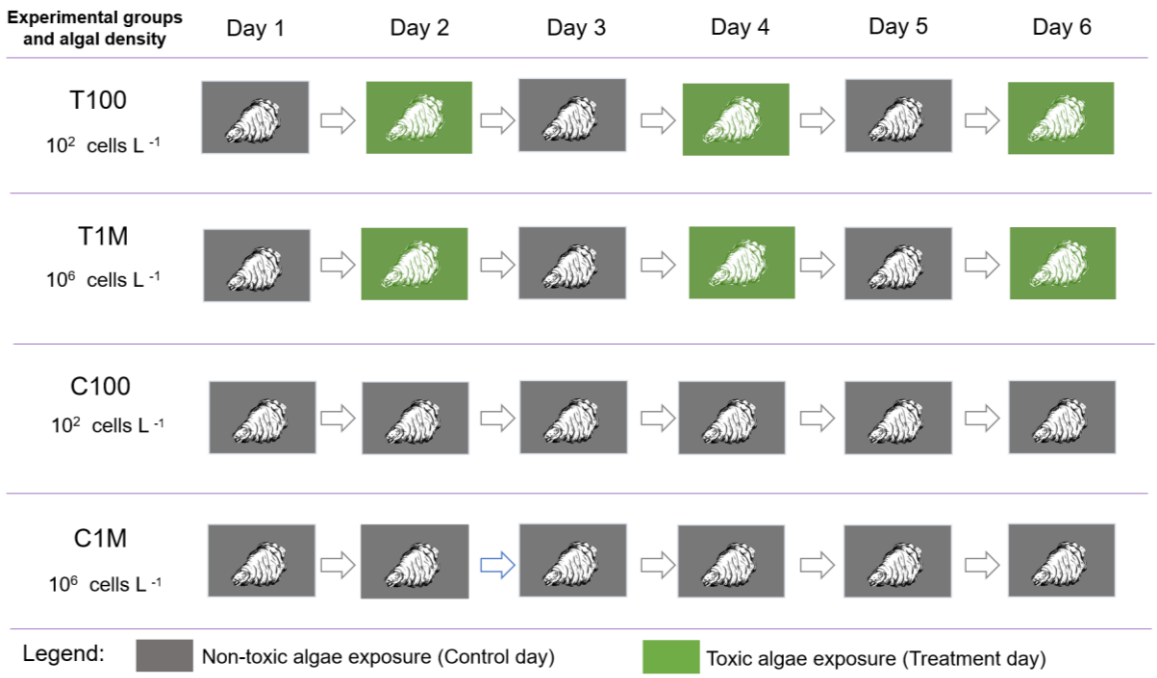


Figure 5.4 *Schematic diagram of the experimental design. Experiments lasted for six days and consisted of four experimental groups. Two experimental groups were the treatment groups:* M.gigas *alternately fed with the non-toxic algae on control days (grey boxes) and* P. lima *on the treatment days (green boxes). One treatment group was fed with 10 ^{2} cells L^{-1} (T100) of the algae corresponding to the threshold levels for* P. lima *in the UK, and the other fed with 10 ^{6} cells L^{-1} (T1M) corresponding to a high concentration of* P. lima *that was recorded in a bloom. The other two experimental groups were the control groups, which were fed with the control algae throughout the study; one was fed with 10 ^{2} cells L^{-1} (C100), and the other was fed with 10 ^{6} cells L^{-1} of the algae (C1M).*

The alternating feeding pattern was intended to facilitate a comparative analysis of the gaping behaviour of the same oysters when subjected to different types of algae. Specifically, the aim was to assess and contrast the oysters' behaviour when fed with toxic algae (*P. lima*) versus when exposed to non-toxic control algae (*Surirella sp*.). The underlying assumption in adopting this approach was that any discernible changes in gaping behaviour could be directly attributed to the specific algae consumed during each feeding period. This design allowed for a direct examination of the effects of toxic algae compared to the control algae on the oysters' gaping behaviour.

This experimental setup was motivated by the findings of a previous study by Romero-Geraldo et al. (2016), which highlighted a distinct behavioural pattern exhibited by adult *M. gigas* oysters. According to their observations, oysters exposed to *P. lima* displayed a tendency to remain narrowly open solely within the first three hours of exposure. In contrast, this behaviour was not observed in oysters exposed to the control algae *I. galbana*. Based on these previous findings, it was posited that any effects on the oysters' behaviour resulting from exposure to the toxic algae would likely be confined to the initial three-hour period.

5.2.5. Data analysis

Data analyses and visualisations were conducted using R (R Core Team, 2021) and the package ggplot2 (Wickham, 2016). The valve behaviour for the whole exposure day, the first, middle, and last-three hours of exposure days were analysed. Plots of

opening amplitude through time for each oyster were constructed and inspected for trends (see *Appendix G*). The distribution of gaping amplitude per day were also constructed to evaluate the behavioural patterns of oysters (see *Appendix H*).

After inspection of the opening amplitude through time and gaping distribution, four specific valve behaviours were used to determine whether *P. lima* exposure induces change in *M. gigas* valve behaviour: **1.** percentage of experimental time when the oysters were widely open (opening amplitude ≥ 0.9), **2.** percentage of experimental time the oysters were closed (opening amplitude ≤ 0.1), **3.** the opening amplitude, and **4.** the number of microclosures. This study defind a microclosure as at least a 3% but not more than a 30% decrease in opening amplitude per second.

For the analysis of the percentage time spent closed, open, and the number of microclosures, Generalised Linear Mixed Effects Models were employed using the "glmmTMB" package in R. GLMMs can handle non-normal response variables by specifying appropriate distributional assumptions, such as binomial (for binary data), Poisson (for count data), or Tweedie (for skewed and zero-inflated data). Due to the positively skewed and zero-inflated nature of the data on the percentage time spent open and closed, the Tweedie distribution was utilised in the GLMM. Similarly, the Poisson distribution was applied to the number of microclosures as it represents a count variable that measures the frequency of microclosures over a given period. As for the analysis of gaping amplitude, the Gaussian distribution was specified in the model.

In order to address the issue of autocorrelation, the models utilised the AR(1) covariance structure, which specifically accounted for the autocorrelation associated with the "day" variable in the experiment. To explore the impact of exposure to toxic algae on the oysters in T100 (alternately fed with 100 cells L^{-1} toxic and control algae) and T1M (alternately fed with 1×10^{6} cells L^{-1} toxic and control algae), the fixed effect "exposure" (control or treatment day) was included. This allows for a comparison between the days when oysters were exposed to the control algae and when they were exposed to the toxic algae, enabling the assessment of the effects of different algal conditions on the same oysters. Meanwhile, in the models aimed at analysing the differences in behaviour among the four experimental groups during treatment day, the fixed effect "treatment group" was incorporated.

In order to account for individual variation among oysters and potential discrepancies in experimental blocks, the random effects "oyster id " and "experimental block" were incorporated. Random effects play a crucial role in modelling individual differences and capturing the nested structure of the data. They also help reduce the influence of potential confounding factors and control for unobserved heterogeneity.

Post-hoc analyses explored pairwise differences in behavioural parameters between experimental groups. The emmeans package was utilised, and Tukey's post-hoc test was applied to determine significant differences between the groups (Lenth , 2023).

5.3. Results

This study was conducted to explore the behaviour of *M. gigas* in response to exposure to the DST-producer *P. lima* and assessed its potential as a biosensor for *P. lima* blooms. The experiment consisted of four distinct experimental groups. Two treatment groups were alternately exposed to *P. lima* (on treatment days) and a non-toxic control (on control days) at concentrations of 10^2 (T100) and 10^6 algal cells L^{-1} (T1M) over six-days. Two control groups were continuously fed with the non-toxic algal control at concentrations of 10^2 (C100) and 10^6 algal cells L^{-1} (C1M).

Four specific behaviours were used to assess the impact of *P. lima* exposure on *M. gigas* valve behaviour: **1.** Percentage of experimental time when the oysters were widely open (opening amplitude ≥ 0.9), **2.** Percentage of experimental time the oysters were closed (opening amplitude ≤ 0.1), **3.** opening amplitude, and **4.** Number of microclosures.

Figure 5.5 shows examples of gaping amplitude plots for T1M oysters, both on a control day and treatment days. For an overview of the the valve gaping behaviour across the entire oyster population during their exposure to both the control and toxic algae in all experimental days, please consult Appendix G.

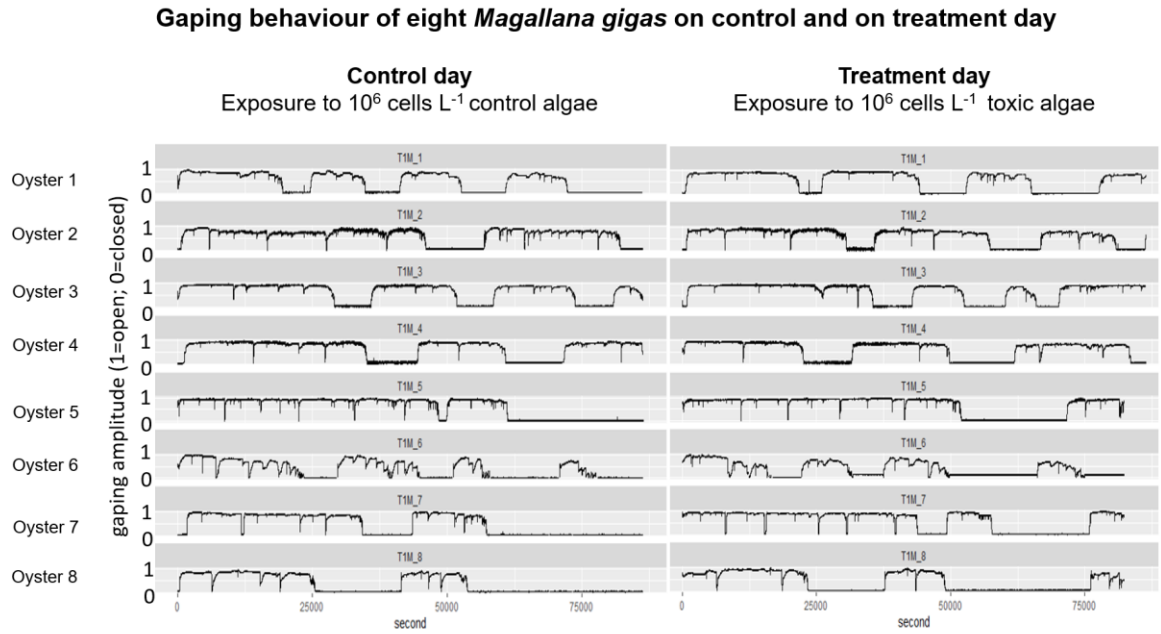


Figure 5.5. *Gaping Amplitude Plots for Eight T1M Oysters on a Control Day (Exposure to 10 ^{6} cells L^{-1} Control Algae,* Surirella sp*) and a Treatment Day (Exposure to 10 ^{6} cells L^{-1} Toxic Algae,* Prorocentrum lima*). In this representation, a value of 0 signifies complete closure of the oysters, while a value of 1 indicates fully open oyster aperture.*

5.3.1. Percentage of time spent wide open

The hypothesis proposed in the introduction suggested that oysters would spend less time widely open following exposure to toxic algae *P. lima.* The results from the study affirm this hypothesis, as oysters exposed to 10 ^{6} cells L^{-1} of the toxic algae *P. lima* (T1M) exhibited a significant decrease in the percentage of time spent widely open on the treatment day compared to the control day when the same oysters were exposed to the non-toxic control algae (C1M) ($\chi 2=4.61$, df=1, p=0.03). This effect was particularly pronounced during the first three hours (control day: 34.5 ± 5.89%; treatment day: 21.2 ± 5.61%) (Fig 5.6 B) of observation and remained significant when analysing the entire twenty-four-hour experimental day (control day: 10.9 ±

2.12% ; treatment day: 7.6 ± 2.01%) (Fig 5.6). Notably, no such trend was observed in the oysters exposed to 10^{2} *P. lima* cells L^{-1} on treatment day (T100).

Comparing the behaviour of the oysters between different experimental groups, Figures 5.6 A to D reveals that oysters exposed to 10^{2} *P. lima* cells L^{-1} on treatment day (T100) consistently spent more time wide open than those exposed to the same concentration of the control algae (C100). However, it is important to note that this difference was not statistically significant, indicating that the toxic algae's impact on oyster behaviour at this concentration may not be conclusive.

Oysters exposed to 10^{6} *P. lima* cells L^{-1} (T1M) did not exhibit a clear behaviour pattern or differences compared to those exposed to the same concentration of control algae (C1M). However, during the middle three hours of observation, it was noteworthy that T1M oysters spent significantly less time widely open (5.7 ± 1.78%) than T100 oysters (24.9 ± 4.80%) (T100) ($\chi 2=1.46$, df=3, p=0.01) (Fig 5.5 C).

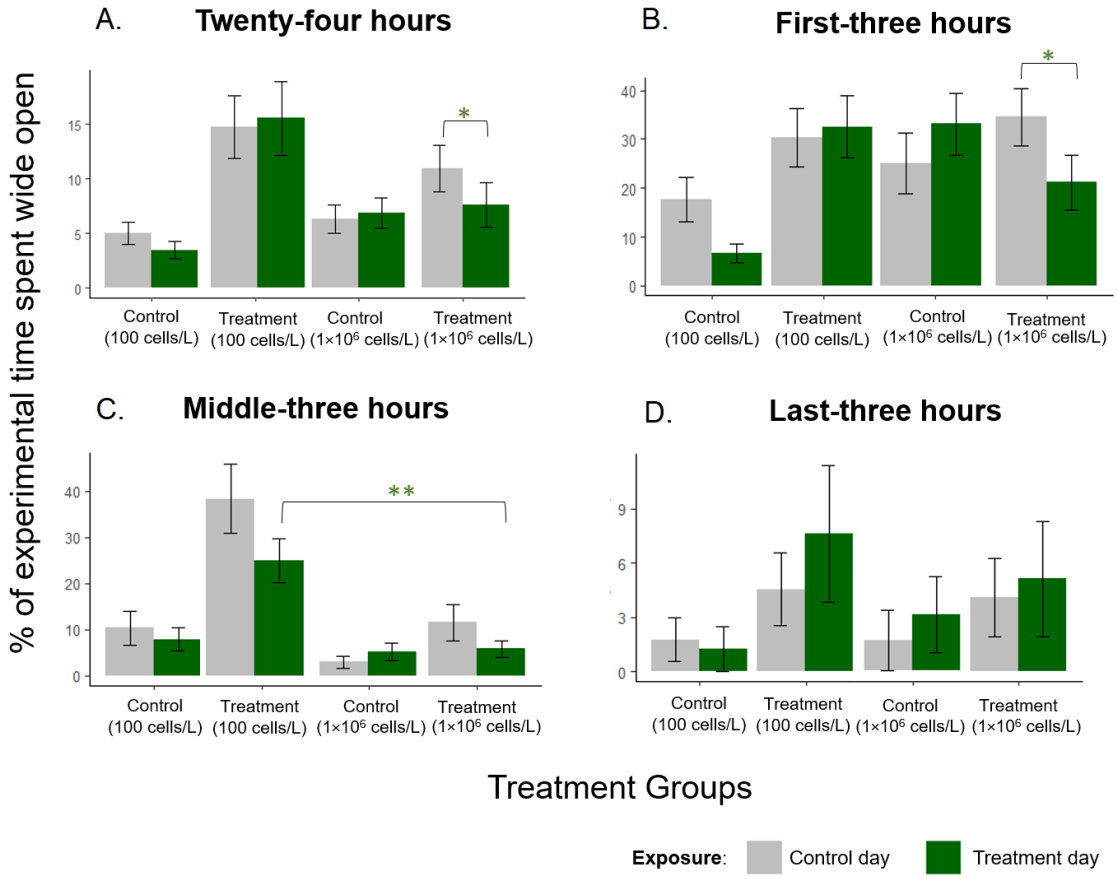


Figure 5.6. *The percentage of time that* M. gigas *spent widely open during different time intervals of the experimental day: 24 hours (**A**), first three hours (**B**), middle-three hours (**C**), and last three hours (**D**) (mean ± 1 standard error).* M. gigas *were exposed to four different treatments: alternate feeding with non-toxic algae (control day) and* P. lima *(treatment day) (10^2 cells L^{-1} and 10^6 cells L^1), and two control groups fed with non-toxic* Surirella *sp. (10^2 cells L^{-1} and 10^6 cells L^{-1}). Grey bars represent control days, and green bars indicate treatment days (n=8).*

5.3.2. Percentage of time spent closed

The response of oysters exposed to toxic algae on the treatment day did not demonstrate a significant change in the time spent closed compared to the days when they were exposed to the control algae. Examining Figure 5.7, no clear pattern can be observed in this behaviour for both concentrations of algae at 10^2 and 10^6 cells L^{-1}.

When comparing the behaviour of oysters among different experimental groups on treatment day, no definitive pattern emerges between C100 and T100. On the other hand, oysters in T1M exhibited a higher mean percentage of time spent closed compared to those exposed to C1M in the twenty-four-hour, first-three-hour, and midlde-three-hours, but not in the last three hours of observations. This trend agrees with the hypothesis presented in the introduction. Nevertheless, it is crucial to note that these differences are not statistically significant.

