Investigating spat mortality in Scottish farmed blue mussels (*Mytilus edulis*)

This thesis is submitted for the degree of Doctor of Philosophy

By

Chelsea Caprice Broughton

MSc. Aquatic Pathobiology, BSc. (Hons) Marine Science

Institute of Aquaculture

Faculty of Natural Sciences, University of Stirling

February 2024

Declaration

This thesis has been composed in its entirety by the candidate. Except where specifically acknowledged, the work described in this thesis has been conducted independently and has not been submitted for any other degree.

Acknowledgements

I am sincerely grateful to Dr Stefano Carboni, the visionary behind this project, for entrusting me with the opportunity to be a part of this remarkable journey. My heartfelt appreciation goes out to my dedicated supervisors, Dr Darren Green and Prof. Trevor Telfer, whose unwavering support and guidance have been instrumental throughout the final years of my project. I am indebted to the entire team at the Institute of Aquaculture for their consistent encouragement and assistance, both during my thesis and beyond. A special acknowledgment is reserved for my dear friend Teresa Grobler, whose presence and camaraderie have made every step of this journey enjoyable. I am also profoundly grateful to my family for their unwavering support, providing strength and encouragement during both the highs and lows of this endeavour. Lastly, I extend my deepest gratitude to my partner Kris, for their unwavering patience and understanding, serving as a constant source of motivation and encouragement.

Abstract

Mussel spat in Loch Eil, Scotland, experiences alarmingly high mortality rates. This study investigates the underlying causes through field surveys, environmental monitoring, and controlled experiments. Mortality in Loch Eil reached 68.3%, significantly higher than the near-zero mortality observed in Loch Sunart. Environmental analyses revealed distinct differences between the lochs, including variations in temperature, salinity, and heavy metal concentrations. Potential pathogens, including *Photobacterium* spp. and *Vibrio* spp., were identified. Controlled experiments demonstrated significant impacts of water quality, salinity fluctuations, and UV exposure on spat survival. Cohabitation experiments highlighted the potential role of genetic variability in determining spat resilience. This research provides crucial insights into the complex factors driving high mortality in Loch Eil mussel spat.

Key words: Mussel spat, Mortality dynamics, *Mytilus edulis*, Environmental influences, Loch Eil, Scotland, Field surveys, Controlled experiments, Temperature, Salinity, Heavy metals, Food availability, Pathogens, Environmental stressors, Genetic variety, Aquaculture, Shellfish

Table of Contents

List of Figures

List of Tables

List of Appendices

Chapter 1

General Introduction

Global shellfish industry

The demand for shellfish has steadily increased due to their affordability, nutritional value, and sustainability, which has led to a notable trend driven by growing consumer preferences for healthy and environmentally friendly seafood options (Azra et al., 2021; Yang et al., 2016). As a result, shellfish aquaculture has expanded in various regions worldwide, supporting the livelihoods of coastal communities and contributing to the global seafood supply (Yang et al., 2016). As depicted in Figure 1.1**,** global shellfish aquaculture production surged dramatically from 2.76 million metric tons in 1985 to 27 million tonnes (mega tonnes) in 2018, representing a tenfold increase over this period (Azra et al., 2021).

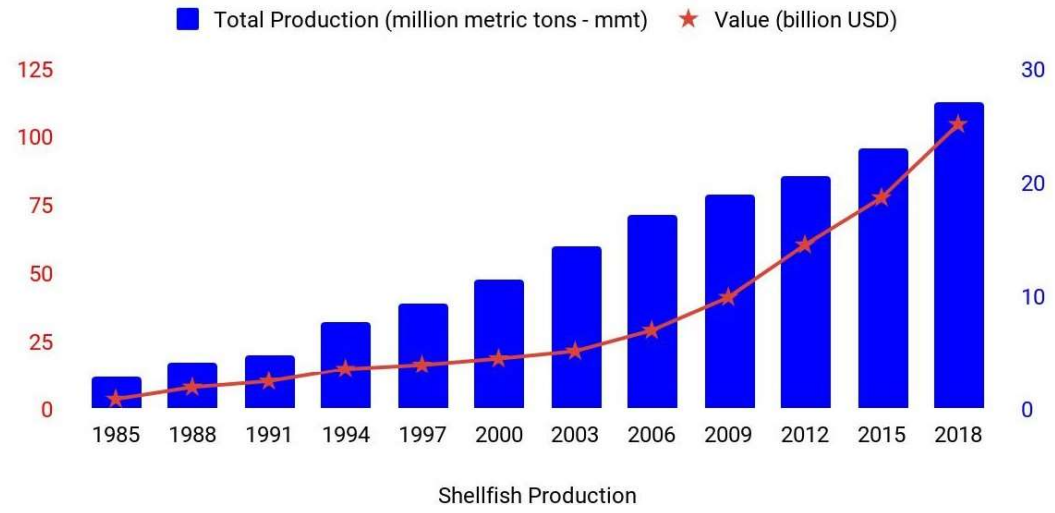

Figure1.1: **Global shellfish aquaculture production and value from 1985 – 2018** Global shellfish aquaculture production (mega tonnes) from 1985 to 2018 is presented as a bar graph, while its economic value (billion USD) is depicted as a line graph (adapted from Azra et al., 2021)

The global shellfish industry plays a vital role in global food security and economic prosperity (Azra et al., 2021) Aquaculture within this sector encompasses a diverse range of species, with marine shrimp and molluscs being key components. Bivalves are crucial in several countries (Figure 1.2) , including New

Zealand (86.9%), France (75.4%), Spain (74.8%), the Republic of Korea (69.7%), Italy (61.6%), and Japan (51.8%), exceeding the global average bivalve contribution of 18.4%, whereby China stands as the foremost producer of shellfish, with a reported output exceeding 870 mega tonnes per annum (Mtpa), underscoring the global reach and importance of the industry (FAO, 2022).