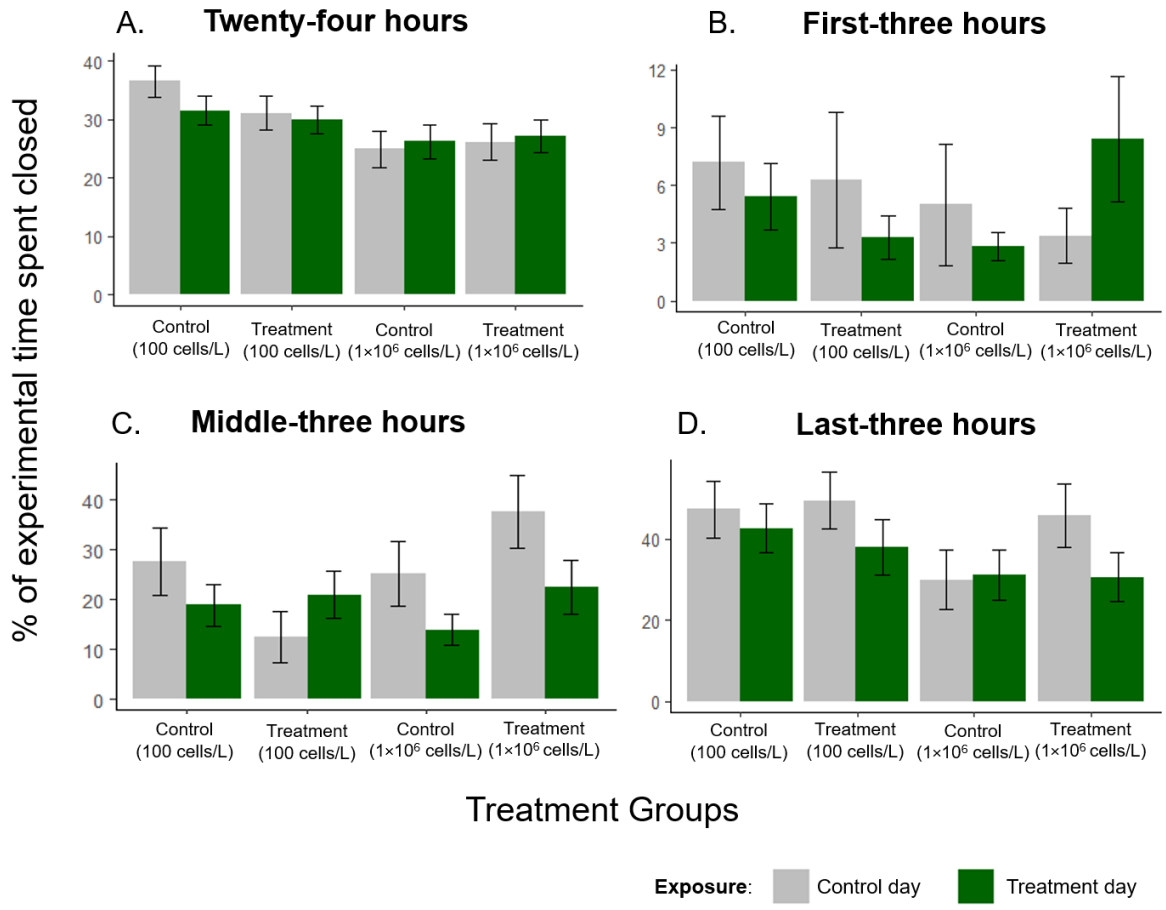


Figure 5.7 *The percentage of time that* M. gigas *spent closed during different time intervals of the experimental day: 24 hours (**A**), first-three hours (**B**), middle-three hours (**C**), and last-three hours (**D**) (mean ± 1 standard error).* M. gigas *were exposed to four different treatments: alternate feeding with non-toxic algae (control day) and* P. lima *(treatment day) (10^2 cells L^{-1} and 10^6 cells L^1), and two control groups fed with non-toxic* Surirella *sp. (10^2 cells L^{-1} and 10^6 cells L^{-1}). Grey bars represent control days, and green bars indicate treatment days (n=8).*

5.3.3. Mean Gaping Amplitude

The mean gaping amplitude across all oysters ranged from 0.28 to 0.84. As hypothesised, it was expected that oysters exposed to toxic algae would display a lower mean gaping amplitude than when exposed to the control algae. However, the analysis of gaping amplitude during both control and treatment days did not reveal any clear patterns or significant differences. Likewise, no notable patterns or differences were observed when comparing the experimental groups exposed to toxic algae and the control algae at both concentrations (Fig. 5.8).

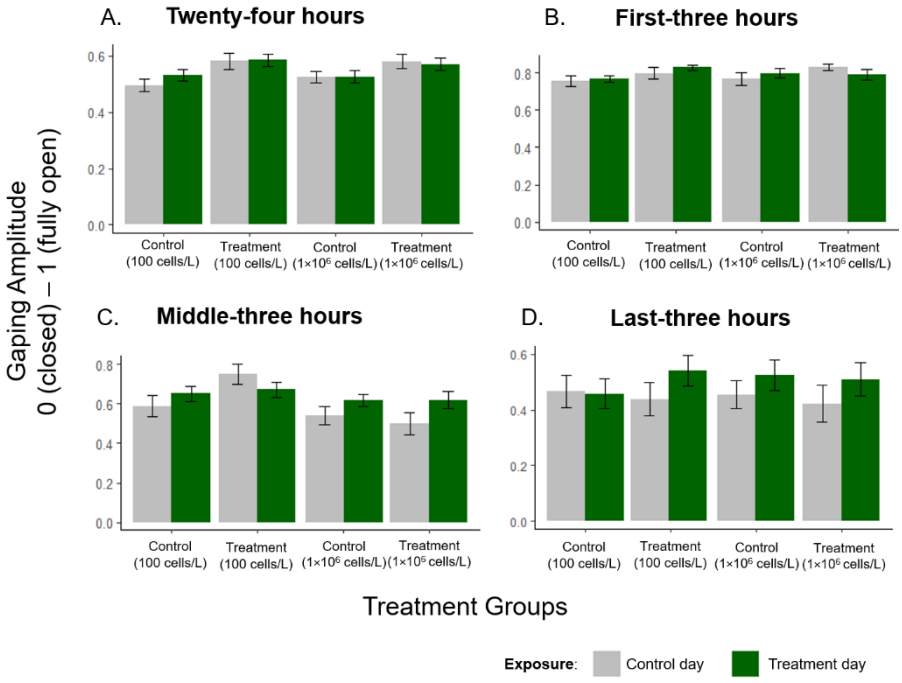


Figure 5.8 *The gaping amplitude of* M. gigas *during different time intervals of the experimental day: 24 hours (**A**), first three hours (**B**), middle three hours (**C**), and last three hours (**D**) (mean ± 1 standard error). Zero (0) is when the oysters are entirely closed, while One (1) is when the oysters are completely open. M. gigas were exposed to four different treatments: alternate feeding with non-toxic algae (control day) and* P. lima *(treatment day) (10^2 cells L^{-1} and 10^6 cells L^1), and two control groups fed with non-toxic* Surirella *sp. (10^2 cells L^{-1} and 10^6 cells L^{-1}). Grey bars represent control days, and green bars indicate treatment days (n=8).*

5.3.4. *Number of microclosures*

As observed in Figure 5.9, no discernible patterns can be deduced from the mean number of microclosures when comparing treatment and control days, as well as between the experimental groups. However, an interesting observation is that the least microclosures occurred during the last three hours of exposure across all experimental groups. This phenomenon might not be solely attributed to the treatment's effects but instead could be a result of exposure to food or algae, regardless of its toxicity.

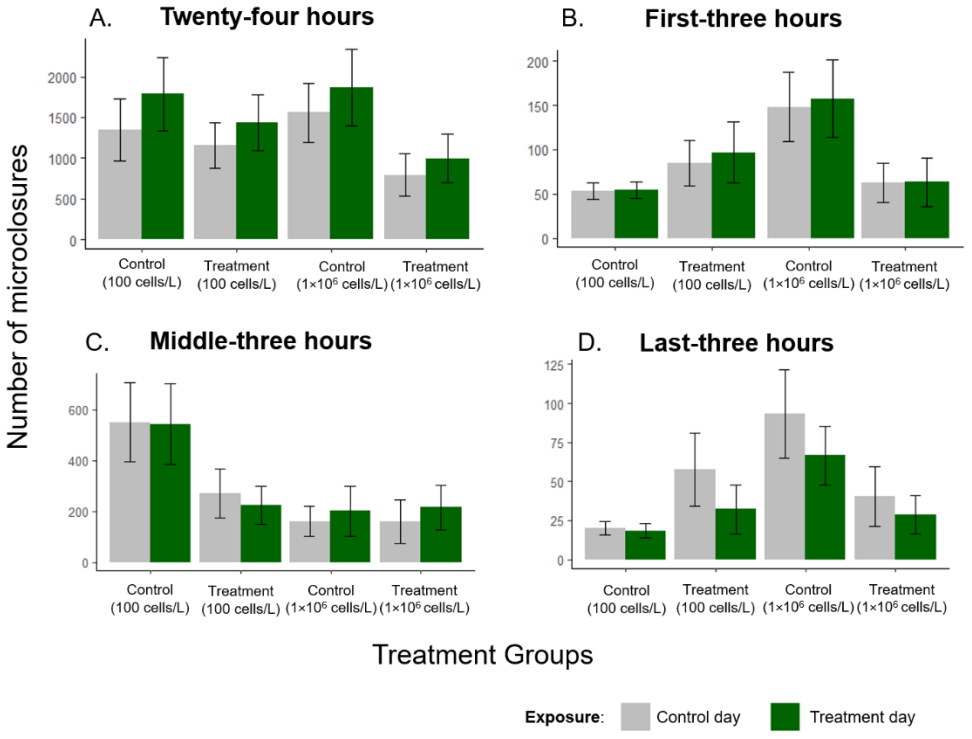


Figure 5.9 *The number of microclosures of* M. gigas *during different time intervals of the experimental day: 24 hours (**A**), first three hours (**B**), middle three hours (**C**), and last three hours (**D**) (mean ± 1 standard error). Zero (0) is when the oysters are entirely closed, while One (1) is when the oysters are completely open. M. gigas were exposed to four different treatments: alternate feeding with non-toxic algae (control day) and* P. lima *(treatment day) (10^2 cells L^{-1} and 10^6 cells L^1), and two control groups fed with non-toxic* Surirella *sp. (10^2 cells L^{-1} and 10^6 cells L^{-1}). Grey bars represent control days, and green bars indicate treatment days (n=8).*

5.4. Discussion

The present study builds upon earlier research that has established bivalves as promising biosensors for detecting the presence of toxic algae. Previous investigations have primarily focused on the bivalve gaping response to PSP-producing algae blooms (Comeau et al., 2019; Coquereau et al., 2016; Durier et al., 2022; Haberkorn et al., 2011; Lavaud et al., 2021, 2021; May et al., 2010; Tran et al., 2010). Notably, Haberkorn et al. (2011) and Tran et al. (2010) observed that *M. gigas* exhibit altered gaping behaviour, including increased opening duration and microclosures, as well as decreased opening amplitude, in response to exposure to *Alexandrium minutum*, a PSP producer.

Less attention is given to other harmful algal bloom (HAB) events, such as diarrhetic shellfish-poisoning (DSP)-associated blooms. DSP-associated algal blooms pose a serious threat to both human health and shellfish production, potentially leading to diarrhetic shellfish poisoning and increasing the incidence of colorectal cancer (Lopez-Rodas et al., 2006) Furthermore, these blooms are a leading cause of summer closures of shellfish production sites in the European Atlantic Arc (Fernandes-Salvador et al., 2021).

Given the importance of addressing DSP-associated HABs, this study aims to investigate the potential of *M. gigas* as an indicator species for the presence of the DSP-producer, *Prorocentrum lima*. Specific bivalve behaviours were closely

examined, including the amount of time spent widely open and closed, the amplitude of the opening, and the occurrence of microclosures following exposure to *Prorocentrum lima*.

Among the four behavioural parameters investigated, the duration of time spent in a wide-open state emerged as a potential indicator of the presence of *P. lima*. When exposed to a concentration of 10^6 cells L^{-1} of *P. lima*, *M. gigas* exhibited a significant decrease in the time spent in a wide-open state compared to the days when they were exposed to the equivalent concentration of the non-toxic control. This behaviour change was consistently observed at all time points, except for the last three hours of exposure. The statistical analysis revealed significant differences in this behaviour during the initial three hours and the 24-hours.

Notably, this behaviour shift was not observed in *M. gigas* exposed to a lower concentration of toxic algae (10^2 cells L^{-1}). This observation suggests a potential cell-concentration dependency of the behavioural response. It appears that *M. gigas* responds to higher concentrations of toxic algae (10^6 cells L^{-1}) with a decreased duration of wide opening, which may serve as a sensitive indicator of the presence of harmful algae. Furthermore, during the middle three hours of exposure, oysters exposed to the higher concentration of toxic algae (10^6 cells L^{-1}) spent significantly less time in a wide-open state than those exposed to the lower concentration (10^2 cells L^{-1}) of toxic algae.

These findings indicate that the time spent widely open by *M. gigas* is a valuable behavioural parameter for detecting the presence of *P. lima*. However, the lack of a significant difference in gaping behaviour during the last three hours of exposure suggests potential habituation or adaptation to the toxic algae over time. Further investigation into the underlying mechanisms of this behavioural pattern, particularly regarding potential physiological or cellular adjustments in *M. gigas*, is warranted to fully comprehend the implications of prolonged exposure to toxic algae on bivalve behaviour.

This study validates the initial hypothesis proposed in the introduction and builds upon the findings of Romero-Geraldo et al. (2016). The previous study reports visual observations of adult *M. gigas* exhibiting a narrow opening behaviour (with no specific measurement of gaping amplitude) during the first three hours after exposure to *P. lima*, which was not observed in oysters exposed to the control algae *I. galbana* (Romero-Geraldo et al., 2016).

The observed decrease in the duration of time spent wide open by *M. gigas* in response to a concentration of 10^6 cells L^{-1} of *P. lima* suggests a significant alteration in the bivalves' gaping behaviour when exposed to this toxic alga. This behavioural change is a critical aspect of the study as it potentially indicates the presence of harmful algal blooms.

Several factors may contribute to this change in gaping behaviour. Firstly, bivalves possess sensory mechanisms that enable them to detect the presence of harmful

substances in their environment (Balbi et al., 2021). In this case, the toxic compounds released by *P. lima* may trigger a defensive response in *M. gigas*, leading them to minimise the time their shells are wide open. By spending less time in a wide-open state, the bivalves could effectively bereducing the chances of ingesting or coming into contact with the toxic cells and their associated toxins, thereby protecting themselves from potential harm.

Secondly, exposure to high concentrations of toxic algae like *P. lima* might induce stress in *M. gigas*. In response to stressors, bivalves may exhibit behavioural changes as part of a stress response mechanism. Spending less time widely open could be a manifestation of this stress response.

Moreover, the presence of *P. lima* and its toxins have direct physiological effects on *M. gigas*, influencing their gaping behaviour. Current knowledge suggests physiological alterations in bivalves induced by DST, such as infiltration of haemocytes, inhibition of phagocytosis, reduction in the tubule area in the digestive gland, increased expression of genes involved in the cell cycle (p21, cafp55, p53), cytoskeleton (tub, act), and immune and inflammatory processes (casp1) (Chi et al., 2016, 2017; Galimany et al., 2008; Hégaret et al., 2009; Huang et al., 2015; Manfrin et al., 2010; Neves et al., 2019; Romero-Geraldo et al., 2016; Romero-Geraldo & Hernández-Saavedra, 2014; Simões et al., 2015; Suarez-Ulloa et al., 2015). These physiological changes might be associated with alterations in bivalves' behaviour, including the observed decrease in wide-opening behaviour in response to *P. lima*. However, further research is needed to understand the underlying mechanisms and

the specific role of these physiological changes in shaping the bivalves' behavioural responses fully.

Although no significant differences were found in the other behaviours, they are still worth exploring, as this lack of statistical difference might be attributed to the relatively low sample size (n=8). The absence of a significant difference at 10^{2} cells L^{-1} may imply that *M. gigas* cannot effectively detect the presence of *P. lima* at regulatory levels set by European countries.

In the future, conducting experiments with oysters exposed to varying cell concentrations is worthwhile. This will help determine the specific cell concentration at which *M. gigas* can exhibit a significant difference in behavioural patterns. By investigating a broader range of cell concentrations, we can better understand the oysters' responsiveness to *P. lima* and potentially identify the threshold at which their behaviour changes significantly. This information could have valuable implications for regulatory monitoring and management of harmful algal blooms in European waters.

The deployment of valve behaviour sensors should be done in areas known to have regular occurrence of DST-associated algal blooms, such as the areas shown in Fig 5.1. The resulting field recordings can then be used to confirm further if these oysters can be used as an effective biomonitoring tool for DST-associated HAB and

eventually develop a system wherein they can be used as an early warning for *P. lima's* presence in the water.

5.5. Conclusion

In conclusion, this study represents a pioneering effort in the field of harmful algal bloom (HAB) research, as it appears to be the first investigation to examine the effects of diarrhetic shellfish-poisoning (DSP) toxins on bivalve gaping behaviour, specifically focusing on the Pacific oyster, *M. gigas*, as a potential biosensor for DSP-associated algal blooms. While previous research has suggested that bivalves may serve as promising indicators for toxic algae, it is essential to acknowledge that our knowledge on this specific aspect, particularly concerning DSP-associated blooms, has been limited, with most studies concentrating on paralytic shellfish-poisoning (PSP)-producing algae blooms.

The key findings of this study indicate that *M. gigas* demonstrates a noticeable increase in the duration of time spent in a wide-open state when exposed to the DSP-producer, *Prorocentrum lima*. This behavioural change may serve as a potentially sensitive indicator of of harmful algae, specifically DSP-associated HABs. Additionally, the observed alterations in bivalve behaviour imply that *M. gigas* might be employing a defensive mechanism to protect itself from the hazardous substances released by *P. lima*.

While these initial findings offer intriguing insights, it is crucial to acknowledge the need for further research to validate and confirm the role of *M. gigas* as an effective biomonitoring tool for early detection of DSP-associated HABs. As the first study exploring the link between *M. gigas'* valve gaping response and DSP toxins, our findings may pave the way for future investigations in harmful algal bloom ecology.

The discovery of *M. gigas'* potential behavioural response to DSP-associated HABs opens up new avenues for further exploration, and understanding the underlying physiological processes triggered by DSP toxins merits additional investigation. Looking ahead, conducting experiments with oysters exposed to varying cell concentrations of *P. lima* is worthwhile, as this will help determine the specific cell concentration at which *M. gigas* can exhibit a significant difference in behavioural patterns. Investigating a broader range of cell concentrations can provide a deeper understanding of the oysters' responsiveness to *P. lima* and potentially identify the threshold at which their behaviour changes significantly. These efforts could have valuable implications for regulatory monitoring and management of harmful algal blooms in European waters.

In summary, this study presents *M. gigas* as a potentially promising biomonitoring tool for the early detection of DSP-associated HABs, offering valuable insights for environmental monitoring and contributing to the sustainable management of marine ecosystems and human health. The tentative pioneering nature of being one of the first investigations to explore the correlation between *M. gigas'* valve gaping response and DSP toxins adds an intriguing dimension to our understanding of

harmful algal bloom dynamics and the potential role of bivalves as bioindicators in environmental protection endeavours.

5.6. References

Andrade, H., Massabuau, J.-C., Cochrane, S., Ciret, P., Tran, D., Sow, M., & Camus, L. (2016). High Frequency Non-invasive (HFNI) Bio-Sensors As a Potential Tool for Marine Monitoring and Assessments. Frontiers in Marine Science, 3. https://www.frontiersin.org/article/10.3389/fmars.2016.00187

Balbi, T., Auguste, M., Ciacci, C., & Canesi, L. (2021). Immunological Responses of Marine Bivalves to Contaminant Exposure: Contribution of the -Omics Approach. Frontiers in Immunology, 12, 618726. https://doi.org/10.3389/fimmu.2021.618726

Basti, L., Nagai, K., Segawa, S., Tanaka, Y., Suzuki, T., & Nagai, S. (2019). Harmful algal blooms and shellfish aquaculture in changing environment—United States-Japan Natural Resources Panel on Aquaculture (NOAA-FRA) [Conference paper]. The 46th UJNR Scientific Symposium “Marine Aquaculture in a Changing Environment.” Hiroshima, Japan.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), Article 1. https://doi.org/10.18637/jss.v067.i01

Blanco, J. (2018). Accumulation of Dinophysis Toxins in Bivalve Molluscs. Toxins, 10(11), E453. https://doi.org/10.3390/toxins10110453

Butzke, D., Grune, B., Kugler, J., Oelgeschläger, M., Seiler, A., Sittner, D., Liebsch, M., & Luch, A. (2013). Chapter 3—The Advent of the Golden Era of Animal Alternatives. In P. M. Conn (Ed.), Animal Models for the Study of Human Disease (pp. 49–73). Academic Press. https://doi.org/10.1016/B978-0-12-415894-8.00003-8

Chen, T., Xu, X., Wei, J., Chen, J., Miu, R., Huang, L., Zhou, X., Fu, Y., Yan, R., Wang, Z., Liu, B., & He, F. (2013). Food-Borne Disease Outbreak of Diarrhetic Shellfish Poisoning Due to Toxic Mussel Consumption: The First Recorded Outbreak in China. PLOS One, 8(5), e65049. https://doi.org/10.1371/journal.pone.0065049

Chi, C., Giri, S. S., Jun, J. W., Kim, H. J., Kim, S. W., Yun, S., & Park, S. C. (2017). Effects of algal toxin okadaic acid on the non-specific immune and antioxidant response of bay scallop (*Argopecten irradians*). Fish & Shellfish Immunology, 65, 111–117. https://doi.org/10.1016/j.fsi.2017.03.031

Chi, C., Giri, S. S., Jun, J. W., Kim, H. J., Yun, S., Kim, S. G., & Park, S. C. (2016). Marine Toxin Okadaic Acid Affects the Immune Function of Bay Scallop (*Argopecten irradians*). Molecules, 21(9), Article 9. https://doi.org/10.3390/molecules21091108

Clements, J., & Comeau, L. (2019). Use of high-frequency, non-invasive electromagnetic biosensors to detect ocean acidification effects on shellfish behaviour. Journal of Shellfish Research, 38, 811–818. https://doi.org/10.2983/035.038.0330

Coates, L., Perks, R., Swan, S., Davidson, K., Turner, A. D., Maskrey, B., Bickerstaff, L., Ford, C., & Panton, S. W. (2019). Annual report on the results of

the Shellfish Official Control Monitoring Programmes for Scotland—2019 [Technical Report]. UK Centre for Environment, Fisheries, and Aquaculture Science. 76.

Coates, L., Stubbs, B., Turner, A. D., Williams, O., Milligan, S., & Algoet, M. (2014). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England & Wales—2014 [Technical Report]. UK Centre for Environment, Fisheries, and Aquaculture Science.146.

Coates, L., Wilkinson, T., Stubbs, B., Turner, A. D., Milligan, S., & Algoet, M. (2015). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England and Wales—2015 [Technical Report]. UK Centre for Environment, Fisheries, and Aquaculture Science.150.

Comeau, L. A., Babarro, J. M. F., Riobó, P., Scarratt, M., Starr, M., & Tremblay, R. (2019). PSP-producing dinoflagellate *Alexandrium minutum* induces valve microclosures in the mussel *Mytilus galloprovincialis*. Aquaculture, 500, 407–413. https://doi.org/10.1016/j.aquaculture.2018.10.025

Coquereau, L., Jolivet, A., Hégaret, H., & Chauvaud, L. (2016). Short-Term Behavioural Responses of the Great Scallop *Pecten maximus* Exposed to the Toxic Alga *Alexandrium minutum* Measured by Accelerometry and Passive Acoustics. PLOS One, 11(8), e0160935. https://doi.org/10.1371/journal.pone.0160935

David, H., Laza-Martínez, A., Kromkamp, J. C., & Orive, E. (2018). Physiological response of *Prorocentrum lima* (Dinophyceae) to varying light intensities. FEMS Microbiology Ecology, 94(1), fix166. https://doi.org/10.1093/femsec/fix166

Durier, G., Nadalini, J.-B., Comeau, L. A., Starr, M., Michaud, S., Tran, D., St-Louis, R., Babarro, J. M. F., Clements, J. C., & Tremblay, R. (2022). Use of valvometry as an alert tool to signal the presence of toxic algae *Alexandrium catenella* by *Mytilus edulis*. Frontiers in Marine Science, 9. https://www.frontiersin.org/articles/10.3389/fmars.2022.987872

FAO/WHO (2016). FAO/WHO Technical Paper on Toxicity Equivalency Factors for Marine Biotoxins Associated with Bivalve Molluscs. FAO/WHO. https://www.fao.org/documents/card/es/c/89196cd6-d970-49ee-8823-61f3a866fd64/

Fernandes-Salvador, J. A., Davidson, K., Sourisseau, M., Revilla, M., Schmidt, W., Clarke, D., Miller, P. I., Arce, P., Fernández, R., Maman, L., Silva, A., Whyte, C., Mateo, M., Neira, P., Mateus, M., Ruiz-Villarreal, M., Ferrer, L., & Silke, J. (2021). Current Status of Forecasting Toxic Harmful Algae for the North-East Atlantic Shellfish Aquaculture Industry. Frontiers in Marine Science, 8. https://www.frontiersin.org/article/10.3389/fmars.2021.666583

Fu, L., Zhao, X., Ji, L., & Xu, J. (2019). Okadaic acid (OA): Toxicity, detection and detoxification. Toxicon, 160, 1–7. https://doi.org/10.1016/j.toxicon.2018.12.007

Fujiki, H., & Suganuma, M. (1993). Tumour promotion by inhibitors of protein phosphatases 1 and 2A: The okadaic acid class of compounds. Advances in Cancer Research, 61, 143–194. https://doi.org/10.1016/s0065-230x(08)60958-6

Galimany, E., Sunila, I., Hégaret, H., Ramón, M., & Wikfors, G. H. (2008). Experimental exposure of the blue mussel (*Mytilus edulis*, L.) to the toxic dinoflagellate *Alexandrium fundyense*: Histopathology, immune responses, and

recovery. Harmful Algae, 5(7), 702–711. https://doi.org/10.1016/j.hal.2008.02.006

Guterres, B. V., Junior, J. N. J., Guerreiro, A. S., Fonseca, V. B., Botelho, S. S. C., & Sandrini, J. Z. (2020). Intelligent Classifiers on the Construction of Pollution Biosensors Based on Bivalves Behavior. In R. Cerri & R. C. Prati (Eds.), Intelligent Systems (pp. 588–603). Springer International Publishing. https://doi.org/10.1007/978-3-030-61380-8_40

Haberkorn, H., Tran, D., Massabuau, J.-C., Ciret, P., Savar, V., & Soudant, P. (2011). Relationship between valve activity, microalgae concentration in the water and toxin accumulation in the digestive gland of the Pacific oyster *Magallana gigas* exposed to *Alexandrium minutum*. Marine Pollution Bulletin, 62(6), 1191–1197. https://doi.org/10.1016/j.marpolbul.2011.03.034

Hanana, H., Talarmin, H., Pennec, J.-P., Droguet, M., Morel, J., & Dorange, G. (2012). Effect of okadaic acid on cultured clam heart cells: Involvement of MAPkinase pathways. Biology Open, 1(12), 1192–1199. https://doi.org/10.1242/bio.20122170

Harris, C. (2020). This Polish city is using mussels to monitor water quality. Retrieved November 15, 2022, from https://www.awa.asn.au/resources/latest-news/technology/innovation/polish-city-using-mussels-monitor-water-quality

Harrison, K., Walton, A., Coates, L., Turner, A., & Algoet, M. (2017). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England & Wales – 2017 [Technical Report]. UK Centre for Environment, Fisheries, and Aquaculture Science. 32.

Harrison, K., Wilkinson, T., Coates, L., Turner, A. D., Milligan, S., & Algoet, M. (2016). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England & Wales—2016 [Techical Report]. . UK Centre for Environment, Fisheries, and Aquaculture Science 43.

He, Z.-B., Duan, G.-F., Liang, C.-Y., Li, H.-Y., Liu, J.-S., & Yang, W.-D. (2019). Up-regulation of Nrf2-dependent antioxidant defenses in *Perna viridis* after exposed to *Prorocentrum lima*. Fish & Shellfish Immunology, 90, 173–179. https://doi.org/10.1016/j.fsi.2019.05.003

Hégaret, H., da Silva, P. M., Sunila, I., Shumway, S. E., Dixon, M. S., Alix, J., Wikfors, G. H., & Soudant, P. (2009). Perkinsosis in the Manila clam *Ruditapes philippinarum* affects responses to the harmful-alga, *Prorocentrum minimum*. Journal of Experimental Marine Biology and Ecology, 371(2), 112–120. https://doi.org/10.1016/j.jembe.2009.01.016

Huang, L., Zou, Y., Weng, H., Li, H.-Y., Liu, J.-S., & Yang, W.-D. (2015). Proteomic profile in *Perna viridis* after exposed to *Prorocentrum lima*, a dinoflagellate producing DSP toxins. Environmental Pollution, 196, 350–357. https://doi.org/10.1016/j.envpol.2014.10.019

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software, 82, 1–26. https://doi.org/10.18637/jss.v082.i13

Lavaud, R., Durier, G., Nadalini, J.-B., Filgueira, R., Comeau, L. A., Babarro, J. M. F., Michaud, S., Scarratt, M., & Tremblay, R. (2021). Effects of the toxic dinoflagellate *Alexandrium catenella* on the behaviour and physiology of the

blue mussel *Mytilus edulis*. Harmful Algae, 108, 102097. https://doi.org/10.1016/j.hal.2021.102097

Lee, T. C.-H., Fong, F. L.-Y., Ho, K.-C., & Lee, F. W.-F. (2016). The Mechanism of Diarrhetic Shellfish Poisoning Toxin Production in *Prorocentrum* spp.: Physiological and Molecular Perspectives. Toxins, 8(10). https://doi.org/10.3390/toxins8100272

Leite, I. do P., Sandrini-Neto, L., Squella, F. L., Alves, T. P., Schramm, M. A., Calado, S. L. de M., Silva de Assis, H. C., & Mafra, L. L. (2020). Toxin accumulation, detoxification and oxidative stress in bivalve (*Anomalocardia flexuosa*) exposed to the dinoflagellate *Prorocentrum lima*. Aquatic Toxicology (Amsterdam, Netherlands), 232, 105738. https://doi.org/10.1016/j.aquatox.2020.105738

Lenth R (2023). emmeans: Estimated Marginal Means, aka Least-Squares Mean. R package version 1.8.6, <https://CRAN.R-project.org/package=emmeans>.

Lindegarth, S., Torgersen, T., Lundve, B., & Sandvik, M. (2009). Differential Retention of Okadaic Acid (OA) Group Toxins and Pectenotoxins (PTX) in the Blue Mussel, *Mytilus edulis* (L.), and European Native oyster, *Ostrea edulis* (L.). Journal of Shellfish Research, 28(2), 313–323. https://doi.org/10.2983/035.028.0213

Lopez-Rodas, V., Maneiro, E., Martinez, J., Navarro, M., & Costas, E. (2006). Harmful algal blooms, red tides and human health: Diarrhetic shellfish poisoning and colorectal cancer. Anales de la Real Academia Nacional de Farmacia. 72(3):391-408

Manfrin, C., Dreos, R., Battistella, S., Beran, A., Gerdol, M., Varotto, L., Lanfranchi, G., Venier, P., & Pallavicini, A. (2010). Mediterranean Mussel Gene Expression Profile Induced by Okadaic Acid Exposure. Environmental Science & Technology, 44(21), 8276–8283. https://doi.org/10.1021/es102213f

May, S. P., Burkholder, J. M., Shumway, S. E., Hégaret, H., Wikfors, G. H., & Frank, D. (2010). Effects of the toxic dinoflagellate *Alexandrium monilatum* on survival, grazing and behavioral response of three ecologically important bivalve molluscs. Harmful Algae, 9(3), 281–293. https://doi.org/10.1016/j.hal.2009.11.005

Nascimento, S. M., Salgueiro, F., Menezes, M., Oliveira, F. de A., Magalhães, V. C. P., De Paula, J. C., & Morris, S. (2016). *Prorocentrum lima* from the South Atlantic: Morphological, molecular and toxicological characterization. Harmful Algae, 57, 39–48. https://doi.org/10.1016/j.hal.2016.05.006

Neves, R. A. F., Santiago, T. C., Carvalho, W. F., Silva, E. dos S., da Silva, P. M., & Nascimento, S. M. (2019). Impacts of the toxic benthic dinoflagellate *Prorocentrum lima* on the brown mussel *Perna perna*: Shell-valve closure response, immunology, and histopathology. Marine Environmental Research, 146, 35–45. https://doi.org/10.1016/j.marenvres.2019.03.006

Parks, R., Bear, E., Coates, L., & Maskrey, B. (2019). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring Programmes for England & Wales – 2019 [Technical Report]. UK Centre for Environment, Fisheries, and Aquaculture Science. 39.

Parks, R., Walton, A., Coates, L., & Maskrey, B. (2018). Cefas Annual report on the results of the Biotoxin and Phytoplankton Official Control Monitoring

Programmes for England & Wales – 2018 [Technical Report]. UK Centre for Environment, Fisheries, and Aquaculture Science. 37.

Romero-Geraldo, R. de J., García-Lagunas, N., & Hernández-Saavedra, N. Y. (2016). *Magallana gigas* exposure to the dinoflagellate *Prorocentrum lima*: Histological and gene expression effects on the digestive gland. Marine Environmental Research, 120, 93–102. https://doi.org/10.1016/j.marenvres.2016.07.011

Romero-Geraldo, R. de J., & Hernández-Saavedra, N. Y. (2014). Stress Gene Expression in *Magallana gigas* (Thunberg, 1793) in response to experimental exposure to the toxic dinoflagellate *Prorocentrum lima* (Ehrenberg) Dodge, 1975. Aquaculture Research, 45(9), 1512–1522. https://doi.org/10.1111/are.12100

Rossignoli, A. E., Fernández, D., Regueiro, J., Mariño, C., & Blanco, J. (2011). Esterification of okadaic acid in the mussel *Mytilus galloprovincialis*. Toxicon: Official Journal of the International Society on Toxinology, 57(5), 712–720. https://doi.org/10.1016/j.toxicon.2011.02.003

Shakspeare, A., Moore, H., Service, M., Wilson, C., Ahmed, H., Cameron, T. C., & Steinke, M. (2023). Gaping behaviour of Blue mussels (*Mytilus edulis*) in relation to freshwater runoff risks. Aquaculture Reports, 33, 101719. https://doi.org/10.1016/j.aqrep.2023.101719

Simões, E., Vieira, R. C., Schramm, M. A., Mello, D. F., Pontinha, V. D. A., Silva, P. M. da, & Barracco, M. A. (2015). Impact of harmful algal blooms (*Dinophysis acuminata*) on the immune system of oysters and mussels from Santa

Catarina, Brazil. Journal of the Marine Biological Association of the United Kingdom, 95(4), 773–781. https://doi.org/10.1017/S0025315414001702

Suarez-Ulloa, V., Fernandez-Tajes, J., Aguiar-Pulido, V., Prego-Faraldo, M. V., Florez-Barros, F., Sexto-Iglesias, A., Mendez, J., & Eirin-Lopez, J. M. (2015). Unbiased high-throughput characterization of mussel transcriptomic responses to sublethal concentrations of the biotoxin okadaic acid. PeerJ, 3, e1429. https://doi.org/10.7717/peerj.1429

Suganuma, M., Fujiki, H., Suguri, H., Yoshizawa, S., Hirota, M., Nakayasu, M., Ojika, M., Wakamatsu, K., Yamada, K., & Sugimura, T. (1988). Okadaic acid: An additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter. Proceedings of the National Academy of Sciences of the United States of America, 85(6), 1768–1771. https://doi.org/10.1073/pnas.85.6.1768

Talarmin, H., Droguet, M., Pennec, J. P., Schröder, H. C., Muller, W. E. G., Gioux, M., & Dorange, G. (2008). Effects of a phycotoxin, okadaic acid, on oyster heart cell survival. Toxicological & Environmental Chemistry, 90(1), 153–168. https://doi.org/10.1080/02772240701382131

Torgersen, T., Sandvik, M., Lundve, B., & Lindegarth, S. (2008). Profiles and levels of fatty acid esters of okadaic acid group toxins and pectenotoxins during toxin depuration. Part II: Blue mussels (*Mytilus edulis*) and native oyster (*Ostrea edulis*). Toxicon: Official Journal of the International Society on Toxinology, 52(3), 418–427. https://doi.org/10.1016/j.toxicon.2008.06.011

Tran, D., Haberkorn, H., Soudant, P., Ciret, P., & Massabuau, J.-C. (2010). Behavioral responses of *Magallana gigas* exposed to the harmful algae

Alexandrium minutum. Aquaculture, 298(3), 338–345. https://doi.org/10.1016/j.aquaculture.2009.10.030

Türkoglu, M. (2016). First harmful algal bloom record of tycoplanktonic dinoflagellate *Prorocentrum lima* (Ehrenberg) F. Stein, 1878 in the Dardanelles (Turkish Straits System, Turkey). Journal of Coastal Life Medicine, 4, 765–774. https://doi.org/10.12980/jclm.4.2016J6-184

Whyte, C., Swan, S., & Davidson, K. (2014). Changing wind patterns linked to unusually high Dinophysis blooms around the Shetland Islands, Scotland. Harmful Algae, 39, 365–373. https://doi.org/10.1016/j.hal.2014.09.006

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Ye, M.-H., Li, D.-W., Cai, Q.-D., Jiao, Y.-H., Liu, Y., Li, H.-Y., & Yang, W.-D. (2022). Toxic Responses of Different Shellfish Species after Exposure to *Prorocentrum lima*, a DSP Toxins Producing Dinoflagellate. Toxins, 14(7), 461. https://doi.org/10.3390/toxins14070461

CHAPTER SIX

General Discussion and Conclusion

6.1. Introduction

In this concluding chapter, I synthesise and discuss the key findings, overarching themes, and policy implications emerging from this research on bivalve aquaculture and climate change resilience. This comprehensive discussion seeks to provide valuable insights and contributions to both scientific understanding and practical applications in aquaculture and environmental management.

Our journey began with a systematic review, highlighting gaps in existing research and setting the stage for subsequent investigations. We explored the thermal performance of *Ostrea edulis*, examined the effects of heatwaves and toxic algal blooms on *Magallana* (*Crassostre*a) *gigas*, and assessed the potential of these bivalves as biomonitoring tools for harmful algal blooms.