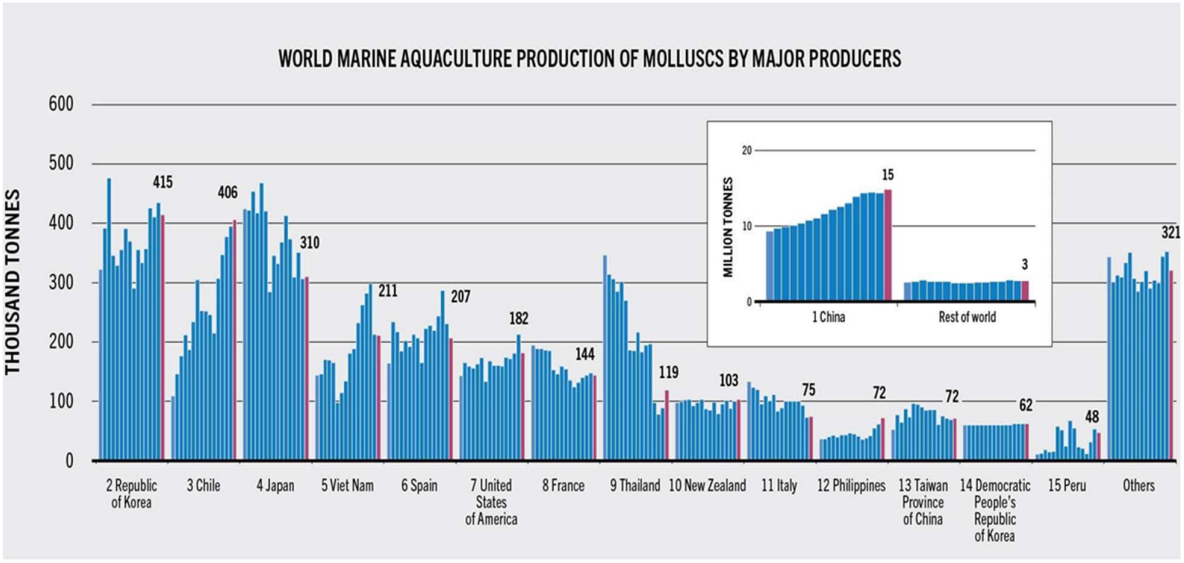

Figure 1.2: **Global production chart of farmed marine mollusc producers and their total yield) from 2005 – 2020**. The production is clearly dominated by China, producing 15 mega tonnes (Mt) compared to the combined production of 3 Mt by the rest of the farmed marine mollusc producers around world (highlighted in the embedded graph). The data in the graphs are expressed in live weight equivalent. (Figure from FAO, 2022).

Molluscs constitute a significant portion of global aquaculture, representing the second largest category by both quantity and value, accounting for 21% of all global aquaculture production by weight in 2016 (Botta et al., 2020; FAO, 2018). Scallops, clams, oysters, and mussels are among the most important bivalve molluscs for international trade (European Commission, 2024) with mussels emerging as key players in global aquaculture due to their ecological resilience and economic viability (Wijsman et al., 2019)

Moreover, advancements in shellfish farming techniques, coupled with sustainable practices, have propelled production levels, further cementing the industry's position as a cornerstone of global aquaculture (Wijsman et al., 2019).

Shellfish industry in Europe and Scotland

Within Europe, the cultivation of shellfish has been a longstanding tradition, contributing substantially to the region's food supply and economy (Wijsman et al., 2019; FAO, 2022).

Molluscs and crustaceans comprise approximately 49% of farmed seafood in the EU (Breuer & Twisk, 2024). Mussels, oysters, and clams are the dominant molluscan species, accounting for over 99% of bivalve production (European Commission, 2024). Production fluctuated, with a decline in 2020 (531.697 tonnes) followed by a recovery in 2022 (522.019 tonnes) and a 5-year peak in value (EUR 1.30 billion) (European Commission, 2024) The EU is a leading global producer of scallops, particularly Great Atlantic scallops, accounting for 93% of global production in 2021 (European Commission, 2024).

Furthermore, Europe is a significant contributor to global mussel production, accounting for over a third of the total output (FAO, 2022) with mussels playing a prominent role in European aquaculture and cuisine, representing approximately one-third of all aquaculture products sold within the EU (FAO, 2022; FAO, 2019, Monfort, 2014). This high consumption level reflects a notable degree of self-sufficiency within the EU with Spain, Italy, France, and the Netherlands being key producers (European Commission, 2024).

Scotland has emerged as a significant contributor to the European shellfish market, with mussel aquaculture being a major driver (Murphy & Munro, 2024) While mussels dominate production, the sector also cultivates Pacific oysters, native oysters, and various scallop species as seen in Figure 1.3 (Murphy & Munro, 2023). Mussel production has seen substantial growth, reaching nearly 8 Mtpa in 2017 from just below 6 Mtpa in 2008 (Dias et al., 2011; Munro & Wallace, 2018). In 2022, production reached a record high of 9 Mtpa, with Shetland contributing a significant portion (79%) (Findlay, 2018; Murphy & Munro, 2024).

For the table	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	% change 21-22
Pacific oyster (000s)	1,891	3,392	2,693	3,534	5,034	4,031	4,393	2,863	4,853	4,087	-16
Native oyster (000s)	260	242	200	201	200	142	103	35	8	109	>100
Queen (000s)	33	18	33	155	273	18	18	0.5	0.5	0.6	20
Scallop (000s)	40	48	30	35	47	31	26	19	27	39	44
Mussel (tonnes)	6,757	7,683	7,270	7,732	8,232	6,874	6,699	5,661	8,590	9,092	6

For on-growing	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	% change 21-22
Pacific oyster (000s)	6,216	6,792	5,864	4,584	3,849	4,240	2,405	1,663	3,528	3,400	-4
Native oyster (000s)	1,015	749	13	323	481	344	327	10	41	8	-81
Queen (000s)	1,490	500	900	17	300	0	0	0	0	0	0
Scallop (000s)	1,470	136	49	23	9	4	0	0	0	0	0
Mussel (tonnes)	1,281	1,263	1,841	2,619	4,437	2,137	3,493	4,127	3,253	2,506	-23

Figure 1.3: **Shellfish production data for Scotland from 2013 to 2022.**The figure is divided into two tables: The top illustrates the shellfish production for the table i.e. market sale, and the production for further on-growing is shown at the bottom, whereby the last column in each table shows the comparison of production mass from 2021 to 2022 in percent change. The graph was extracted from Scottish Shellfish Farm Production Survey Report 2022 (Murphy & Munro, 2023).

Despite this growth, the number of active mussel farms has decreased since 2015, attributed to factors like remote locations, high operating costs, and challenges with spat collection (Munro, 2019; Munro & Wallace, 2018; Murphy & Munro, 2023). The 2022 Scottish Shellfish Farm Production Survey reported 300 active sites, with production varying due to economic fluctuations, logistical challenges, the impact of the pandemic, and environmental factors such as harmful algal blooms (HABs) (Adamson et al., 2018; De Rijcke et al., 2015; Murphy & Munro, 2024; Murray et al., 2022). While mussels dominate, Scotland also cultivates Pacific oysters (*Magallana gigas* Thunberg, 1793) native oysters (*Ostrea edulis* Linnaeus, 1758), king scallops (*Pecten maximus* Linnaeus,1758) and queen scallops (*Aequipecten opercularis* Linnaeus,1758) (Mcleod & Mcleod, 2019). Mussel cultivation primarily utilises long-line systems due to the flexibility of the system structure in response to wave movement (Stevens et al., 2008). Offshore culture is gaining traction in the UK, offering potential for increased production and typically involves deploying seeded ropes, although recent

advancements have focused on collecting spat directly offshore, potentially reducing reliance on onshore hatcheries (Buck, 2007; Langan & Horton, 2003).

Hatchery production of mussels in the UK faces several challenges, hindering its widespread adoption. Mussel hatchery production involves conditioning adult mussels to induce spawning, rearing the larvae in controlled environments, and transferring the juvenile mussels to nursery systems for growth before moving them to outdoor grow-out systems (Goulletquer, 2009).

While some hatcheries exist, such as the oyster hatchery in Morecambe Bay operated by Loch Fyne Oysters, their output is currently limited (Adamson et al., 2018). Factors contributing to this include the high costs of establishing and operating hatcheries, the technical complexities of mussel larval rearing, and the potential for disease outbreaks (Murray et al., 2022). Additionally, the development of robust and cost-effective hatchery techniques specifically tailored to Scottish mussel species remains an area of ongoing research and development.

Mussel cultivation and production cycle

Cultivating mussels is notably advantageous due to their rapid growth to marketable size in less than 2 to 3 years (Gosling, 2015a) and their ability to anchor onto various surfaces using byssal threads. This makes mussel farming more practical compared to other mollusc species. The cultivation process (Figure 1.4) typically involves the following stages (Gosling, 2003a, 2015a):

- Spawning: Farmers closely monitor water temperature and other environmental cues to predict spawning events to strategically deploy collectors during peak spawning periods to maximise the capture of settling larvae.
- Seed collection: While less common than in the past, mussel seed can be obtained by either dredging natural mussel beds, or the more common and sustainable method nowadays, via spat collection ropes. To overcome the challenges of relying solely on natural seed sources, European countries such as France, Spain, the Netherlands (Robert et al., 2013) and the UK (Adamson et al., 2018) have invested in mollusc hatcheries, providing a more sustainable and stable supply for on growing bivalve aquaculture.

- Relocation and on-growing: Following collection, mussel seed is typically relocated to suitable on-growing sites equipped with structures like Buouchots, longlines or rafts. In some methods, seed is transferred into netlon tubes or similar structures attached to these systems. These sites are carefully selected to provide optimal growth conditions.
- Grading involves separating mussels by size using specialised machinery, while declumping involves manually or mechanically separating individual mussels from clusters that form during growth. Regular grading, declumping, and thinning of mussels on ropes ensure adequate space and resources for individual mussels to grow optimally, ultimately leading to higher yields.
- Harvesting techniques vary depending on the culture system. Raft and longline systems typically involve hauling ropes or lines onto vessels and manually detaching mussels. Mechanical harvesters may be used. Post-harvest, mussels undergo depuration in clean seawater tanks to remove toxins before being transported for processing or market distribution (Aypa, 1990; FAO, 2022).

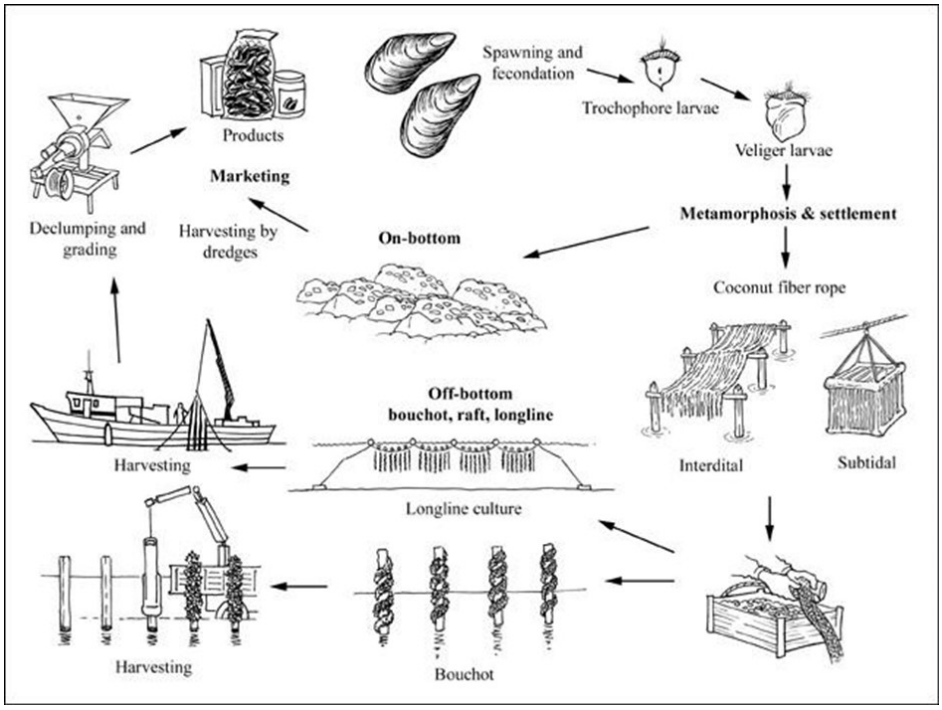

Figure 1.4: **Schematic representation of mussel aquaculture**, illustrating the main stages from spawning and larval development to harvesting and marketing. Different culture methods, including on-bottom, Bouchot, raft, and longline, are depicted. (Figure from FAO, Goulletquer, 2009)

Common farming methods based on tidal and subtidal techniques are described by FAO, 2019 and are depicted in Figure 1.5: on-bottom culture, Bouchot culture, raft culture, and longline culture.

On-bottom culture, a method widely used in the Netherlands, involves relocating naturally settled spat to more suitable areas such as fixed tidal or subtidal culture plots on the seabed, to optimise growth and achieve marketable size (Aypa, 1990).

Raft culture (Figure 1.5a) is a long-standing practice in Spanish mussel farming, where mussel seeds naturally settle on collector ropes suspended from various structures, ranging from traditional wooden boats with frameworks to modern catamarans or simpler raft designs with floats and anchors. Raft culture is highly productive, as the mussels are periodically thinned and transferred to longer ropes as they grow (Aypa, 1990; Figueiras et al., 2002; Pérez-Camacho et al., 2013).

Bouchot culture (Figure 1.5b), primarily practiced in France, utilises vertical wooden poles, driven into the intertidal mudflats. Mussel seeds are collected on

coconut fibre ropes attached to these poles. The spat are then transferred to netlon tubes and spiralled around the poles for growth to market size (Aypa, 1990; Goulletquer & Heral, 1997).

Longline culture (Figures 1.5c and 1.5d), also known as rope culture, is a newer technique effective method for mussel farming in areas with significant wave action, whereby spat collector ropes are suspended from floating lines and buoys, allowing for flexibility in response to wave movement (Aypa, 1990; Karayücel et al., 2003; Stevens et al., 2008).