As we delve into the general discussion, I aim to connect the dots between these chapters, extract common themes, and provide a holistic perspective on the challenges and opportunities in bivalve aquaculture amid a changing climate. This research expands the boundaries of scientific knowledge and offers practical implications for aquaculturists, policymakers, and conservation managers.

This chapter serves as a platform to reflect on the broader significance of this work in addressing global food security and ecosystem resilience. It also identifies limitations

and suggests future research directions, emphasising the need for continued exploration in this critical field. In doing so, I contribute to the ongoing efforts to understand, adapt to, and mitigate the impacts of climate change on bivalve aquaculture and the environment.

6.2. Key Findings and Discussion

6.2.1. Systematic Review of Climate Change Stressors

The research journey embarked upon in this study initiated with a systematic review (Chapter 2), revealing critical knowledge gaps that underscore the pressing need for more comprehensive investigations in the realm of bivalve research. Historically, most research has been channelled towards a limited selection of bivalve species, such as *Crassostrea virginica* and *Mytilus galloprovincialis*. However, it has become increasingly evident that a broader exploration is imperative, encompassing ecologically significant bivalve species that offer vital ecosystem services and hold the potential for future economic benefits. Among these overlooked species are the giant clam *Tridacna squamosa*, the common cockle *Cerastoderma edule*, the Pacific rock oysters, and the European native oysters (Carss et al., 2020; Clements & Comeau, 2019; Coen et al., 2007; Gentry et al., 2020; Grabowski & Peterson, 2007; Neo et al., 2015).

Moreover, Chapter 2 underscores the glaring paucity of research on understudied bivalve life stages, particularly the critical early stages of embryos and larvae. These stages play an indispensable role in shaping overall population dynamics, and their responses to environmental stressors can ripple through time, profoundly impacting future adult populations (Talmage & Gobler, 2011). Comprehending how environmental factors influence these crucial life stages is pivotal for advancing bivalve aquaculture practices and assuring stable production levels.

The chapter also delves into the intricate challenges and far-reaching implications of multiple stressor studies in bivalve research. It underscores the underexplored dimension of stressors like harmful algal blooms (HABs) despite their potential to significantly disrupt aquaculture operations and the safety of bivalve products (Griffith & Gobler, 2017). Furthermore, it highlights the formidable challenge of replicating ecologically realistic stressor fluctuations within laboratory experiments. The review brings to the forefront the noticeable emphasis on specific response parameters, such as survival rates, biochemical indicators, and heavy metal accumulation, while other equally vital aspects, like bivalve reproductive traits and behaviours, remain relatively uncharted territories (Grazer & Martin, 2012; Van Colen et al., 2020).

Beyond the confines of the laboratory, these findings accentuate the imperative for collaboration among a diverse spectrum of stakeholders, including scientists, policymakers, industry leaders, and local communities. Equitable resource allocation is pivotal, especially in supporting research efforts in low-income nations. This is

crucial in light of the global ramifications of climate change on food security and ecological stability. The prevailing concentration of studies in high-income countries, notably the United States, Portugal, and Italy, among others, as observed in the comprehensive list of references, can be attributed to the more significant financial resources available in these regions, facilitating extensive research into the impacts of climate change on bivalves (Acharya & Pathak, 2019). Recognising the budgetary constraints low-income nations face becomes paramount, potentially leaving information gaps concerning native, non-invasive bivalve species in these regions.

In conclusion, this chapter has uncovered a compelling narrative that beckons for more inclusive, holistic, and globally conscious bivalve research. The findings accentuate the need for an expanded focus on overlooked bivalve species, a deeper exploration of critical life stages and a more comprehensive understanding of the intricate web of stressors that affect them. Moreover, it underscores the necessity of fostering international collaboration and addressing resource disparities to ensure a robust and equitable response to the challenges posed by climate change on bivalve ecosystems, food security, and ecological stability. This research serves as a clarion call for the bivalve research community to collectively navigate the uncharted waters of knowledge, stewarding these vital organisms toward a resilient and sustainable future.

6.2.2. Thermal Performance of Ostrea edulis

In this study, we delved into the thermal responses of European native oysters, specifically *Ostrea edulis*, to unravel their essential thermal requirements and the consequent implications for bivalve aquaculture management. Our exploration unfolded through two key experiments, each shedding light on critical aspects of *O. edulis*' thermal sensitivity.

In Experiment 1, my observations unveiled a notable stability in the metabolic rate (MO_2) of *O. edulis* within a temperature range of 9-21°C, in line with prior research by Hutchinson & Hawkins (1992). However, a pivotal revelation occurred beyond 24°C, as I observed a significant and abrupt surge in MO_2, concomitant with initial mortality at 27°C. This temperature-dependent response underscores a distinct physiological reaction of *O. edulis* to elevated temperatures, imparting vital information directly relevant to aquaculture practices.

Experiment 2, which incorporated heart rate measurements, corroborated the MO_2 increase at 28°C while instigating a decline in heart rate. Here, I determined an Arrhenius Break Point Temperature (ABT) of 24.88 ± 1.25°C, unequivocally signifying a critical thermal threshold for *O. edulis*, as Eymann et al. (2020) documented. These findings closely align with earlier research, notably the work of Eymann et al. (2020), which pinpointed the thermal optimum for *O. edulis* within the

range of 18 to 24°C. Importantly, Eymann et al. (2020) also underscored 26°C as a critical temperature, beyond which performance limitations such as reduced filtration rates and arrhythmia become evident.

This chapter's standout contribution is identifying 24°C as the upper thermal optimum for *O. edulis*. This conclusion gains robust support from the synthesis of findings from both experiments and alignment with prior studies (Eymann et al., 2020). As temperatures rise, *O. edulis*, like many aquatic ectotherms, experiences an increase in metabolic rate (Giomi & Poertner, 2013). However, it is essential to recognise that there are limits to this adaptation. Beyond an estimated upper critical limit, ranging between 33 and 36°C identified in this study, *O. edulis* can only endure short-term exposure. This critical response arises from breaking weak protein bonds, leading to protein unfolding and a subsequent heat shock response characterised by upregulated heat shock proteins (HSPs). However, this protective mechanism costs overall performance, underscoring the importance of maintaining optimal water temperatures in bivalve aquaculture (Miller & Stillman, 2012).

The ramifications of these findings extend far beyond the confines of aquaculture. With global temperatures rising and heatwaves becoming increasingly common, *O. edulis* populations face mounting challenges. Frequent exposure to temperatures exceeding the thermal optimum triggers physiological and behavioural changes, disrupting metabolic processes and compromising essential functions such as growth, reproduction, and immune response (Abele et al., 2002; Anestis et al., 2010; Arcus et al., 2016; Artigaud et al., 2014; Beiras et al., 1995; Brown & Hartwick, 1988;

Bylenga et al., 2015; Carss et al., 2020; Talmage & Gobler, 2011). The heightened metabolic demands for homeostasis and cellular repair under such conditions divert resources away from vital functions, jeopardising overall well-being. Consequently, *O. edulis* may be compelled to migrate towards higher latitudes in search of suitable conditions. However, this shift can result in habitat fragmentation, decreased connectivity, and intensified resource competition, further imperilling population survival.

In summation, this chapter serves as a beacon illuminating critical insights into the thermal requirements and responses of *O. edulis*, yielding valuable implications for both bivalve aquaculture and broader ecological considerations in the context of climate change. The pinpointing of 24°C as the upper thermal optimum for *O. edulis* carries profound significance for aquaculture management, emphasising the importance of continuous temperature monitoring and control as we navigate the dynamic challenges of a changing climate.

6.2.3. Impact of Heatwaves and Algal Blooms on Magallana gigas

This chapter represents a deep dive into the combined effects of a heatwave and a *Prorocentrum lima* algal bloom on Pacific oysters (*Magallana gigas*), yielding invaluable insights into their responses to these simultaneous stressors. Several key

findings emerged, shedding light on the intricate interactions between these environmental challenges.

First and foremost, it was observed that exposure to *P. lima*, either alone or in conjunction with the concurrent heatwave, resulted in the accumulation of diarrhetic shellfish toxins (DSTs), primarily okadaic acid (OA), within the soft tissues of the oysters. Alarmingly, within a mere five-day exposure, the total OA concentration exceeded the safety thresholds for shellfish consumption in Europe and other regions. This alarming revelation underscores the substantial public health risks associated with toxic algal blooms, particularly in shifting environmental conditions (Anderson et al., 2012).

Regarding metabolic responses, it is customary for Pacific oysters to exhibit increased metabolic activity in response to elevated temperatures, mirroring a common trend among aquatic ectotherms (Rubalcaba et al., 2020). However, the oysters displayed an unexpected dampening of this metabolic response when exposed to both the heatwave and *P. lima*. This intriguing phenomenon suggests that toxic algae may disrupt the oysters' typical metabolic reactions to heat stress, potentially compromising their ability to cope with rapidly changing environments (Turner et al., 2019). This observed interaction between the stressors, where their combined impact on metabolic activity deviated from the mere sum of their individual effects, underscores the complexity of these interactions, necessitating a more nuanced understanding that goes beyond the study of individual stressors in isolation.

Additionally, evaluating the condition index (CI) as a tool for assessing bivalve health in response to these stressors revealed limited effectiveness. Unlike prior studies that demonstrated CI changes in response to heat stress or toxic algae (Hiebenthal et al., 2012; Lannig et al., 2006), this investigation found no immediate impact on CI. However, it is crucial to acknowledge that the timing of the experiment, conducted post-reproduction, may have influenced these outcomes. This underscores the importance of considering the reproductive cycle's influence on CI in bivalves subjected to stressors (Lucas & Beninger, 1985).

Finally, the study examined immune parameters in Pacific oysters, including total haemocyte count (THC), lysosomal membrane stability measured through Neutral Red uptake (NR), and haemolymph protein concentration (HPC). Notably, THC exhibited an antagonistic response when exposed to both stressors during recovery, indicating a modulation mechanism in haemocyte numbers. This finding contributes to the ongoing discourse on the impact of environmental stressors on bivalve immune responses (Matozzo & Marin, 2011). While NR uptake showed variability between treatment groups, no statistically significant differences were observed, indicating potential short-term effects on lysosomal membrane stability that warrant further investigation. Additionally, HPC remained unchanged under the influence of both stressors, aligning with previous studies on Pacific oysters (Mello et al., 2010). Collectively, these findings enhance our understanding of how Pacific oysters respond to multiple stressors at the immune level.

This chapter illuminates the intricate interplay between heatwaves, toxic algal blooms, and Pacific oysters, offering critical insights into toxin accumulation, metabolic responses, and immune parameters. These findings underscore the imperative of considering the collective impacts of multiple stressors and the urgent need for comprehensive monitoring and management strategies in the face of environmental change, safeguarding the health and resilience of marine ecosystems.

6.2.4. Oyster Valve Behaviour as a Biomonitoring Tool

In this chapter, the research aimed to assess the feasibility of employing the Pacific oyster, *M. gigas*, as a biosensor for detecting *Prorocentrum lima*, a DSP-producing algae. This investigation extended the existing body of knowledge, which predominantly focused on PSP-producing algae blooms, by exploring bivalve responses to a broader spectrum of harmful algal bloom (HAB) events. The primary findings underscore that *M. gigas* undergoes a significant modification in its gaping behaviour when exposed to *P. lima*. Specifically, there is a noteworthy decrease in the time the oysters spend in a wide-open state, a change particularly pronounced at a concentration of 10^6 cells L^{-1} of *P. lima*. This behavioural response, consistently observed at most time intervals during exposure, suggests a potential dependence on the concentration of toxic algae cells and, consequently, offers promise as a sensitive indicator for the presence of DSP-producing algae. These outcomes are in accordance with previous research emphasising the potential utility of bivalves as indicators of the presence of toxic algae. Nevertheless, this study extends the applicability of this approach to DSP-associated HABs, which have received

comparatively less attention in previous research predominantly focused on PSP-producing algae (Comeau et al., 2019; Coquereau et al., 2016; Durier et al., 2022; Haberkorn et al., 2011; Lavaud et al., 2021; May et al., 2010; Tran et al., 2010).

I explored several potential explanations to comprehend the mechanisms underlying this behavioural change in *M. gigas*. One hypothesis posits that bivalves possess sensory mechanisms to detect harmful substances in their environment (Balbi et al., 2021). Alternatively, the observed behavioural alterations might be associated with stress responses induced by exposure to high concentrations of toxic algae. Additionally, DSP toxins from *P. lima* could directly influence the physiological state of *M. gigas*, potentially leading to alterations in gaping behaviour as a defensive strategy against ingestion or contact with toxic cells and their associated toxins. While this study provides compelling initial insights, further research must delve deeper into the underlying mechanisms responsible for these behavioural changes and the physiological adjustments occurring within *M. gigas* (Balbi et al., 2021).

Furthermore, the absence of significant behavioural differences at a lower cell concentration (10^2 cells L^{-1}) implies that *M. gigas* may not effectively detect *P. lima* at regulatory levels stipulated by European countries. To address this limitation, future experiments encompassing a broader spectrum of cell concentrations should be conducted to pinpoint the threshold at which significant behavioural changes manifest. This knowledge has the potential to be invaluable for regulatory monitoring and management of harmful algal blooms in European waters. In addition, the prospect of deploying valve behaviour sensors in regions with a historical prevalence

of DST-associated algal blooms holds promise for generating field data that can affirm the efficacy of *M. gigas* as biomonitoring for DSP-associated HABs. This deployment may also establish a practical early warning system for *P. lima* in aquatic environments, contributing to proactive environmental protection and public health safety.

In summary, this chapter constitutes a valuable contribution to the evolving domain of HAB research by exploring the link between *M. gigas*' valve gaping response and DSP toxins. These findings strongly suggest that *M. gigas* can function as a highly sensitive biosensor for DSP-associated HABs, thereby broadening our understanding of harmful algal bloom dynamics and the potential role of bivalves as bioindicators in environmental conservation efforts. This pioneering endeavour adds a novel dimension to the field. It underscores the importance of further investigations to validate and refine the utility of *M. gigas* as a biomonitoring tool for DSP-producing algae in the interest of safeguarding marine ecosystems and public health (Comeau et al., 2019; Coquereau et al., 2016; Durier et al., 2022; Haberkorn et al., 2011; Lavaud et al., 2021; May et al., 2010; Tran et al., 2010).

6.3. Overarching Themes and Insights

6.3.1. Bivalve Vulnerability to Climate Change

In this thesis, I have undertaken a comprehensive examination of bivalves' susceptibility to the impacts of climate change, addressing a matter of utmost significance. Bivalves have exhibited a remarkable degree of sensitivity to environmental shifts, with increasing sea temperatures occupying a central role in this vulnerability. My research has further discussed a significant association between elevated sea temperatures and the proliferation of harmful algal blooms. Chapter 3 vividly illustrates how heightened temperatures can induce metabolic stress, cardiac arrests, and even mortality in *O. edulis*, emphasising this issue's severity. Meanwhile, the findings in Chapter 4 underscore that exposure to toxic algae, like *P. lima*, suppresses oysters' metabolic responses, potentially impeding their ability to adapt to abrupt temperature fluctuations, which are crucial for survival amidst changing environmental conditions.

Chapter 5 reveals notable alterations in the behaviour of *M. gigas* when confronted with *P. lima* blooms, highlighting that they are indeed affected by this toxic algal bloom, prompting changes in their behaviour. These findings underscore the pressing need for implementing climate change mitigation measures to curb further warming of coastal waters. Failing to do so could have devastating consequences for

bivalve populations and the associated ecosystems that play an indispensable role in bivalve aquaculture.

6.3.2. Holistic Research Approach

One of the most critical lessons from this dissertation is the necessity of adopting a holistic research approach when studying the effects of climate change on bivalves. Historically, research in this field has often concentrated on a limited set of bivalve species, predominantly adult stages, and a narrow selection of response parameters. However, the dissertation emphasises broadening the scope to encompass a broader range of bivalve species, including lesser-studied varieties like Pacific rock oysters and European native oysters and early life stages. By doing so, researchers can gain a more comprehensive understanding of bivalve vulnerabilities, enabling more effective conservation and management strategies.

6.3.3. Environmental Resilience

The theme of environmental resilience runs as a common thread throughout the dissertation. Bivalves are integral to coastal ecosystems, contributing to water filtration, nutrient cycling, and overall ecosystem health. The research underscores that the resilience or vulnerability of bivalve populations can have far-reaching effects on these ecosystems. For instance, Chapter 4 delves into the interplay between heatwaves and toxic algal blooms and reveals how bivalves like *M. gigas*

are highly sensitive to these stressors. This sensitivity emphasises the need to prioritise ecosystem resilience and consider the broader ecological implications of bivalve responses to climate change.

6.3.4. Behaviour as a Biomonitoring Tool

A groundbreaking discovery from this research is the potential of bivalve behaviour, particularly valve behaviour, as a biomonitoring tool. Chapter 5 demonstrates how *M. gigas* responds to DSP-associated algal blooms by altering its gaping behaviour. Specifically, changes in the duration of valve openness indicate the bloom of DSP-associated toxic algae. This innovative approach has significant implications for the early detection of harmful algal blooms, benefiting aquaculture and ecosystem management. It signifies a shift from conventional monitoring methods to more dynamic and responsive approaches, potentially revolutionising the field.

6.3.5. Food Safety Concerns

The dissertation raises critical concerns regarding food safety in the context of toxic algal blooms. Chapter 4 highlights how the co-occurrence of heatwaves and toxic algal blooms can lead to elevated toxin levels in bivalves, surpassing regulatory limits for human consumption. This finding underscores the importance of rigorous monitoring and management practices to ensure consumers' safety of shellfish products. It also emphasises the economic repercussions for the aquaculture

industry, as contaminated shellfish can lead to market closures and financial losses. Aquaculture management practices should consider harvest timing to mitigate potential hazards related to diarrhetic shellfish toxin (DST) contamination in shellfish. Delaying the harvest of shellfish like *M. gigas* for a suitable period after exposure to harmful algal blooms, such as *P. lima*, can aid in lowering the levels of DST in their soft tissues. This precautionary step adheres to food safety guidelines and ensures the safety of shellfish for consumption.

6.3.6. Interdisciplinary Collaboration

Lastly, the research underscores the necessity of interdisciplinary collaboration to address the multifaceted challenges climate change poses on bivalves and coastal ecosystems. These challenges extend beyond scientific inquiry and require the involvement of various stakeholders, including scientists, policymakers, industry leaders, and local communities. The findings emphasise the need for a concerted effort to develop and implement effective climate change mitigation and adaptation strategies and equitable resource allocation to support research in low-income nations facing similar challenges.