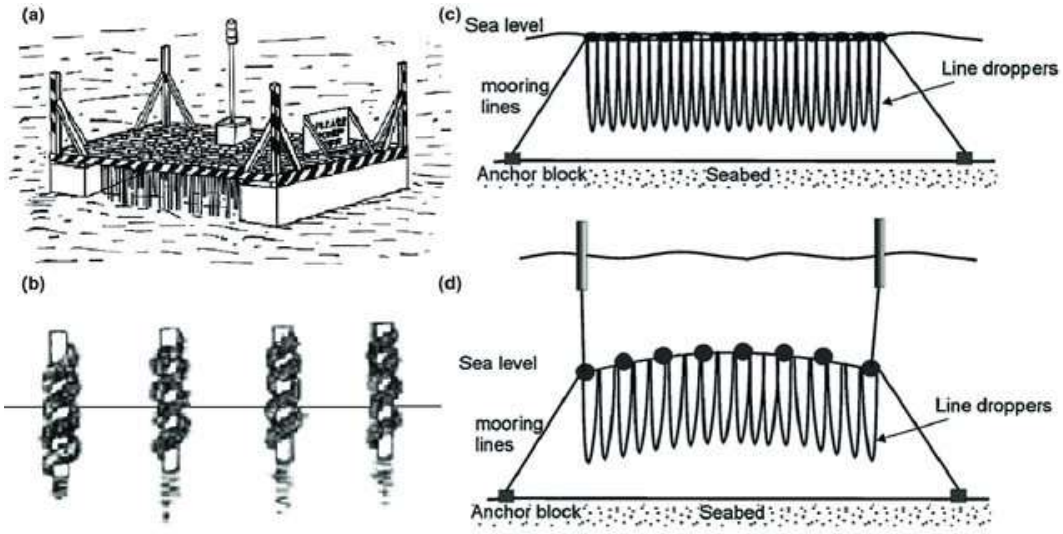

Figure 1.5: **Methods of mussel cultivation.**Common shellfish farming methods (in Europe) include (a) raft culture (Aypa, 1990; Figueiras et al., 2002; Pérez-Camacho et al., 2013), (b) Bouchot culture (Aypa, 1990; Goulletquer & Heral, 1997), (c & d) longline culture (Aypa, 1990; Karayücel et al., 2003; Stevens et al., 2008). (Figure from Mascorda Cabre et al., 2021).

Mussel biology and reproduction

Mussels are bivalve molluscs belonging to the Mytilidae family, characterised by their elongated, wedge-shaped shells (Bayne, 1976, Dailianis, 2010).They possess a strong byssus thread, allowing them to attach firmly to various substrates such as rocks, plants, or other shells (Dailianis, 2010). Mussels are filter feeders, drawing water through their incurrent siphon to extract phytoplankton, bacteria, detritus, and other organic particles for consumption (Riisgård et al., 2011).

Mussels inhabit a wide range of environments, from the intertidal zone to shallow subtidal areas, in both marine and brackish waters (Dailianis, 2010) They are euryhaline, tolerating a wide range of salinities, and eurythermal, able to withstand significant temperature fluctuations (Gosling, 2003b, 2015b; McDonald & Koehn, 1988). Optimal growth occurs in environments with clean, well-oxygenated water, moderate salinities (20 – 35 ‰), and temperatures between 5 – 20°C (Bayne, 1976; Rayssac et al., 2010). Adequate nutrient availability, firm substrates for attachment, and moderate wave exposure are essential for healthy mussel populations (Wijsman et al., 2019). They can form dense populations, creating reef-like structures that provide valuable habitat for a diverse range of marine organisms (Bayne, 1976; Gosling, 2003b; Seed & Suchanek, 1992). The Menai Strait mussel beds serve as an excellent example, supporting a rich community of associated species, including fish, invertebrates, and algae.

They are primarily dioecious, with separate sexes and reach sexual maturity after their first year, although hermaphroditism can occur (Fisher & Skibinski, 1990). Reproductive output increases with size, with larger females producing significantly more eggs (Thompson, 1984; Tyler-Walters et al., 2022) Spawning typically occurs during spring and summer, influenced by factors such as water temperature, food availability, and tidal exposure (Myint & Tyler, 1982). Northern populations generally exhibit a later spawning season compared to southern populations (Seed, 1969; Newell et al., 1982; Seed and Suchanek, 1992). In northeast England, for example, gametogenesis occurs throughout the winter, leading to a partial spawning in spring followed by a secondary spawning later in the summer (Seed & Suchanek, 1992).

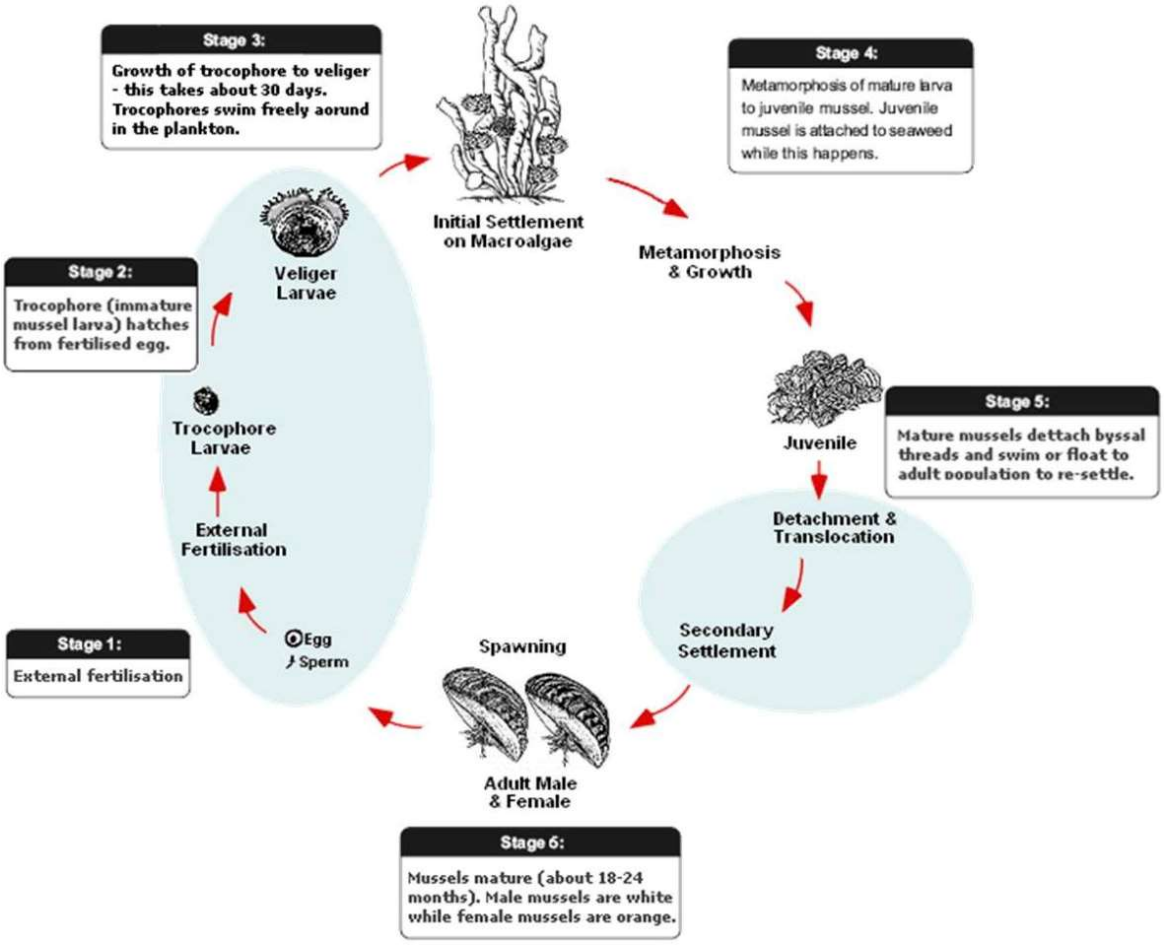

Figure 1.6: **Life cycle of the mussel *P. canaliculus***, showing the stages of development from fertilized egg to adult. Key stages include external fertilization, trochophore and veliger larval stages, settlement on a substrate, metamorphosis, and juvenile growth. (Figure adapted from Young, 2009).