6.4. Policy and Management Implications

The findings of this dissertation hold several critical implications for policy and management in the fields of bivalve aquaculture and climate change resilience. First

and foremost, diversifying bivalve species and life stages is a crucial policy consideration. Policymakers should encourage research and aquaculture practices that prioritise the inclusion of various bivalve species and life stages. This strategic diversification reduces the industry's reliance on a single species and enhances our understanding of vulnerabilities, ultimately contributing to greater aquaculture resilience. In practical terms, aquaculture managers are encouraged to diversify their stock, thereby mitigating risks associated with environmental stressors and promoting a more adaptable industry.

Another vital policy implication arises from our discovery of critical temperature thresholds for bivalve health. Regulatory agencies should consider implementing temperature-based management strategies for bivalve aquaculture and setting guidelines and thresholds for temperature exposure. This proactive approach is essential in safeguarding bivalve populations from the adverse effects of thermal stress. In their management capacity, Aquaculturists should closely monitor water temperatures and enact adaptive measures when thresholds are approached. Such actions could include adjustments in stocking densities, modifications in cultivation methods, and the use of cooling technologies to preserve bivalve health.

Furthermore, this research underscores the importance of monitoring and mitigating harmful algal blooms in bivalve aquaculture regions. Governments and regulatory bodies should establish comprehensive monitoring programs to address this, emphasising the rapid detection and response to toxic algal events and protecting both bivalves and public health, which hinges on timely interventions in the face of

harmful algal blooms. Aquaculture operators should adopt proactive strategies by monitoring water quality and implementing early warning systems to detect harmful algal blooms promptly. Contingency measures such as temporary closures and toxin testing should be readily available to safeguard bivalves and consumers.

This work introduces a novel concept—using bivalves as bioindicators of diarrhetic shellfish poisoning (DSP)-associated algal blooms. Policymakers and environmental agencies should consider incorporating bivalves as bioindicators in coastal monitoring programs, enhancing early detection of DSP-associated algal blooms and improving ecosystem health assessments. Aquaculture and conservation managers can actively engage in this endeavour by exploring the integration of bivalves as bioindicators into their routines. Routine monitoring of bivalve behaviour and health could provide invaluable data for early detection and response to environmental stressors.

Lastly, the broader implications encompass the sustainability of bivalve management and conservation. Governments and international bodies should advocate for sustainable bivalve management practices, emphasising the protection of bivalve habitats, responsible harvesting, and establishing marine protected areas. Aquaculture managers and conservationists should work hand in hand to ensure responsible bivalve cultivation and conservation. Efforts should centre on maintaining healthy bivalve populations and preserving the ecosystems they inhabit, promoting long-term sustainability.

In addition to these recommendations, collaboration is paramount. Governments and funding agencies should actively encourage collaborative research initiatives addressing the challenges in bivalve aquaculture and climate change resilience. The sharing of research findings and best practices should be facilitated among researchers, aquaculture practitioners, and policymakers. This collaborative approach can yield more effective strategies for addressing bivalve vulnerabilities, thereby ensuring the industry's long-term viability. Incorporating these policy and management implications into the decision-making process is essential for bolstering the resilience of bivalve aquaculture, food security, and safeguarding marine ecosystems in the face of climate change and environmental stressors.

6.5. Limitations and Future Research Direction

This dissertation's contributions to understanding bivalve aquaculture and climate change resilience are substantial but not without certain limitations. These limitations, while recognised, provide valuable insights into areas where future research efforts can be focused to build upon the foundation established in this study. One notable limitation in several chapters of this dissertation is the reliance on relatively small sample sizes. For instance, in Chapter 5, the study included a limited number of *M. gigas* specimens (n=8). This limited sample size could have impacted the statistical power of some analyses and potentially restricted the generalizability of findings. The research predominantly concentrated on specific bivalve species, such as *M. gigas* and *O. edulis*, which may exhibit unique responses to climate change stressors. Expanding the scope of future research to encompass a more

extensive range of bivalve species and larger sample sizes could provide a more comprehensive understanding of bivalve vulnerabilities.

Another limitation is the controlled laboratory conditions under which much of the research was conducted. While these controlled settings offer precise control over variables, they may not fully replicate the complex and dynamic conditions that bivalves experience in their natural habitats. Thus, future research could greatly benefit from incorporating more field-based studies that assess bivalve responses to climate change stressors in their natural environments.

Furthermore, many experiments in this research were of relatively short duration, typically ranging from a few days to a month. While these experiments provided valuable insights into acute responses to stressors, they may not capture climate change's long-term, chronic effects on bivalve populations. Therefore, conducting longer-term experiments and monitoring bivalves over extended periods could reveal additional nuances in their responses.

Regarding future research directions, it is imperative to address these limitations and capitalise on the groundwork laid by this dissertation. Long-term monitoring studies could be precious, given the potential for chronic and cumulative effects of climate change stressors. Tracking bivalve populations, their health, and environmental conditions over multiple seasons and years can provide insights into how stressors impact them.

Moreover, expanding research to encompass a broader range of bivalve species can help elucidate species-specific responses to climate change stressors. Comparative studies can identify resilient species and those at higher risk, aiding conservation efforts and aquaculture practices. Field-based studies are essential for understanding how bivalves respond to climate change stressors in their natural habitats. Research conducted in coastal environments can reveal the complex interactions between bivalves, environmental conditions, and stressors.

Additionally, an emerging research area investigates the genetic basis of bivalve responses to climate change stressors. Understanding the genetic adaptations that enable some bivalve populations to thrive under changing conditions can inform breeding programs and conservation strategies.

Furthermore, given the potential of bivalve valve behaviour as an early detection tool for harmful algal blooms, future research can explore the development of automated monitoring systems. These systems could provide real-time data on toxin levels and improve the timeliness of warnings and management actions.

Finally, the impacts of climate change on bivalve aquaculture extend beyond the biological realm. Future research should incorporate socioeconomic and policy dimensions, including assessing the economic costs and benefits of adaptive strategies and evaluating policy frameworks for climate-resilient aquaculture.

6.6. Conclusion

This comprehensive investigation into the intricate relationship between bivalve aquaculture, climate change stressors, and their ecological implications has yielded valuable insights and highlighted the multifaceted challenges confronting these vital marine organisms and their ecosystems. As we navigate an era of unprecedented environmental change, this dissertation contributes significantly to our understanding of bivalve vulnerabilities and resilience strategies.

The overarching theme that emerges from this study is the undeniable vulnerability of bivalve populations to the escalating impacts of climate change. From Chapter 3's exploration of *O. edulis'* response to rising sea-surface temperatures to Chapter 4's revelation of *M. gigas*' heightened susceptibility to heatwaves and toxic algal blooms, the evidence is clear: bivalves are acutely sensitive to shifts in their environmental conditions. These findings underscore the urgent need for adaptive strategies within the aquaculture industry and ecosystem management to safeguard the future of these ecologically and economically significant species.

The innovative potential of bivalves, particularly *M. gigas*, as sentinel organisms for detecting harmful algal blooms is illuminated in Chapter 5. The altered valve behaviour observed in response to the presence of *P. lima* signifies an invaluable tool for early detection and, consequently, risk mitigation. This discovery transcends

the laboratory setting, promising to revolutionise the field of monitoring harmful algal blooms and providing a safeguard not only for bivalve aquaculture but also for human health and the integrity of coastal ecosystems.

The findings presented here resonate globally, transcending the confines of individual species or regions. The implications of climate change are profound. Bivalves, acting as ecosystem engineers, play pivotal roles in nutrient cycling, water filtration, and habitat provisioning, impacting marine biodiversity and the stability of coastal ecosystems. As such, the vulnerabilities and adaptations of these keystone species reverberate through the intricate web of marine life.

In the broader context of global food security, this research sheds light on the challenges bivalve-dependent industries face and the pressing need for sustainable aquaculture practices. With seafood being a crucial protein source for millions worldwide, understanding and mitigating the risks posed by climate change to bivalve aquaculture becomes a matter of economic and nutritional security. The implications of this study extend to policymakers, conservation managers, and industry leaders who must engage collaboratively to navigate the turbulent waters of a changing climate.

In conclusion, this dissertation represents a significant step forward in our comprehension of bivalve aquaculture and climate change resilience. It advances scientific understanding, offers practical solutions, and emphasises the critical importance of equitable resource allocation to facilitate research in low-income

nations. As we stand at the intersection of climate change challenges and aquaculture opportunities, this research provides a guiding light, offering a path toward sustainable coexistence with our oceans' dynamic and invaluable bivalve populations.

6.7. References

Abele, D., Heise, K., Portner, H. O., & Puntarulo, S. (2002). Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. Journal of Experimental Biology, 205(13), 1831–1841.

Acharya, K. P., & Pathak, S. (2019). Applied Research in Low-Income Countries: Why and How? Frontiers in Research Metrics and Analytics, 4, 3. https://doi.org/10.3389/frma.2019.00003

Anderson, D. M., Cembella, A. D., & Hallegraeff, G. M. (2012). Progress in understanding harmful algal blooms (HABs): Paradigm shifts and new technologies for research, monitoring and management. Annual Review of Marine Science, 4, 143–176. https://doi.org/10.1146/annurev-marine-120308-081121

Anestis, A., Poertner, H. O., Karagiannis, D., Angelidis, P., Staikou, A., & Michaelidis, B. (2010). Response of *Mytilus galloprovincialis* (L.) to increasing seawater temperature and to marteliosis: Metabolic and physiological parameters. Comparative Biochemistry and Physiology A-Molecular &

Integrative Physiology, 156(1), 57–66. https://doi.org/10.1016/j.cbpa.2009.12.018

Arcus, V. L., Prentice, E. J., Hobbs, J. K., Mulholland, A. J., Van der Kamp, M. W., Pudney, C. R., Parker, E. J., & Schipper, L. A. (2016). On the Temperature Dependence of Enzyme-Catalyzed Rates. Biochemistry, 55(12), 1681–1688. https://doi.org/10.1021/acs.biochem.5b01094

Artigaud, S., Lacroix, C., Pichereau, V., & Flye-Sainte-Marie, J. (2014). Respiratory response to combined heat and hypoxia in the marine bivalves *Pecten maximus* and *Mytilus* spp. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology, 175, 135–140. https://doi.org/10.1016/j.cbpa.2014.06.005

Balbi, T., Auguste, M., Ciacci, C., & Canesi, L. (2021). Immunological Responses of Marine Bivalves to Contaminant Exposure: Contribution of the -Omics Approach. Frontiers in Immunology, 12, 618726. https://doi.org/10.3389/fimmu.2021.618726

Beiras, R., Camacho, A. P., & Albentosa, M. (1995). Short-term and long-term alterations in the energy budget of young oyster *Ostrea edulis* L. in response to temperature change. Journal of Experimental Marine Biology and Ecology, 186(2), 221–236. https://doi.org/10.1016/0022-0981(94)00159-B

Brown, J. R., & Hartwick, E. B. (1988). Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas*: II. Condition index and survival. Aquaculture, 70(3), 253–267. https://doi.org/10.1016/0044-8486(88)90100-7

Bylenga, C. H., Cummings, V. J., & Ryan, K. G. (2015). Fertilisation and larval development in an Antarctic bivalve, *Laternula elliptica*, under reduced pH and

elevated temperatures. Marine Ecology Progress Series, 536, 187–201. https://doi.org/10.3354/meps11436

Carss, D. N., Brito, A. C., Chainho, P., Ciutat, A., de Montaudouin, X., Fernández Otero, R. M., Filgueira, M. I., Garbutt, A., Goedknegt, M. A., Lynch, S. A., Mahony, K. E., Maire, O., Malham, S. K., Orvain, F., van der Schatte Olivier, A., & Jones, L. (2020). Ecosystem services provided by a non-cultured shellfish species: The common cockle *Cerastoderma edule*. Marine Environmental Research, 158, 104931. https://doi.org/10.1016/j.marenvres.2020.104931

Clements, J. C., & Comeau, L. A. (2019). Nitrogen removal potential of shellfish aquaculture harvests in eastern Canada: A comparison of culture methods. Aquaculture Reports, 13, 100183. https://doi.org/10.1016/j.aqrep.2019.100183

Coen, L. D., Brumbaugh, R. D., Bushek, D., Grizzle, R., Luckenbach, M. W., Posey, M. H., Powers, S. P., & Tolley, S. G. (2007). Ecosystem services related to oyster restoration. Marine Ecology Progress Series, 341, 303–307. https://doi.org/10.3354/meps341303

Comeau, L. A., Babarro, J. M. F., Riobó, P., Scarratt, M., Starr, M., & Tremblay, R. (2019). PSP-producing dinoflagellate *Alexandrium minutum* induces valve microclosures in the mussel *Mytilus galloprovincialis.* Aquaculture, 500, 407–413. https://doi.org/10.1016/j.aquaculture.2018.10.025

Coquereau, L., Jolivet, A., Hégaret, H., & Chauvaud, L. (2016). Short-Term Behavioural Responses of the Great Scallop *Pecten maximus* Exposed to the Toxic Alga *Alexandrium minutum* Measured by Accelerometry and Passive Acoustics. PLOS ONE, 11(8), e0160935. https://doi.org/10.1371/journal.pone.0160935

Durier, G., Nadalini, J.-B., Comeau, L. A., Starr, M., Michaud, S., Tran, D., St-Louis, R., Babarro, J. M. F., Clements, J. C., & Tremblay, R. (2022). Use of valvometry as an alert tool to signal the presence of toxic algae *Alexandrium catenella* by Mytilus edulis. Frontiers in Marine Science, 9. https://www.frontiersin.org/articles/10.3389/fmars.2022.987872

Eymann, C., Götze, S., Bock, C., Guderley, H., Knoll, A. H., Lannig, G., Sokolova, I. M., Aberhan, M., & Pörtner, H.-O. (2020). Thermal performance of the European flat oyster, *Ostrea edulis* (Linnaeus, 1758)—Explaining ecological findings under climate change. Marine Biology, 167(2), 17. https://doi.org/10.1007/s00227-019-3620-3

Gentry, R. R., Alleway, H. K., Bishop, M. J., Gillies, C. L., Waters, T., & Jones, R. (2020). Exploring the potential for marine aquaculture to contribute to ecosystem services. Reviews in Aquaculture, 12(2), 499–512. https://doi.org/10.1111/raq.12328

Giomi, F., & Poertner, H. (2013). A role for haemolymph oxygen capacity in heat tolerance of eurythermal crabs. Frontiers in Physiology, 4, 110. https://doi.org/10.3389/fphys.2013.00110

Grabowski, J. H., & Peterson, C. H. (2007). 15—Restoring Oyster Reefs to Recover Ecosystem Services. In K. Cuddington, J. E. Byers, W. G. Wilson, & A. Hastings (Eds.), Theoretical Ecology Series (Vol. 4, pp. 281–298). Academic Press. https://doi.org/10.1016/S1875-306X(07)80017-7

Grazer, V. M., & Martin, O. Y. (2012). Investigating Climate Change and Reproduction: Experimental Tools from Evolutionary Biology. Biology, 1(2), 411–438. https://doi.org/10.3390/biology1020411

Griffith, A. W., & Gobler, C. J. (2017). Transgenerational exposure of North Atlantic bivalves to ocean acidification renders offspring more vulnerable to low pH and additional stressors. Scientific Reports, 7(1), Article 1. https://doi.org/10.1038/s41598-017-11442-3

Haberkorn, H., Tran, D., Massabuau, J.-C., Ciret, P., Savar, V., & Soudant, P. (2011). Relationship between valve activity, microalgae concentration in the water and toxin accumulation in the digestive gland of the Pacific oyster *Crassostrea gigas* exposed to *Alexandrium minutum*. Marine Pollution Bulletin, 62(6), 1191–1197. https://doi.org/10.1016/j.marpolbul.2011.03.034

Hiebenthal, C., Philipp, E. E. R., Eisenhauer, A., & Wahl, M. (2012). Interactive effects of temperature and salinity on shell formation and general condition in Baltic Sea *Mytilus edulis* and *Arctica islandica*. Aquatic Biology, 14(3), 289–298. https://doi.org/10.3354/ab00405

Lannig, G., Flores, J. F., & Sokolova, I. M. (2006). Temperature-dependent stress response in oysters, *Crassostrea virginica*: Pollution reduces temperature tolerance in oysters. Aquatic Toxicology, 79(3), 278–287. https://doi.org/10.1016/j.aquatox.2006.06.017

Lavaud, R., Durier, G., Nadalini, J.-B., Filgueira, R., Comeau, L. A., Babarro, J. M. F., Michaud, S., Scarratt, M., & Tremblay, R. (2021). Effects of the toxic dinoflagellate *Alexandrium catenella* on the behaviour and physiology of the blue mussel *Mytilus edulis*. Harmful Algae, 108, 102097. https://doi.org/10.1016/j.hal.2021.102097

Lucas, A., & Beninger, P. G. (1985). The use of physiological condition indices in marine bivalve aquaculture. Aquaculture, 44(3), 187–200. https://doi.org/10.1016/0044-8486(85)90243-1

Matozzo, V., & Marin, M. (2011). Bivalve immune responses and climate changes: Is there a relationship? ISJ, 8, 70–77.

May, S. P., Burkholder, J. M., Shumway, S. E., Hégaret, H., Wikfors, G. H., & Frank, D. (2010). Effects of the toxic dinoflagellate *Alexandrium monilatum* on survival, grazing and behavioural response of three ecologically important bivalve molluscs. Harmful Algae, 9(3), 281–293. https://doi.org/10.1016/j.hal.2009.11.005

Mello, D. F., Proença, L. A. de O., & Barracco, M. A. (2010). Comparative Study of Various Immune Parameters in Three Bivalve Species during a Natural Bloom of *Dinophysis acuminata* in Santa Catarina Island, Brazil. Toxins, 2(5), 1166–1178. https://doi.org/10.3390/toxins2051166

Miller, N., & Stillman, J. (2012). Physiological Optima and Critical Limits | Learn Science at Scitable. https://www.nature.com/scitable/knowledge/library/physiological-optima-and-critical-limits-45749376/

Neo, M. L., Eckman, W., Vicentuan, K., Teo, S. L.-M., & Todd, P. A. (2015). The ecological significance of giant clams in coral reef ecosystems. Biological Conservation, 181, 111–123. https://doi.org/10.1016/j.biocon.2014.11.004

Rubalcaba, J. G., Verberk, W. C. E. P., Hendriks, A. J., Saris, B., & Woods, H. A. (2020). Oxygen limitation may affect the temperature and size dependence of

metabolism in aquatic ectotherms. Proceedings of the National Academy of Sciences, 117(50), 31963–31968. https://doi.org/10.1073/pnas.2003292117

Talmage, S. C., & Gobler, C. J. (2011). Effects of Elevated Temperature and Carbon Dioxide on the Growth and Survival of Larvae and Juveniles of Three Species of Northwest Atlantic Bivalves. PLoS ONE, 6(10). https://doi.org/10.1371/journal.pone.0026941

Tran, D., Haberkorn, H., Soudant, P., Ciret, P., & Massabuau, J.-C. (2010). Behavioral responses of *Crassostrea gigas* exposed to the harmful algae *Alexandrium minutum*. Aquaculture, 298(3), 338–345. https://doi.org/10.1016/j.aquaculture.2009.10.030

Turner, L. M., Havenhand, J. N., Alsterberg, C., Turner, A. D., Girisha, S. K., Rai, A., Venugopal, M. N., Karunasagar, I., & Godhe, A. (2019). Toxic Algae Silence Physiological Responses to Multiple Climate Drivers in a Tropical Marine Food Chain. Frontiers in Physiology, 10, 373. https://doi.org/10.3389/fphys.2019.00373

Van Colen, C., Ong, E. Z., Briffa, M., Wethey, D. S., Abatih, E., Moens, T., & Woodin, S. A. (2020). Clam feeding plasticity reduces herbivore vulnerability to ocean warming and acidification. Nature Climate Change, 10(2), Article 2. https://doi.org/10.1038/s41558-019-0679-2

Appendix A

Bibligoraphic details of the 69 studies gathered that investigated the effects of multiple climate change stressors effects on bivalves, listed in the Web of Science as of 4 June 2020.