Mussels undergo a fascinating life cycle, illustrated in Figure 1.6. The mussel life cycle includes three distinct larval stages: trochophore, veliger, and pediveliger (King et al., 1989; Newell et al., 1982). It begins with the release of gametes into the water column during spawning, after which fertilisation occurs externally, and the resulting zygote develops into a free-swimming trochophore larva. This early larval stage is characterised by a band of cilia that enables the larva to swim and rotate (Tyler-Walters, 2008).

Trochophore larva then develop into a veliger larva, characterised by the development of a velum, a ciliated organ that aids in feeding and locomotion. During this stage, the larvae continue to grow and develop, undergoing significant morphological changes (Gosling, 2003c).

The last larval stage is characterised by the development of a foot and is known as the pediveliger stage. This final larval stage marks the onset of settlement as it becomes capable of attaching to a substrate when reaching approximately 250 µm in length (Bayne, 1964; Gosling, 2003c), and undergoing metamorphosis into a juvenile mussel, known as “spat” (Bayne, 1964; Bhagde Rupendra V., 2013; Gosling, 2015c).

Mussels as environmental monitors

Bivalve molluscs, including oysters, mussels, and clams, are widely recognised as valuable bioindicators of environmental pollution, particularly in marine environments (Boening, 1999).

Especially mussels possess several attributes that make them ideal for biomonitoring, such as their wide geographical distribution, abundance, sedentary nature, tolerance to a range of environmental conditions, and mainly their filter-feeding habits, which lead to the accumulation of contaminants in their tissues (Riisgård et al., 2011; Streit, 1998). As sessile organisms, they integrate exposure to pollutants over time, providing a valuable record of environmental contamination (Rodney et al., 2007; Streit, 1998). Furthermore, mussels exhibit a range of physiological responses to environmental stressors (Myint & Tyler, 1982; Tan et al., 2023; Wing & Leichter, 2011), including changes in gene expression (Kerambrun et al., 2016), enzyme activity (Nicholson & Lam, 2005), and cellular integrity (Kolyuchkina & Ismailov, 2011; Langston et al., 2012; Perceval et al., 2004). These biomarkers provide insights into the subcellular effects of pollutants, offering a more comprehensive understanding of their impact on mussel health and the overall ecosystem (Zuykov et al., 2013). Their wide distribution, abundance, and relative ease of sampling make them ideal sentinel organisms for monitoring environmental quality in marine environments.

Furthermore, mussels serve as crucial bioindicators for the presence and accumulation of marine biotoxins, such as those associated with Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), and Amnesic Shellfish Poisoning (ASP) (Viviani et al., 1995). As filter feeders, mussels accumulate toxins produced by harmful algal blooms (HABs) (De Rijcke et al., 2015), including those associated with 'red tides' (Lee, 2003) and 'mucilaginous

aggregates' (Flander-Putrle & Malej, 2008) posing significant risks to human health if consumed (CEFAS, no date; Stobo et al., 2008). By monitoring toxin levels in mussel tissues, public health authorities can effectively identify areas with elevated contamination and implement appropriate measures, such as temporary closures of shellfish harvesting areas, to protect consumers from consuming contaminated seafood (Food Standards Agency FSA, no date). This proactive approach to monitoring ensures the safety of the seafood supply chain and safeguards public health from the potential risks associated with consuming contaminated shellfish.

Speciation and hybridisation

The native mussel species in Scotland is *Mytilus edulis* (Linnaeus 1758). However, the introduction of *M. galloprovincialis* (Lamarck 1819), an invasive species from the Mediterranean, and the presence of *M. trossulus* (Gould 1850) likely a relict population from the post-glacial period, have led to complex hybridisation events in Scottish waters (Dias et al., 2011; Michalek et al., 2016). *M. edulis* is a circumpolar species (Beaumont et al., 2008), widely distributed in both the Northern and Southern Hemispheres (Bayne, 1976). *M. galloprovincialis* has a more southern distribution, while *M. trossulus* is a cold-water species (Boroda et al., 2020) originating in the North Pacific (McDonald & Koehn, 1988). These species exhibit varying thermal tolerances, with *M. trossulus* being the most cold-tolerant and *M. galloprovincialis* displaying the broadest thermal range (Braby & Somero, 2006; Hofmann & Somero, 1995).

The presence of these multiple species and the potential for hybridisation have significant implications for the genetic diversity and ecological interactions within Scottish mussel populations (Beaumont et al., 2008; Dias et al., 2011). While some hybrids may inherit desirable traits such as stronger shells, others may exhibit weaker shells (Mathiesen et al., 2017). This variability can impact the resilience to environmental stressors, susceptibility to predation, and ability to withstand handling during harvesting and processing (Dias et al., 2011).

In the blue mussel complex, consisting of *Mytilus edulis* Linnaeus, 1758; *Mytilus galloprovincialis*, Lamarck, 1819; and *Mytilus trossulus*, Gould, 1850 hybridisation is common in overlapping geographical regions (Beaumont et al., 2008). Rope culturing can potentially enhance hybridisation among sympatric

Mytilus species. While mussels possess a pelagic larval stage with the potential for long-distance dispersal (≥ 30 km; Gilg & Hilbish, 2003), environmental factors and post-settlement selection can influence population dynamics (Gardner & Skibinski, 1991; Dobretsov & Miron, 2001). Rope culture, providing a high surface area for settlement and minimising benthic predation, can increase the likelihood of interbreeding among closely related species. This increased gene flow, while potentially leading to novel genotypes and increased genetic diversity, may also have unintended consequences for aquaculture operations (Michalek, Ventura & Sanders, 2016).

The native mussel species in Scotland is *M. edulis*. However, the introduction of *M. galloprovincialis* from the Mediterranean and the presence of *M. trossulus*, likely a relict population from the post-glacial period, have led to complex hybridisation events in Scottish waters (Beaumont et al., 2008; Dias et al., 2011; Michalek et al., 2016) *M. edulis* is a circumpolar species (Beaumont et al., 2008), widely distributed in both the Northern and Southern Hemispheres (Bayne, 1976). *M. galloprovincialis* has a more southern distribution, while *M. trossulus* is a cold-water species originating in the North Pacific (McDonald and Koehn, 1988). These species exhibit varying thermal tolerances, with *M. trossulus* being the most cold-tolerant and *M. galloprovincialis* displaying the broadest thermal range (Hofmann and Somero, 1995; Braby and Somero, 2006).