1. Artigaud, S., Lacroix, C., Pichereau, V., & Flye-Sainte-Marie, J. (2014). Respiratory response to combined heat and hypoxia in the marine bivalves *Pecten maximus* and *Mytilus* spp. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology, 175, 135–140. https://doi.org/10.1016/j.cbpa.2014.06.005
2. Basso, L., Hendriks, I., Steckbauer, A., & Duarte, C. (2015). Resistance of juveniles of the Mediterranean pen shell, (*Pinna nobilis*) to hypoxia and interaction with warming. Estuarine Coastal and Shelf Science, 165, 199–203. https://doi.org/10.1016/j.ecss.2015.05.016
3. Braga, A. C., Camacho, C., Marques, A., Gago-Martínez, A., Pacheco, M., & Costa, P. R. (2018). Combined effects of warming and acidification on accumulation and elimination dynamics of paralytic shellfish toxins in mussels *Mytilus galloprovincialis*. Environmental Research, 164, 647–654. https://doi.org/10.1016/j.envres.2018.03.045
4. Britto, R. S., Nascimento, J. P., Serode, T., Santos, A. P., Soares, A. M. V. M., Figueira, E., Furtado, C., Lima-Ventura, J., Monserrat, J. M., & Freitas, R. (2019). The effects of co-exposure of graphene oxide and copper under different pH conditions in Manila clam *Ruditapes philippinarum*. Environmental Science and Pollution Research. https://doi.org/10.1007/s11356-019-06643-4
5. Bylenga, C. H., Cummings, V. J., & Ryan, K. G. (2015). Fertilisation and larval development in an Antarctic bivalve, *Laternula elliptica*, under reduced pH and elevated temperatures. Marine Ecology Progress Series, 536, 187–201. https://doi.org/10.3354/meps11436
6. Cameron, L. P., Reymond, C. E., Muller-Lundin, F., Westfield, I., Grabowski, J. H., Westphal, H., & Ries, J. B. (2019). Effects of temperature and ocean acidification on the extrapallial fluid ph, calcification rate, and condition factor of the king scallop *Pecten maximus*. Journal of Shellfish Research, 38(3), 763–777. https://doi.org/10.2983/035.038.0327
7. Castillo, N., Saavedra, L. M., Vargas, C. A., Gallardo-Escárate, C., & Détrée, C. (2017). Ocean acidification and pathogen exposure modulate the immune response of the edible mussel *Mytilus chilensis*. Fish & Shellfish Immunology, 70, 149–155. https://doi.org/10.1016/j.fsi.2017.08.047
8. Cheng, M. C. F., Sara, G., & Williams, G. A. (2018). Combined effects of thermal conditions and food availability on thermal tolerance of the marine bivalve, *Perna viridis*. Journal of Thermal Biology, 78, 270–276. https://doi.org/10.1016/j.jtherbio.2018.10.014

9. Cherkasov, A. S., Ringwood, A. H., & Sokolova, I. M. (2006). Combined effects of temperature acclimation and cadmium exposure on mitochondrial function in eastern oysters *Crassostrea virginica* gmelin (Bivalvia: Ostreidae). Environmental Toxicology and Chemistry, 25(9), 2461–2469. https://doi.org/10.1897/05-584R.1
10. Cherkasov, A. S., Taylor, C., & Sokolova, I. M. (2010). Seasonal variation in mitochondrial responses to cadmium and temperature in eastern oysters *Crassostrea virginica* (Gmelin) from different latitudes. Aquatic Toxicology, 97(1), 68–78. https://doi.org/10.1016/j.aquatox.2009.12.004
11. Coppola, F., Almeida, A., Henriques, B., Soares, A. M. V. M., Figueira, E., Pereira, E., & Freitas, R. (2017). Biochemical impacts of Hg in *Mytilus galloprovincialis* under present and predicted warming scenarios. Science of the Total Environment, 601, 1129–1138. https://doi.org/10.1016/j.scitotenv.2017.05.201
12. Coppola, F., Almeida, A., Henriques, B., Soares, A. M. V. M., Figueira, E., Pereira, E., & Freitas, R. (2018). Biochemical responses and accumulation patterns of *Mytilus galloprovincialis* exposed to thermal stress and Arsenic contamination. Ecotoxicology and Environmental Safety, 147, 954–962. https://doi.org/10.1016/j.ecoenv.2017.09.051
13. Costa, S., Coppola, F., Pretti, C., Intorre, L., Meucci, V., Soares, A. M. V. M., Freitas, R., & Sole, M. (2020). The influence of climate change related factors on the response of two clam species to diclofenac. Ecotoxicology and Environmental Safety, 189, 109899. https://doi.org/10.1016/j.ecoenv.2019.109899
14. Freitas, R., Almeida, Â., Calisto, V., Velez, C., Moreira, A., Schneider, R. J., Esteves, V. I., Wrona, F. J., Figueira, E., & Soares, A. M. V. M. (2016). The impacts of pharmaceutical drugs under ocean acidification: New data on single and combined long-term effects of carbamazepine *on Scrobicularia plana*. Science of The Total Environment, 541, 977–985. https://doi.org/10.1016/j.scitotenv.2015.09.138
15. Freitas, R., Coppola, F., Costa, S., Pretti, C., Intorre, L., Meucci, V., Soares, A. M. V. M., & Solé, M. (2019). The influence of temperature on the effects induced by Triclosan and Diclofenac in mussels. Science of The Total Environment, 663, 992–999. https://doi.org/10.1016/j.scitotenv.2019.01.189
16. Freitas, R., Salamanca, L., Velez, C., Wrona, F. J., Soares, A. M. V. M., & Figueira, E. (2016). Multiple stressors in estuarine waters: Effects of arsenic and salinity on *Ruditapes philippinarum*. Science of The Total Environment, 541, 1106–1114. https://doi.org/10.1016/j.scitotenv.2015.09.149
17. Fuhrmann, M., Delisle, L., Petton, B., Corporeau, C., & Pernet, F. (2018). Metabolism of the Pacific oyster, *Crassostrea gigas*, is influenced by salinity and modulates survival to the Ostreid herpesvirus OsHV-1. Biology Open, 7(2). https://doi.org/10.1242/bio.028134
18. Gobler, C. J., DePasquale, E. L., Griffith, A. W., & Baumann, H. (2014). Hypoxia and Acidification Have Additive and Synergistic Negative Effects on the Growth, Survival, and Metamorphosis of Early Life Stage Bivalves. PLoS ONE, 9(1). https://doi.org/10.1371/journal.pone.0083648

19. Gobler, C. J., Clark, H. R., Griffith, A. W., & Lusty, M. W. (2017). Diurnal Fluctuations in Acidification and Hypoxia Reduce Growth and Survival of Larval and Juvenile Bay Scallops (*Argopecten irradians*) and Hard Clams (*Mercenaria mercenaria*). Frontiers in Marine Science, 3. https://doi.org/10.3389/fmars.2016.00282Goetze, S., Bock, C., Eymann, C.,
20. Lannig, G., Steffen, J. B. M., & Poertner, H.-O. (2020). Single and combined effects of the “Deadly trio” hypoxia, hypercapnia and warming on the cellular metabolism of the great scallop *Pecten maximus*. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology, 243, 110438. https://doi.org/10.1016/j.cbpb.2020.110438
21. Goetze, S., Matoo, O. B., Beniash, E., Saborowski, R., & Sokolova, I. M. (2014). Interactive effects of CO2 and trace metals on the proteasome activity and cellular stress response of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. Aquatic Toxicology, 149, 65–82. https://doi.org/10.1016/j.aquatox.2014.01.027
22. Greco, L., Pellerin, J., Capri, E., Garnerot, F., Louis, S., Fournier, M., Sacchi, A., Fusi, M., Lapointe, D., & Couture, P. (2011). Physiological Effects of Temperature and a Herbicide Mixture on the Soft-Shell Clam *Mya Arenaria* (mollusca, Bivalvia). Environmental Toxicology and Chemistry, 30(1), 132–141. https://doi.org/10.1002/etc.359
23. Green, D. S., Christie, H., Pratt, N., Boots, B., Godbold, J. A., Solan, M., & Hauton, C. (2017). Competitive interactions moderate the effects of elevated temperature and atmospheric CO2 on the health and functioning of oysters. Marine Ecology Progress Series, 582, 93–103. https://doi.org/10.3354/meps12344
24. Hiebenthal, C., Philipp, E. E. R., Eisenhauer, A., & Wahl, M. (2012). Interactive effects of temperature and salinity on shell formation and general condition in Baltic Sea *Mytilus edulis* and *Arctica islandica*. Aquatic Biology, 14(3), 289–298. https://doi.org/10.3354/ab00405
25. Holan, J. R., King, C. K., Proctor, A. H., & Davis, A. R. (2019). Increased sensitivity of subantarctic marine invertebrates to copper under a changing climate—Effects of salinity and temperature. Environmental Pollution, 249, 54–62. https://doi.org/10.1016/j.envpol.2019.02.016
26. Husmann, G., Abele, D., Rosenstiel, P., Clark, M. S., Kraemer, L., & Philipp, E. E. R. (2014). Age-dependent expression of stress and antimicrobial genes in the hemocytes and siphon tissue of the Antarctic bivalve, *Laternula elliptica,* exposed to injury and starvation. Cell Stress and Chaperones, 19(1), 15–32. https://doi.org/10.1007/s12192-013-0431-1
27. Husmann, G., Philipp, E. E. R., Rosenstiel, P., Vazquez, S., & Abele, D. (2011). Immune response of the Antarctic bivalve *Laternula elliptica* to physical stress and microbial exposure. Journal of Experimental Marine Biology and Ecology, 398(1), 83–90. https://doi.org/10.1016/j.jembe.2010.12.013
28. Ivanina, A. V., Dickinson, G. H., Matoo, O. B., Bagwe, R., Dickinson, A., Beniash, E., & Sokolova, I. M. (2013). Interactive effects of elevated temperature and CO_2 levels on energy metabolism and biomineralization of

marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology, 166(1), 101–111. https://doi.org/10.1016/j.cbpa.2013.05.016

29. Ivanina, A. V., Hawkins, C., & Sokolova, I. M. (2016). Interactive effects of copper exposure and environmental hypercapnia on immune functions of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. Fish & Shellfish Immunology, 49, 54–65. https://doi.org/10.1016/j.fsi.2015.12.011
30. Jansson, A., Norkko, J., Dupont, S., & Norkko, A. (2015). Growth and survival in a changing environment: Combined effects of moderate hypoxia and low pH on juvenile bivalve *Macoma balthica*. Journal of Sea Research, 102, 41–47. https://doi.org/10.1016/j.seares.2015.04.006
31. Khan, B., Clinton, S. M., Hamp, T. J., Oliver, J. D., & Ringwood, A. H. (2018). Potential impacts of hypoxia and a warming ocean on oyster microbiomes. Marine Environmental Research, 139, 27–34. https://doi.org/10.1016/j.marenvres.2018.04.018
32. Lannig, G., Flores, J. F., & Sokolova, I. M. (2006). Temperature-dependent stress response in oysters, *Crassostrea virginica*: Pollution reduces temperature tolerance in oysters. Aquatic Toxicology, 79(3), 278–287. https://doi.org/10.1016/j.aquatox.2006.06.017
33. Lardies, M. A., Benitez, S., Osores, S., Vargas, C. A., Duarte, C., Lohrmann, K. B., & Lagos, N. A. (2017). Physiological and histopathological impacts of increased carbon dioxide and temperature on the scallops *Argopecten purpuratus* cultured under upwelling influences in northern Chile. Aquaculture, 479, 455–466. https://doi.org/10.1016/j.aquaculture.2017.06.008
34. Lemasson, A. J., Hall-Spencer, J. M., Kuri, V., & Knights, A. M. (2019). Changes in the biochemical and nutrient composition of seafood due to ocean acidification and warming. Marine Environmental Research, 143, 82–92. https://doi.org/10.1016/j.marenvres.2018.11.006
35. Lesser, M. P., Thompson, M. M., & Walker, C. W. (2019). Effects of Thermal Stress and Ocean Acidification on the Expression of the Retrotransposon Steamer in the Softshell *Mya Arenaria*. Journal of Shellfish Research, 38(3), 535–541. https://doi.org/10.2983/035.038.0304
36. Li, S., Liu, Y., Liu, C., Huang, J., Zheng, G., Xie, L., & Zhang, R. (2015). Morphology and classification of hemocytes in *Pinctada fucata* and their responses to ocean acidification and warming. Fish & Shellfish Immunology, 45(1), 194–202. https://doi.org/10.1016/j.fsi.2015.04.006
37. Lu, Y., Wang, L., Wang, L., Cong, Y., Yang, G., & Zhao, L. (2018). Deciphering carbon sources of mussel shell carbonate under experimental ocean acidification and warming. Marine Environmental Research, 142, 141–146. https://doi.org/10.1016/j.marenvres.2018.10.007
38. Mackenzie, C. L., Lynch, S. A., Culloty, S. C., & Malham, S. K. (2014). Future Oceanic Warming and Acidification Alter Immune Response and Disease Status in a Commercial Shellfish Species, *Mytilus edulis* L. Plos One, 9(6), e99712. https://doi.org/10.1371/journal.pone.0099712
39. Mackenzie, C. L., Ormondroyd, G. A., Curling, S. F., Ball, R. J., Whiteley, N. M., & Malham, S. K. (2014). Ocean Warming, More than Acidification,

Reduces Shell Strength in a Commercial Shellfish Species during Food Limitation. PLOS ONE, 9(1), e86764. https://doi.org/10.1371/journal.pone.0086764

40. Magalhaes, L., de Montaudouin, X., Figueira, E., & Freitas, R. (2018). Trematode infection modulates cockles biochemical response to climate change. Science of the Total Environment, 637, 30–40. https://doi.org/10.1016/j.scitotenv.2018.04.432
41. Matoo, O. B., Ivanina, A. V., Ullstad, C., Beniash, E., & Sokolova, I. M. (2013). Interactive effects of elevated temperature and CO2 levels on metabolism and oxidative stress in two common marine bivalves (*Crassostrea virginica* and *Mercenaria mercenaria*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 164(4), 545–553. https://doi.org/10.1016/j.cbpa.2012.12.025
42. Matozzo, V., Chinellato, A., Munari, M., Bressan, M., & Marin, M. G. (2013). Can the combination of decreased pH and increased temperature values induce oxidative stress in the clam *Chamelea gallina* and the mussel *Mytilus galloprovincialis*? Marine Pollution Bulletin, 72(1), 34–40. https://doi.org/10.1016/j.marpolbul.2013.05.004
43. Moreira, A., Figueira, E., Libralato, G., Soares, A. M. V. M., Guida, M., & Freitas, R. (2018). Comparative sensitivity of *Crassostrea angulata* and *Crassostrea gigas* embryo-larval development to As under varying salinity and temperature. Marine Environmental Research, 140, 135–144. https://doi.org/10.1016/j.marenvres.2018.06.003
44. Moreira, A., Freitas, R., Figueira, E., Volpi Ghirardini, A., Soares, A. M. V. M., Radaelli, M., Guida, M., & Libralato, G. (2018). Combined effects of arsenic, salinity and temperature on *Crassostrea gigas* embryotoxicity. Ecotoxicology and Environmental Safety, 147, 251–259. https://doi.org/10.1016/j.ecoenv.2017.08.043
45. Munari, M., Matozzo, V., Benetello, G., Riedl, V., Pastore, P., Badocco, D., & Marin, M. G. (2020). Exposure to Decreased pH and Caffeine Affects Hemocyte Parameters in the Mussel *Mytilus galloprovincialis*. Journal of Marine Science and Engineering, 8(4), Article 4. https://doi.org/10.3390/jmse8040238
46. Munari, M., Matozzo, V., Chemello, G., Riedl, V., Pastore, P., Badocco, D., & Marin, M. G. (2019). Seawater acidification and emerging contaminants: A dangerous marriage for haemocytes of marine bivalves. Environmental Research, 175, 11–21. https://doi.org/10.1016/j.envres.2019.04.032
47. Munari, M., Matozzo, V., Gagne, F., Chemello, G., Riedl, V., Finos, L., Pastore, P., Badocco, D., & Marin, M. G. (2018). Does exposure to reduced pH and diclofenac induce oxidative stress in marine bivalves? A comparative study with the mussel *Mytilus galloprovincialis* and the clam *Ruditapes philippinarum*. Environmental Pollution, 240, 925–937. https://doi.org/10.1016/j.envpol.2018.05.005
48. Nardi, A., Benedetti, M., d'Errico, G., Fattorini, D., & Regoli, F. (2018). Effects of ocean warming and acidification on accumulation and cellular responsiveness to cadmium in mussels *Mytilus galloprovincialis*: Importance

of the seasonal status. Aquatic Toxicology, 204, 171–179. https://doi.org/10.1016/j.aquatox.2018.09.009