M. trossulus exhibits greater tolerance to low salinity environments compared to *M. edulis* (Kautsky, Johannesson and Tedengren, 1990; Väinölä and Hvilsom, 1991), suggesting a key physiological differentiation (Hilbish, Bayne and Day, 1994). This salinity preference likely contributes to its distribution patterns, particularly in areas with varying salinity levels, such as estuaries. While other environmental factors, such as pH, temperature, and dissolved oxygen, also influence species distribution, salinity appears to be a significant factor in differentiating the distribution of *M. trossulus* from that of *M. edulis* on a global scale (Koehn, 1991; Seed, 1992). The presence of these multiple species and the potential for hybridisation have significant implications for the genetic diversity and ecological interactions within Scottish mussel populations (Beaumont et al., 2008; Dias et al., 2011). While some hybrids may inherit desirable traits such as stronger shells, others may exhibit weaker shells (Mathiesen et al., 2017). This variability can impact the resilience to environmental stressors, susceptibility to

predation, and ability to withstand handling during harvesting and processing (Dias et al., 2011).

Challenges Facing the Mussel Aquaculture Industry

The survival and dynamics of mussel populations are influenced by a complex interplay of factors, including abiotic stressors (temperature, salinity, extreme weather) (Lazo & Pita, 2012), biological interactions (predation, competition) (Wing & Leichter, 2011), and anthropogenic impacts (pollution, disease, climate change) (Chu & Hale, 1994; McLusky et al., 1986; Murray et al., 2022; Stewart-Sinclair et al., 2020; Tan et al., 2023).

i. ***Temperature, salinity and other abiotic factors***

Successful mussel farming relies on specific environmental conditions conducive to optimal growth and health. Clean and unpolluted water with moderate salinity levels between 20 to 35 parts per thousand (ppt, ‰) (Maar et al., 2015) and temperatures ranging from 5 to 20°C is fundamental (Bayne, 1976; Rayssac et al., 2010). Adequate nutrient availability, including phytoplankton, supports mussel growth and reproduction (Page & Hubbard, 1987; Wong & Levinton, 2004). Firm substrates for attachment, shallow coastal waters with sufficient water exchange, and moderate wave exposure are also necessary (Wijsman et al., 2019). Well-oxygenated water and effective predator management are crucial, while compliance with environmental regulations ensures sustainable farming practices (Tyler-Walters, 2008; Tyler-Walters et al., 2022).

In terms of current and emerging risks for shellfish, both globally and in Scotland, several factors are significant. These include environmental changes such as ocean acidification and warming waters, which can impact shellfish growth, reproduction, and survival (Stewart-Sinclair et al., 2020). Shellfish are susceptible to contamination from pollutants such as heavy metals, chemicals, and microplastics present in the water. During feeding, bivalves ingest suspended particles from their environment, including non-nutritious organic or inorganic particles and potentially toxic elements (PTEs), such as heavy metals, leading to their bioaccumulation in various tissue types (Rodney et al., 2007). Factors such as the metabolic rate of the mussel, element concentration in water, and duration of exposure affect material uptake and subsequent accumulation (Mubiana &

Blust, 2007; Baines & Fisher, 2008; Deruytter et al., 2015). Salinity and temperature also influence water chemistry, impacting bioaccumulation dynamics (Kumar et al., 2015; McLusky et al., 1986). Furthermore, climate change exacerbates many of these risks, amplifying stressors on shellfish populations and requiring adaptive management strategies to ensure their sustainability. Rising sea temperatures, changing ocean currents, and extreme weather events associated with climate change can disrupt shellfish habitats and affect their growth, reproduction, and survival (Maar et al., 2015; Murray et al., 2022). In addition to other factors, inadequate food availability, such as insufficient phytoplankton blooms, can lead impact the survival and growth of mussel spat, particularly during periods of high larval density and intense competition for limited resources (Seed, 1968; White et al., 2022).

ii. ***Predation***

Predation is a significant factor influencing the dynamics and mortality of mussel populations. Predators include a range of organisms, such as the dogwhelk (*Nucella lapillus*), starfish (*Asterias rubens*), crabs (*Carcinus maenas*, *Cancer pagurus*), fish, and various bird species including oystercatchers, eiders and gulls (Seed, 1993; Seed & Suchanek, 1992). Predation pressure can vary depending on predator abundance and the size of the mussel (Tyler-Walters, 2008). Bird predation, particularly by oystercatchers (*Haematopus ostralegus*) and eiders (*Somateria mollissima*) (Hilgerloh, 1997), can have a substantial impact on mussel populations, with significant mortality rates observed in some areas (Hamilton, 2000). In addition to predation, other factors such as disease outbreaks (Paillard et al., 2004; Travers et al., 2015; Zannella et al., 2017), anthropogenic pollutants such as heavy metals (McLusky et al., 1986; Tyler-Walters, 2008), organic pollutants (Deruytter et al., 2015; Nadella et al., 2009), and the impacts of climate change (Murray et al., 2022; Stewart-Sinclair et al., 2020) pose significant threats to mussel survival and population health.

iii. ***Invasive species***

The introduction and spread of marine and coastal non-native species are primarily driven by human activities, including global shipping (Carlton, 1996). Key pathways for the introduction of non-native species include ballast water exchange, hull fouling, and aquaculture (Naylor et al., 2001; O'Shaughnessy et al., 2023). Recreational boating also plays a significant role in their secondary spread (Acosta & Forrest, 2009; Clarke Murray et al., 2011; O'Shaughnessy et al., 2023). Once introduced, non-native species can outcompete native species for resources, impacting biodiversity (Lengyel, 2009; Stachowicz et al., 1999). Aquaculture activities can facilitate the spread of associated species (Naylor et al., 2001). Invasive species can significantly impact mussel aquaculture. Biofouling impedes growth, while competition for resources and increased mortality due to predation or disease reduce yields (Fitridge et al., 2012; Forrest & Atalah, 2017; Murray et al., 2020).

According to a recent publication by the Scottish Government (2023), the introduction of non-native molluscs, such as zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*), poses a significant threat to native mollusc populations in Scotland. These invasive species can outcompete native mussels for food and space, leading to a decline in their abundance and diversity. Zebra and quagga mussels can also filter large volumes of water, removing nutrients and plankton that native species rely on. This can disrupt the entire food web and negatively impact other aquatic organisms (Scottish Government, 2023).

Another significant threat to Scottish mussel aquaculture is the invasive *M. trossolus,* which had been identified in Loch Etive in 2004 (Beaumont et al., 2008). It has been reported to negatively impact mussel aquaculture by exhibiting traits undesirable for commercial production, including thin, fragile shells (Beaumont et al., 2008; Michalek et al., 2021; Penney et al., 2007), leading to increased breakage during harvest and processing, reduced meat yield, and shortened shelf life. As discussed in Michalek et al., (2021), *M. trossulus* and its hybrids subsequently dominated mussel farms in Loch Etive, leading to a significant decline in production. Mussel production plummeted severely, rendering mussel farming in the area economically unviable. Due to these

detrimental impacts, *M. trossulus* is now classified as a commercially damaging species in Scotland under the Aquaculture and Fisheries (Scotland) Act 2013, requiring mandatory reporting to mitigate future industry impacts (Michalek et al., 2021).

iv. ***Disease***

Disease outbreaks pose a significant threat to mussel populations, both in aquaculture and in the wild. Bacterial pathogens, particularly *Vibrio* species such as *V. harveyi* and *V. splendidus*, can cause significant mortalities, especially in high-density aquaculture settings (Paillard et al., 2004). These bacteria can thrive in warm water temperatures and high salinities, contributing to increased disease prevalence (Benabdelmouna & Ledu, 2016; Dégremont et al., 2019). Viral infections, such as those caused by Ostreid herpesvirus 1 (OsHV-1), can also have devastating impacts on mussel populations, leading to mass mortalities and significant economic losses in aquaculture (Faury et al., 2014; Renault et al., 2014). Furthermore, the emergence of transmissible cancers, such as "mussel leukaemia," highlights the vulnerability of mussel populations to infectious diseases (Baez-Ortega & Murchison, 2022; Benadelmouna et al., 2018; Metzger et al., 2015). These diseases, coupled with other stressors such as environmental changes and anthropogenic impacts, pose significant challenges to the sustainability of mussel aquaculture.

v. ***Spat shortage***

The EU mussel aquaculture sector has experienced recent fluctuations in production, primarily driven by a decline in natural spat availability. In 2020, EU mussel production decreased by 10%, reaching 406,910 tonnes, with a corresponding 9% decline in value (European Commission, 2024). This decline was particularly evident in Spain, the largest producer, with a 10% drop in production and a 13% decrease in value (European Commission, 2024). Furthermore, per capita consumption of mussels in the EU decreased slightly in 2022 compared to the previous year, indicating a decline in market demand. This decrease in production can be attributed to several factors, including a lack of sufficient mussel seed due to fluctuations in natural spatfall. These fluctuations, often unpredictable and influenced by environmental factors, pose significant

challenges to the industry's sustainability and profitability (European Commission, 2024).

According to Avdelas et al.,(2021) the unpredictable availability of wild mussel seed worsened by competition from invasive species such as the Pacific oyster (*Crassostrea gigas*).

The Scottish shellfish aquaculture industry faces significant challenges, with a notable decline in natural spat settlement posing a major threat to production (Murphy & Munro, 2024). A 2011 survey conducted by the Marine Directorate in response to industry reports of poor spat settlement and mortality revealed that while not widespread, insufficient spat settlement significantly impacted some Scottish mussel farmers.

This shortage of naturally occurring spat, the juvenile stage of mussels, has significant implications for the sector's sustainability and highlights the vulnerability of the industry to fluctuations in natural spatfall, a crucial component for sustainable production.

A subsequent survey in 2013, which included a specific question on spat collection, found that while 41% of surveyed sites were used for spat collection, only 57% of these sites reported sufficient spat settlement for production purposes. This reliance on natural spat collection, which can be highly variable and unpredictable, presents several challenges (Murphy & Munro, 2024). Firstly, the variability and unpredictability of natural spatfalls (Boudry et al., 2013) can lead to significant fluctuations in production, impacting industry profitability and long-term sustainability. Secondly, while mussel seed hatcheries offer a potential solution to the challenges posed by variable natural spatfall, current production levels remain relatively low (Adamson et al., 2018). The insufficient spat settlement has necessitated the movement of mussels between sites, increasing the risk of disease transmission and potentially impacting the long-term health of the mussel populations and the overall sustainability of the Scottish shellfish aquaculture sector (Adamson et al., 2018; Murphy & Munro, 2024).

vi. ***Spat mortalities***

Mass mortality events in European mussels have become increasingly frequent, impacting both *M. edulis* and *M. galloprovincialis* (Mandić et al., 2024). Notable examples include the 2015/2016 and 2019 mortality events of marine mussels in

the Oosterschelde, Netherlands, where mortality rates reached 40-50% on culture plots and a devastating 100% in wild seed beds during the first event (Capelle et al., 2021). In France, significant mortalities occurred in 2014 and 2016 along the Atlantic and English Channel coasts, affecting both juveniles and adults (Benabdelmouna & Ledu, 2016; Charles et al., 2020). These events often lack a single, clear cause, with researchers pointing to a combination of factors, such as high spawning activity, algal blooms, and the development of granulocytomas in the Oosterschelde, as potential contributors to weakened mussel health. Furthermore, previous research has demonstrated that genetic factors can significantly influence blue mussel mortality rates, while environmental conditions primarily impact growth variability (Benabdelmouna et al., 2018; Myrand et al., 2000). This aligns with findings from other studies that observed variations in mortality rates among different mussel stocks, even within similar environments (Benabdelmouna et al., 2018; Fuentes et al., 1992; Myrand & Gaudreault, 1995; Tremblay et al., 1998). These findings are supported by extensive evidence of genetic differentiation among mussel populations across various geographic scales (Fuentes et al., 1992; Gosling & McGrath, 1990; Koehn et al., 1984, 1976; Koehn & Mitton, 1972; Levinton & Suchanek, 1978).

These events underscore the vulnerability of mussel populations to a range of stressors and the urgent need for further research to understand and mitigate their impacts.

Recent observations have raised concerns regarding an alarming increase in spat mortality rates on the West coast of Scotland, particularly within key source areas for mussel larvae such as Loch Eil (Corrochano-Fraile et al., 2024), posing a significant threat to local mussel farming operations.

Aims and objectives

i. ***Aims***

Given the recent decline in natural spat availability across Europe, including a significant decrease in spatfall within Scottish mussel farms, and the observed escalation of spat mortality rates, this research aims to investigate the factors contributing to these declines.

Loch Eil, known for its high level of spat mortality, was chosen as a case study to investigate the factors influencing spat mortality within this key source area for mussel larvae.

The primary aim of this thesis is to conduct a multidisciplinary analysis of spat mortality dynamics in Loch Eil, investigating the patterns and rates of spat death within this system and comparing the results to an unaffected control site (Figure 1.7). To achieve, the study will focus on three key objectives:

ii. ***Objectives***

1. **Characterising environmental factors influencing spat mortality:** This will involve a thorough investigation of environmental variables such as temperature, salinity, water quality and chlorophyll as a proxy for nutrient levels, and their potential impact on spat survival at the study site.
2. **Investigating the role of pathogens:** This objective will focus on identifying and characterising potential pathogens affecting spat survival, including bacterial and viral infections at the study site. Results will be compared to a control site where no significant spat mortality has been observed. This chapter, focusing on the investigation of pathogens affecting spat survival, was not included in the final thesis.
3. **Integrate environmental and biological factors:** This objective will involve conducting in situ experiments to test the interactive effects of identified environmental factors and potential pathogens on spat survival under controlled conditions.

By integrating the findings from these three objectives, the study will attempt to provide a comprehensive understanding of the key factors driving spat mortality in Loch Eil.