49. Nardi, A., Benedetti, M., Fattorini, D., & Regoli, F. (2018). Oxidative and interactive challenge of cadmium and ocean acidification on the smooth scallop *Flexopecten glaber*. Aquatic Toxicology, 196, 53–60. https://doi.org/10.1016/j.aquatox.2018.01.008
50. Ong, E. Z., Briffa, M., Moens, T., & Van Colen, C. (2017). Physiological responses to ocean acidification and warming synergistically reduce condition of the common cockle *Cerastoderma edule.* Marine Environmental Research, 130, 38–47. https://doi.org/10.1016/j.marenvres.2017.07.001
51. Pirone, G., Coppola, F., Pretti, C., Soares, A. M. V. M., Solé, M., & Freitas, R. (2019). The effect of temperature on Triclosan and Lead exposed mussels. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 232, 42–50. https://doi.org/10.1016/j.cbpb.2019.02.007
52. Prado, P., Roque, A., Pérez, J., Ibáñez, C., Alcaraz, C., Casals, F., & Caiola, N. (2016). Warming and acidification-mediated resilience to bacterial infection determine mortality of early *Ostrea edulis* life stages. Marine Ecology Progress Series, 545, 189–202. https://doi.org/10.3354/meps11618
53. Range, P., Chícharo, M. A., Ben-Hamadou, R., Piló, D., Fernandez-Reiriz, M. J., Labarta, U., Marin, M. G., Bressan, M., Matozzo, V., Chinellato, A., Munari, M., El Menif, N. T., Dellali, M., & Chícharo, L. (2014). Impacts of CO2-induced seawater acidification on coastal Mediterranean bivalves and interactions with other climatic stressors. Regional Environmental Change, 14(1), 19–30. https://doi.org/10.1007/s10113-013-0478-7
54. Rodríguez-Romero, A., Jiménez-Tenorio, N., Basallote, M. D., De Orte, M. R., Blasco, J., & Riba, I. (2014). Predicting the impacts of CO_2 leakage from subseabed storage: Effects of metal accumulation and toxicity on the model benthic organism *Ruditapes philippinarum*. Environmental Science & Technology, 48(20), 12292–12301. https://doi.org/10.1021/es501939c
55. Sara, G., Romano, C., Widdows, J., & Staff, F. J. (2008). Effect of salinity and temperature on feeding physiology and scope for growth of an invasive species (*Brachidontes pharaonis* - Mollusca: Bivalvia) within the Mediterranean sea. Journal of Experimental Marine Biology and Ecology, 363(1–2), 130–136. https://doi.org/10.1016/j.jembe.2008.06.030
56. Serra-Compte, A., Maulvault, A. L., Camacho, C., Alvarez-Munoz, D., Barcelo, D., Rodriguez-Mozaz, S., & Marques, A. (2018). Effects of water warming and acidification on bioconcentration, metabolization and depuration of pharmaceuticals and endocrine disrupting compounds in marine mussels (*Mytilus galloprovincialis*). Environmental Pollution, 236, 824–834. https://doi.org/10.1016/j.envpol.2018.02.018
57. Sokolova, I. M. (2004). Cadmium effects on mitochondrial function are enhanced by elevated temperatures in a marine poikilotherm*, Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). Journal of Experimental Biology, 207(15), 2639–2648. https://doi.org/10.1242/jeb.01054

58. Speights, C. J., Silliman, B. R., & McCoy, M. W. (2017). The effects of elevated temperature and dissolved ρCO_2 on a marine foundation species. Ecology and Evolution, 7(11), 3808–3814. https://doi.org/10.1002/ece3.2969
59. Stevens, A. M., & Gobler, C. J. (2018). Interactive effects of acidification, hypoxia, and thermal stress on growth, respiration, and survival of four North Atlantic bivalves. Marine Ecology Progress Series, 604, 143–161. https://doi.org/10.3354/meps12725
60. Sui, Y., Hu, M., Shang, Y., Wu, F., Huang, X., Dupont, S., Storch, D., Poertner, H.-O., Li, J., Lu, W., & Wang, Y. (2017). Antioxidant response of the hard shelled mussel *Mytilus coruscus* exposed to reduced pH and oxygen concentration. Ecotoxicology and Environmental Safety, 137, 94–102. https://doi.org/10.1016/j.ecoenv.2016.11.023
61. Sui, Y., Kong, H., Shang, Y., Huang, X., Wu, F., Hu, M., Lin, D., Lu, W., & Wang, Y. (2016). Effects of short-term hypoxia and seawater acidification on hemocyte responses of the mussel *Mytilus coruscus*. Marine Pollution Bulletin, 108(1), 46–52. https://doi.org/10.1016/j.marpolbul.2016.05.001
62. Talmage, S. C., & Gobler, C. J. (2011). Effects of Elevated Temperature and Carbon Dioxide on the Growth and Survival of Larvae and Juveniles of Three Species of Northwest Atlantic Bivalves. Plos One, 6(10), e26941. https://doi.org/10.1371/journal.pone.0026941
63. Taylor, A. M., Maher, W. A., & Ubrihien, R. P. (2017). Mortality, condition index and cellular responses of *Anadara trapezia* to combined salinity and temperature stress. Journal of Experimental Marine Biology and Ecology, 497, 172–179. https://doi.org/10.1016/j.jembe.2017.09.023
64. Thorp, J. H., Alexander, J. E., Bukaveckas, B. L., Cobbs, G. A., & Bresko, K. L. (1998). Responses of Ohio River and Lake Erie dreissenid molluscs to changes in temperature and turbidity. Canadian Journal of Fisheries and Aquatic Sciences, 55(1), 220–229. https://doi.org/10.1139/cjfas-55-1-220
65. Turner, L. M., Alsterberg, C., Turner, A. D., Girisha, S. K., Rai, A., Havenhand, J. N., Venugopal, M. N., Karunasagar, I., & Godhe, A. (2016). Pathogenic marine microbes influence the effects of climate change on a commercially important tropical bivalve. Scientific Reports, 6(1), Article 1. https://doi.org/10.1038/srep32413
66. Turner, L. M., Havenhand, J. N., Alsterberg, C., Turner, A. D., Girisha, S. K., Rai, A., Venugopal, M. N., Karunasagar, I., & Godhe, A. (2019). Toxic Algae Silence Physiological Responses to Multiple Climate Drivers in a Tropical Marine Food Chain. Frontiers in Physiology, 10, 373. https://doi.org/10.3389/fphys.2019.00373
67. Velez, C., Figueira, E., Soares, A. M. V. M., & Freitas, R. (2016). Combined effects of seawater acidification and salinity changes in *Ruditapes philippinarum*. Aquatic Toxicology, 176, 141–150. https://doi.org/10.1016/j.aquatox.2016.04.016
68. Watson, S.-A., Southgate, P. C., Miller, G. M., Moorhead, J. A., & Knauer, J. (2012). Ocean acidification and warming reduce juvenile survival of the fluted giant clam, *Tridacna squamosa*. Molluscan Research, 32(3), 177–180.

69. Zhao, L., Schöne, B. R., Mertz-Kraus, R., & Yang, F. (2017). Insights from sodium into the impacts of elevated pCO2 and temperature on bivalve shell formation. Journal of Experimental Marine Biology and Ecology, 486, 148–154. https://doi.org/10.1016/j.jembe.2016.10.009

Appendix B

Complete list of responses and number of studies per response recorded among the multi-stressor studies collected.

	Responses	Number of studies
1	survival	26
2	antioxidant and biotransformation enzyme (CAT, SOD, GSTs, GPx)	18
3	energy related markers (ET, PROT, GLY, ATP, ADO or AMP)	17
4	indicators of cellular damage (LPO, GSH, GSSG, DNA strand break formation, PC)	14
5	heavy metal accumulation	11
6	immunological responses (THC, phagocytosis activity, phagocytosis efficiency, ROS)	10
7	shell calcification rate	10
8	respiration rate	9
9	condition index	8
10	clearance rate	6
11	shell morphometrics and composition (dry weight, length, width, height, thickness, calcite: aragonite, Na/CA)	5
12	tissue growth	4
13	pharmaceuticals and personal care products accumulation	4
14	bacterial growth/quantification	4
15	development/metamorphosis	4
16	histopathological analysis	3
17	lysosomal membrane stability	3
18	prevalence of parasites	3
19	standard metabolic rate	3
20	burrowing activity	3
21	proximate composition	3
22	abnormalities	2
23	Arrhenius breakpoint temperature	2
24	mitochondrial respiration	2
25	Na and K concentration	2
26	neurotoxicity biomarker	2
27	scope for growth	2

28	total antioxidant capacity (TAOC)	2
29	toxic algae toxin quantification	2
30	extrapallial fluid and haemolymph and pH	2
31	shell mechanical properties (hardness, fracture resistance, microhardness, crack radius), carbonic hydrase (CA) activity),	2
32	lipofuscin accumulation (stress indicator)	1
33	accumulation/elimination dynamics of HAB-toxins in shellfish	1
34	bioconcentration, metabolization, and depuration of contaminants	1
35	calcium content in haemocyte and haemolymph	1
36	cellular metabolism (metabolites, pathway analysis of gills)	1
37	dry tissue weight	1
38	dynamic energy budget model stimulation of energy reserves	1
39	embryotoxicity	1
40	fertilization	1
41	haemocyte and siphon gene expression	1
42	haemocyte morphology and classification	1
43	hepatosomatic index	1
44	immune related gene expression	1
45	macro and micro minerals	1
46	mitochondrial density	1
47	mitochondrial function	1
48	mitochondrial membrane potential	1
49	moisture content	1
50	mRNA expression of genes for metal binding proteins, stress, and ubiquitin-proteasome pathway	1
51	oxidative metabolism	1
52	oxidative stress biomarkers	1
53	pathogen level	1
54	pathological conditions	1
55	reproductive status (ACTcase activity, Gonadosomatic index)	1
56	retrotransposons (steamer) expression	1
57	RNA sequence	1
58	RNA/DNA ratio	1
59	sources of DIC/calcifying fluid chemistry	1
60	thermal tolerance range	1
61	adsorption efficiency	1

Appendix C

Pairwise comparison of oxygen consumption rates of *O. edulis* in mL O_2 h^{-1} g $AFDW^{-1}$ between temperatures in Experiment 1.

Contrast (Temperature(C))		estimate	SE	df	t ratio	p value
9	12	0.09627	0.0585	156	1.646	0.7778
9	15	0.06551	0.0585	156	1.12	0.9704
9	18	-0.00379	0.0585	156	-0.065	1
9	21	0.05442	0.0585	156	0.93	0.9909
9	24	-0.04565	0.0585	156	-0.78	0.9973
9	27	-0.13584	0.0593	156	-2.292	0.3537
9	30	-0.07776	0.0593	156	-1.312	0.9265
9	**33**	**-0.28738**	**0.0601**	**157**	**-4.779**	**0.0001**
12	15	-0.03077	0.0585	156	-0.526	0.9998
12	18	-0.10006	0.0585	156	-1.711	0.7392
12	21	-0.04186	0.0585	156	-0.716	0.9985
12	24	-0.14192	0.0585	156	-2.426	0.2773
12	**27**	**-0.23211**	**0.0593**	**156**	**-3.916**	**0.0042**
12	30	-0.17403	0.0593	156	-2.936	0.0882
12	**33**	**-0.38365**	**0.0601**	**157**	**-6.38**	**<.0001**
15	18	-0.06929	0.0585	156	-1.185	0.9587
15	21	-0.01109	0.0585	156	-0.19	1
15	24	-0.11116	0.0585	156	-1.9	0.6145
15	**27**	**-0.20134**	**0.0593**	**156**	**-3.397**	**0.0238**
15	30	-0.14327	0.0593	156	-2.417	0.2824
15	**33**	**-0.35288**	**0.0601**	**157**	**-5.868**	**<.0001**
18	21	0.0582	0.0585	156	0.995	0.9859
18	24	-0.04187	0.0585	156	-0.716	0.9985
18	27	-0.13205	0.0593	156	-2.228	0.3932
18	30	-0.07397	0.0593	156	-1.248	0.9443
18	**33**	**-0.28359**	**0.0601**	**157**	**-4.716**	**0.0002**
21	24	-0.10007	0.0585	156	-1.711	0.7391
21	**27**	**-0.19025**	**0.0593**	**156**	**-3.209**	**0.0417**
21	30	-0.13218	0.0593	156	-2.23	0.392
21	**33**	**-0.34179**	**0.0601**	**157**	**-5.684**	**<.0001**
24	27	-0.09018	0.0593	156	-1.521	0.8439
24	30	-0.03211	0.0593	156	-0.542	0.9998
24	**33**	**-0.24172**	**0.0601**	**157**	**-4.02**	**0.0028**
27	30	0.05808	0.0601	157	0.967	0.9883
27	33	-0.15154	0.0609	157	-2.488	0.246
30	**33**	**-0.20962**	**0.0608**	**156**	**-3.449**	**0.0202**

Appendix D

Pairwise comparison of heart rates of *O. edulis* in bpm between temperatures in Experiment 2.

Contrast (Temperature)		estimate	SE	df	t ratio	p value
18	20	-0.6462	1.41	32.3	-0.459	0.9998
18	22	-3.5884	1.81	33.7	-1.988	0.5043
18	**24**	**-4.552**	**1.29**	**31.2**	**-3.539**	**0.0248**
18	**26**	**-5.3675**	**1.41**	**32.2**	**-3.814**	**0.0121**
18	28	-4.6112	1.62	33.2	-2.847	0.1174
18	30	-2.0691	1.5	32.8	-1.382	0.8587
18	32	-2.0378	1.41	32.4	-1.447	0.8286
20	22	-2.9422	1.87	32.9	-1.577	0.7598
20	24	-3.9058	1.41	32.3	-2.774	0.1374
20	26	-4.7213	1.51	32.6	-3.127	0.0641
20	28	-3.965	1.69	32.4	-2.348	0.2999
20	30	-1.4228	1.6	33.1	-0.891	0.9849
20	32	-1.3916	1.51	32.5	-0.922	0.9816
22	24	-0.9636	1.81	33.7	-0.534	0.9994
22	26	-1.7791	1.9	34.5	-0.934	0.9804
22	28	-1.0228	2.03	33.7	-0.503	0.9996
22	30	1.5193	1.96	34.2	0.776	0.9933
22	32	1.5506	1.9	34.5	0.814	0.9911
24	26	-0.8155	1.41	32.2	-0.579	0.9989
24	28	-0.0592	1.62	33.2	-0.037	1
24	30	2.4829	1.5	32.8	1.659	0.7123
24	32	2.5142	1.41	32.4	1.785	0.6339
26	28	0.7563	1.69	32.6	0.447	0.9998
26	30	3.2984	1.6	33.2	2.065	0.4568
26	32	3.3297	1.51	32.6	2.206	0.3747
28	30	2.5422	1.74	32	1.458	0.8229
28	32	2.5735	1.69	32.4	1.524	0.789
30	32	0.0313	1.57	31.8	0.02	1

Appendix E

Pairwise comparison of oxygen consumption rates of *O. edulis* in mL O_2 h^{-1} g $AFDW^{-1}$ between temperatures in Experiment 2.

Contrast (Temperature)		estimate	SE	df	t ratio	p value
18	20	0.03817	0.0503	49	0.758	0.9945
18	22	0.01004	0.0503	49	0.199	1
18	24	0.00462	0.0503	49	0.092	1
18	26	-0.00732	0.0503	49	-0.145	1
18	28	-0.03782	0.0503	49	-0.751	0.9948
18	30	-0.1117	0.0503	49	-2.219	0.3586
18	**32**	**-0.20207**	**0.0503**	**49**	**-4.015**	**0.0047**
20	22	-0.02813	0.0503	49	-0.559	0.9992
20	24	-0.03355	0.0503	49	-0.667	0.9975
20	26	-0.04549	0.0503	49	-0.904	0.9843
20	28	-0.07599	0.0503	49	-1.51	0.7986
20	30	-0.14987	0.0503	49	-2.978	0.079
20	**32**	**-0.24024**	**0.0503**	**49**	**-4.773**	**0.0004**
22	24	-0.00542	0.0503	49	-0.108	1
22	26	-0.01736	0.0503	49	-0.345	1
22	28	-0.04786	0.0503	49	-0.951	0.9791
22	30	-0.12174	0.0503	49	-2.419	0.2555
22	**32**	**-0.21211**	**0.0503**	**49**	**-4.214**	**0.0025**
24	26	-0.01194	0.0503	49	-0.237	1
24	28	-0.04244	0.0503	49	-0.843	0.9895
24	30	-0.11632	0.0503	49	-2.311	0.3086
24	**32**	**-0.20669**	**0.0503**	**49**	**-4.107**	**0.0035**
26	28	-0.0305	0.0503	49	-0.606	0.9986
26	30	-0.10439	0.0503	49	-2.074	0.4454
26	**32**	**-0.19476**	**0.0503**	**49**	**-3.869**	**0.0072**
28	30	-0.07389	0.0503	49	-1.468	0.8202
28	**32**	**-0.16426**	**0.0503**	**49**	**-3.263**	**0.0389**
30	32	-0.09037	0.0503	49	-1.795	0.626

Appendix F

Areas in England and Wales where either Azaspiracids, Domoic acid, Okadaic acid group and Saxitons were detected above the maximum permitted levels in shellfish from 2014 to 2019 (Data from Cefas report: Coates et al., 2014, 2015; Harrison et al., 2016, 2017; Parks et al., 2018, 2019).

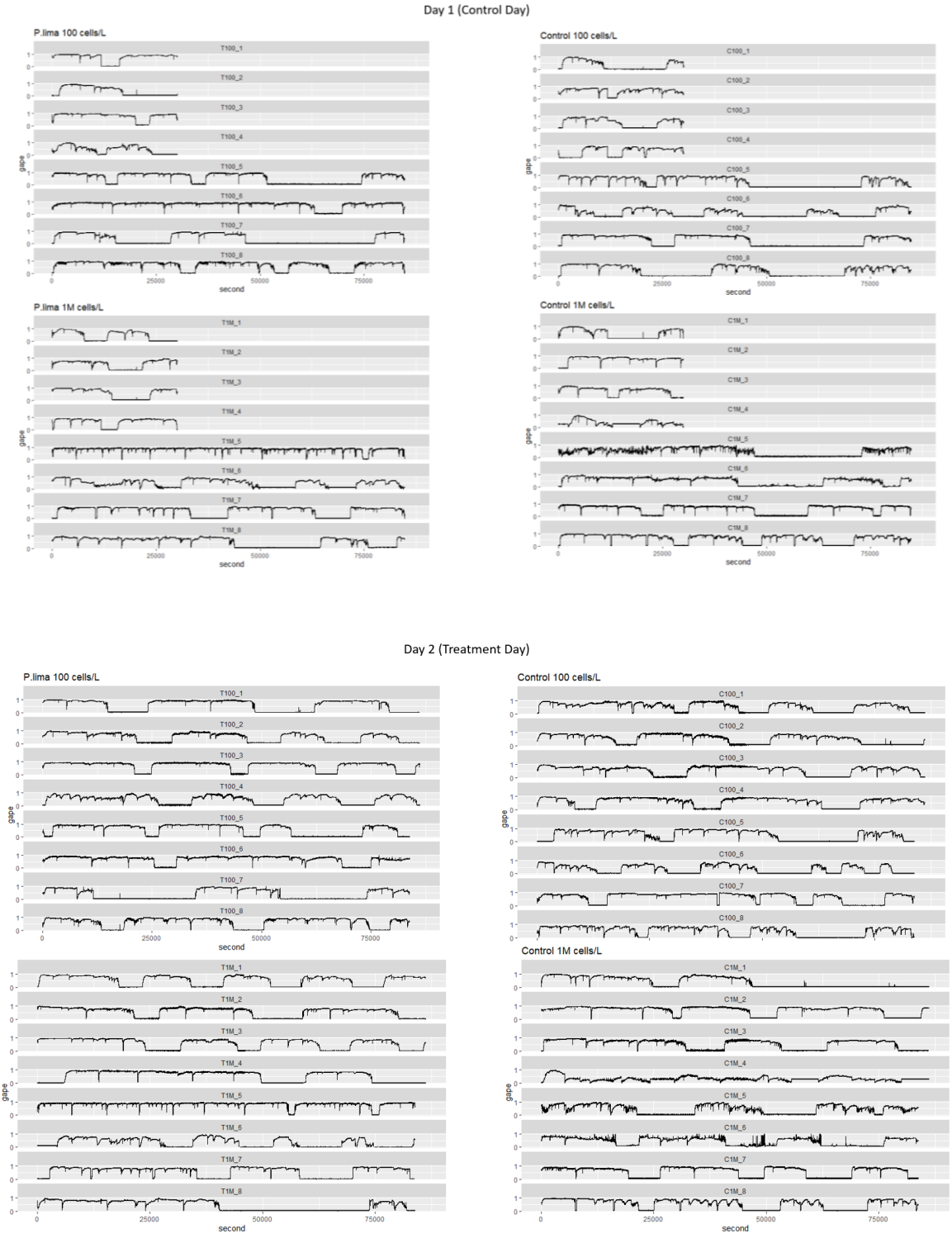
Local Authority	Production Area & Site	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2014													
Weymouth PHA	Portland: North Eastern Breakwater												
Cornwall PHA	St. Austell Bay: Ropehaven Outer												
	Fowey: Pont Pill												
Torridge DC	Taw/Torridge: Spratt Ridge East												
2015													
Cornwall PHA	St. Austell Bay: Ropehaven Outer												
	Fowey: Pont Pill												
	Lantivet Bay: Sandheap Point												
Torridge DC	Taw/Torridge: Spratt Ridge East												
West Somerset Council	Porlock: Porlock Beach												
Torbay BC	Lyme Bay: Site 1												
2016													
Cornwall PHA	St. Austell Bay: Ropehaven Outer												
	Fowey: Pont Pill												
	Fowey: Wisemeans												
	Lantivet Bay: Sandheap Point												
Torridge DC	Taw/Torridge: Spratt Ridge East												
Torbay BC	Brixham: Fishcombe SW corner												
	Lyme Bay: Site 1												
South Hams DC	Salcombe: Geese Quarries												
Swansea PHA	Swansea: Queen's Dock												
2017													
Torbay BC	Lyme Bay: Site 1												
Cornwall PHA	Fowey: Pont Pill												
South Hams DC	Salcombe: Geese Quarries												
2018													
Torbay BC	Lyme Bay: Site 1												
Cornwall PHA	Mylor Creek												
	Pont Pill												
	Porthallow North												
	Ropehaven Outer												
	Sandheap Point												
2019													
Cornwall PHA	Sandheap Point												
	Porthallow North												
	Ropehaven Outer												
	Pont Pill												

Legend:

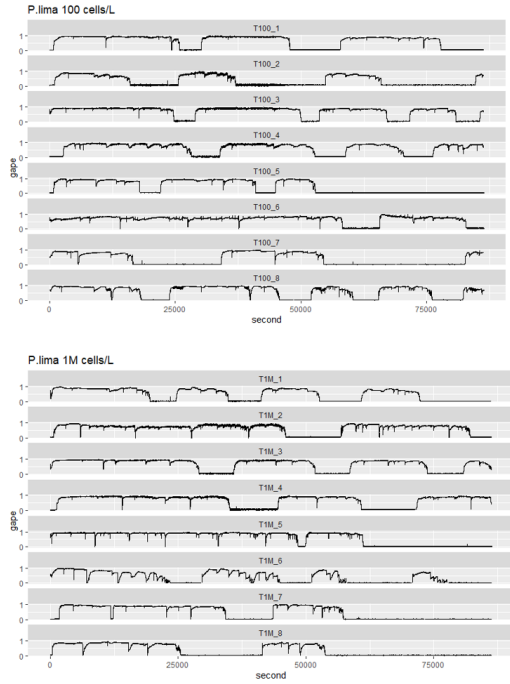
- Domoic acid
- Okadaic acid group of toxins
- Azaspiracids
- Saxitoxins

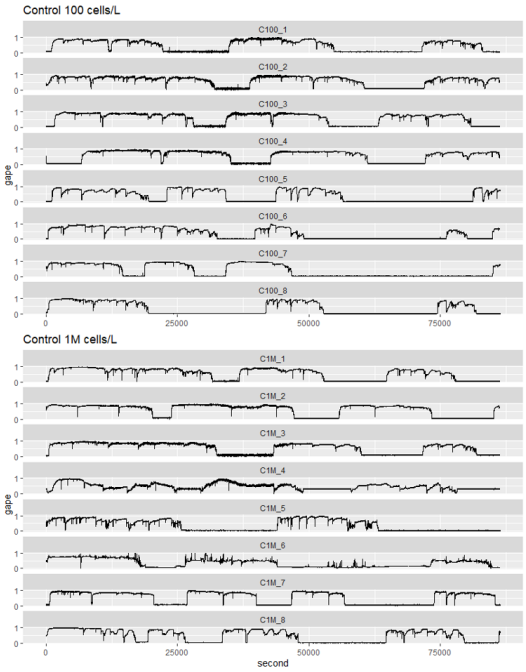
Appendix G

Daily gaping amplitude plots of oysters exposed to *Prorocentrum lima* and *Surirella* sp. at 10^2 and 10^5 cells L^{-1}.

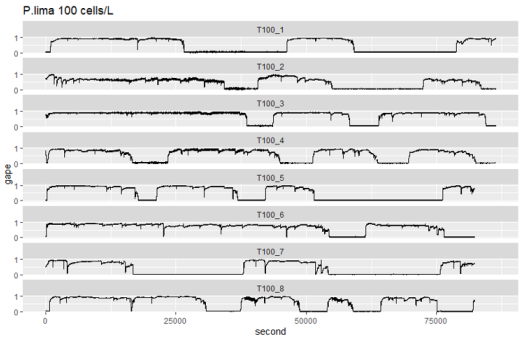


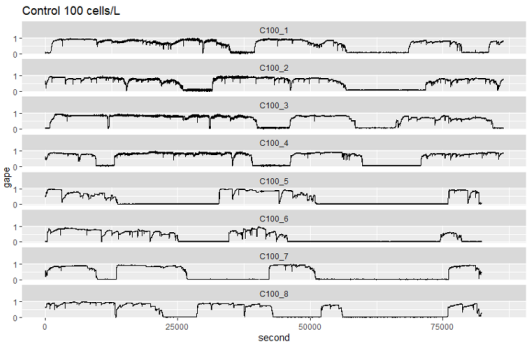
Day 3 (Control Day)

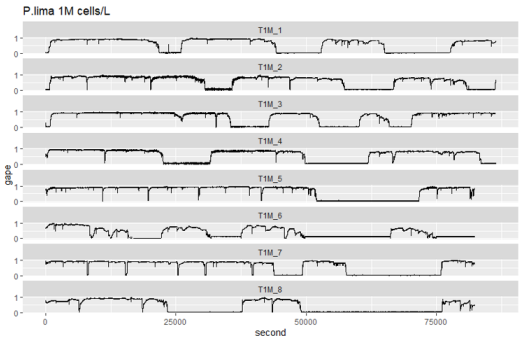


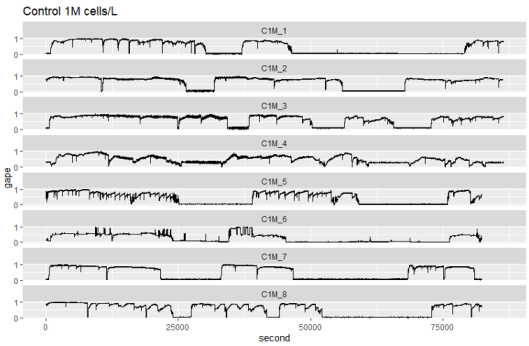


Day 4 (Treatment Day)

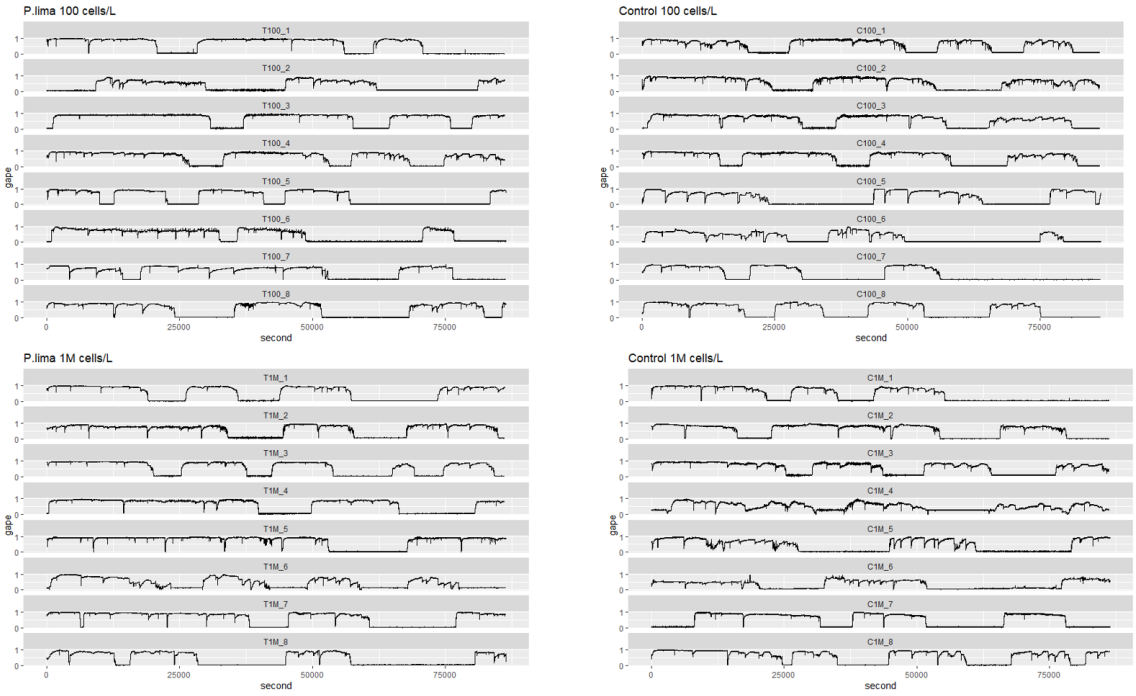




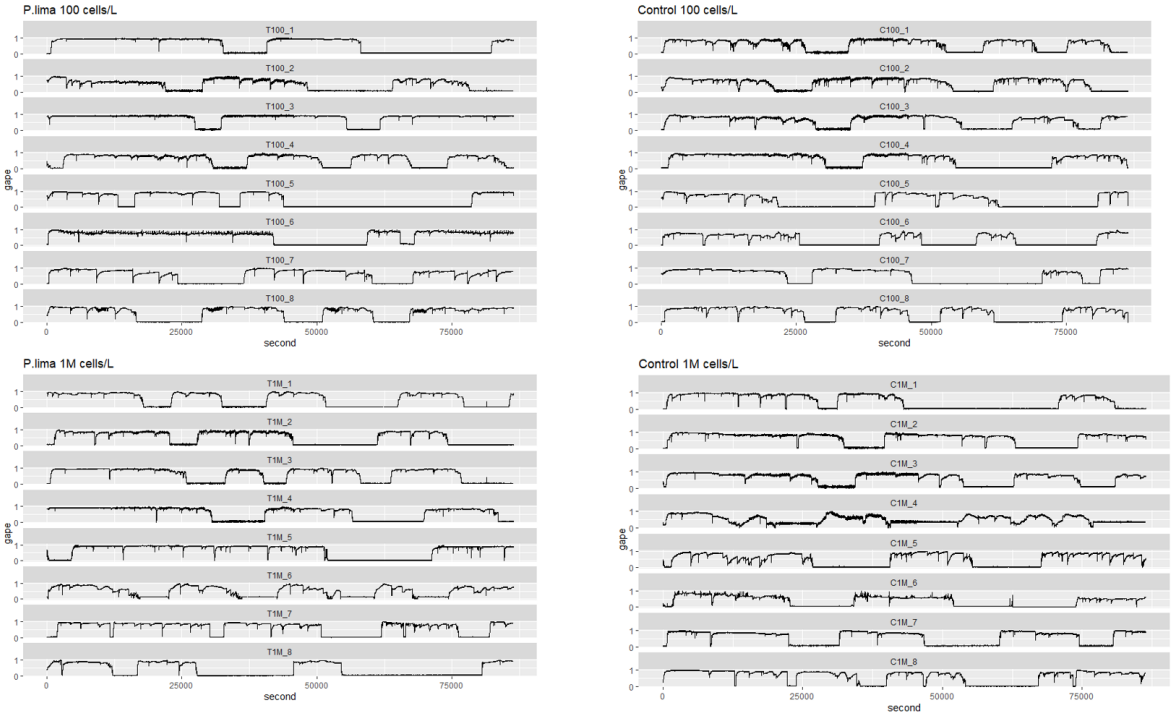




Day 5 (Control Day)



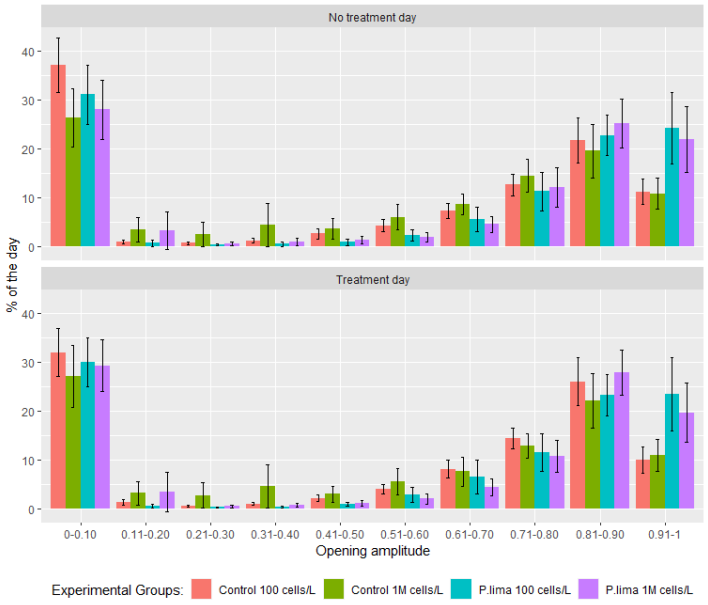
Day 6 (Treatment Day)



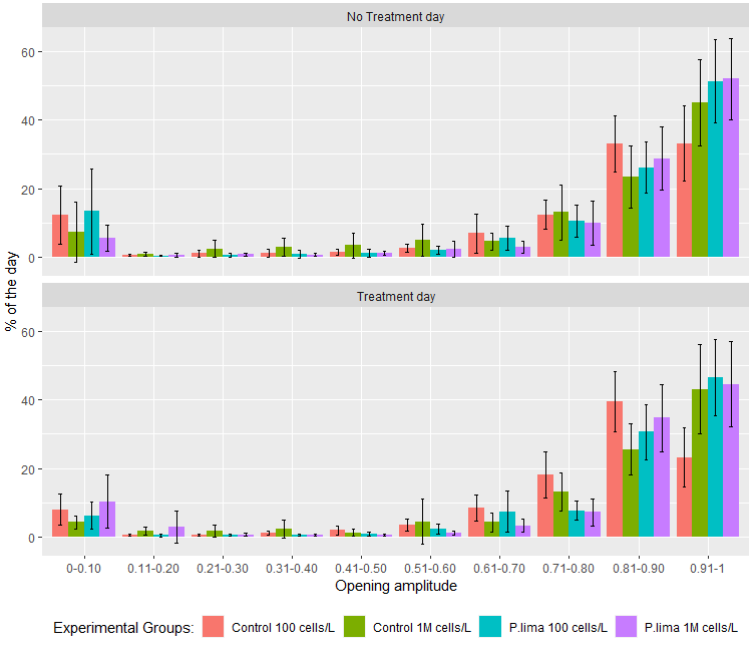
Appendix H

Daily mean ± se opening amplitude distribution of oysters exposed to *Prorocentrum lima* and *Surirella* sp. at 10^2 and 10^5 cells L^{-1} (n=8).

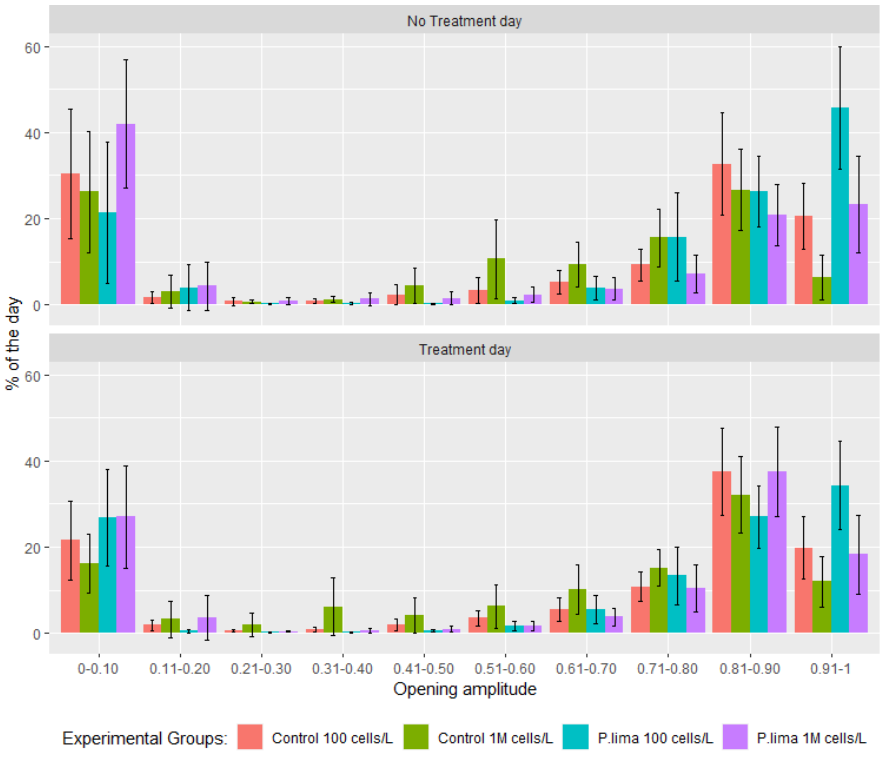
1. Whole day recordings



2. First three hours of exposure days



3. Middle-three hours of exposure days



4. Last-three hours of exposure days