Google Maps, 2019

Figure 1.7: **Map of the West Coast of Scotland highlighting the locations of the study site (Loch Eil) and the control site (Loch Sunart).** Loch Eil is located south of Fort William, while Loch Sunart lies to the south of Loch Eil and is connected to the North Atlantic Ocean via the Sound of Mull. (Figure created using Google Maps)

Chapter 2

Understanding mortality dynamics: Observational studies on mussel spat (*Mytilus edulis*) in Loch Eil

Abstract

This study investigated high spat mortality rates observed in Loch Eil, a key source area for mussel larvae in Scotland. Field observations revealed significant differences in mortality rates between Loch Eil and a control site in Loch Sunart, with Loch Eil experiencing mortality rates as high as 68.3% compared to a maximum of 0.9% in Loch Sunart. Further analysis revealed distinct temporal patterns of mortality in Loch Eil, with peak mortality occurring early in the observation period. These findings highlight the presence of site-specific factors influencing spat survival within Loch Eil and emphasise the need for further investigation into the underlying causes of these observed mortality events.

Keywords: Mortality dynamics; mussel spat; shellfish farming; environmental factors; mortality events; environmental parameters; spat survival; sustainable aquaculture

2.1 Introduction

Mortality dynamics refer to the patterns and rates of death within a population over time. As per reports by Munro and Wallace (2018) and by Adamson Syvret and Woolmer (2018), the mortality dynamics of mussel spat in aquaculture settings represent a critical area of study essential for sustaining shellfish farming industries. Therefore, understanding the factors influencing mortality events is vital for sustaining shellfish industries.

Spat mortality can be influenced by a range of factors, including environmental variables such as ocean acidification (Murray et al., 2022; Stewart-Sinclair et al., 2020), temperature fluctuations (Incze et al., 1980; Lazo & Pita, 2012), changes in salinity (Kautsky, 1982; Maar et al., 2015) and nutrient levels (Seed, 1968; White et al., 2022). Biological factors also play a significant role, with predation (Hamilton, 2000; Hilgerloh, 1997), competition for space and resources (Lengyel, 2009; Stachowicz et al., 1999), and disease outbreaks (Faury et al., 2014; Metzger & Goff, 2016; Paillard et al., 2004; Renault et al., 2014; Travers et al., 2015) posing significant threats to spat survival. Furthermore, anthropogenic activities such as pollution from land-based sources (Artiola et al., 2019; Rodney et al., 2007) and disturbances from shipping traffic (Acosta & Forrest, 2009; Clarke Murray et al., 2011; van der Gaag et al., 2016) can exacerbate these pressures and contribute to increased spat mortality.

Importantly, previous research has demonstrated that genetic factors, particularly the origin of mussel stocks, can significantly influence mortality rates, while environmental conditions primarily impact growth variability (Benabdelmouna et al., 2018; Fuentes et al., 1992; Gosling & McGrath, 1990; Koehn et al., 1984, 1976; Koehn & Mitton, 1972; Levinton & Suchanek, 1978; Myrand & Gaudreault, 1995; Tremblay et al., 1998).

The motivation for this research arose from the observations of local shellfish farmers who have noted significant mortality events impacting mussel spat populations, mirroring mass mortality events previously documented in Europe (Mandić et al., 2024), such as those observed in the Oosterschelde in the Netherlands (Capelle et al., 2021) and along the French coasts (Charles et al., 2020).

Recent observations have raised concerns regarding an increase in spat mortality rates within Loch Eil in Scotland, posing a significant threat to local mussel farming operations. Loch Eil serves as an important site for mussel farming, renowned for its pristine waters and ideal environmental conditions conducive to robust shellfish cultivation (Edwards et al., 1980; Gosling, 2015a, 2015b). Situated in the Scottish Highlands, its coastal waters boast moderate salinity levels, optimal temperatures, and nutrient-rich currents, providing an ideal habitat for mussel spat development and growth.

This research aimed to investigate and narrow down the scope of the factors contributing to spat mortality in Loch Eil by examining the complex interplay between environmental factors, site-specific conditions, and spat health building upon the famers previous findings and observations.

2.2 Materials and methods

Farmer interviews and observations

This study incorporated valuable insights from local mussel farmers. Interviews were conducted to gather information on the historical context of mussel farming in Loch Eil, their observations regarding spat mortality events, and their perspectives on potential contributing factors, including environmental changes, biological interactions, and anthropogenic influences.

Study sites and hydrography

i. ***Study site***

Loch Eil, a key source of mussel larvae (Corrochano-Fraile et al., 2024), is located near Fort William, north-west of the popular tourist destination and is home to industries including fish farming, forestry, aluminium works, distilling and agriculture. Loch Eil's position resembles a what has been described as a 'dog leg' or an 'arm' on the northern-end of the adjacent water body, Loch Linnhe, due to its transitional shape and connection (SEPA, 2011).The shape of Loch Eil is detailed in (Galbraith et al., 2012) and its hydrography in (Milne, 1972), stating a length of approximately 12-13 km and describing its shape as quite uniform varying only between 0.9 and 1.2 km within the majority of the loch. According to the Scottish Sanitary Survey Programme (2012) and a report by SEPA (2005), the loch is very sheltered and possesses a maximum depth of 71 metres. One of the two shallow sills in the loch is positioned near the west end and measures 31 metres in depth, which is roughly the location where my research was conducted. The other sill sits on the eastern end of The Narrows, also known as The Annat Narrows. These are situated near Corpach, east of Loch Eil, and have been measured to be between 200 – 300 metres in width, whereas the main channel within the narrows has been measured to be less than 6 metres deep (Galbraith et al., 2012). Despite the two sills within the loch, most research and analyses were conducted presuming the loch as one large basin (Edwards et al., 1980; Galbraith et al., 2012).

ii. ***Control site***

Loch Sunart, chosen as the control site for this investigation, is located within a 40 km radial distance from Loch Eil, roughly 30 km south-west of Fort William. This loch is considered among the longest sea lochs in the country, reaching nearly 31 km in length, approximately 1.5 km in width with a measured maximum depth of 124 metres. It is surrounded by small villages along the northern shore, very few individual residences on the southern shore, with the main town, Strontian, with a population of ca. 350, situated north easterly of the upper basin. Not unlike Loch Eil, the bathymetry of Loch Sunart includes six subtle sills ranging from 6 to 70 metres deep (CEFAS, 2014). Due to its length and narrow width, the loch is partially sheltered near the head, whereas the outer reaches sustain more exposure to wind and accompanying waves. The loch receives a large influx of freshwater from surrounding rivers, however, compared to the tidal flow, the freshwater input near the surface does not seem to influence the salinity of the loch (Austin & Inall, 2002; CEFAS, 2014). According to Gillibrand et al. (1995), Sunart follows a semi-diurnal tidal regime which promotes a more rapid renewal of its bottom waters, in contrast to Loch Eil. However, as half of the freshwater enters the loch near the upper basin towards the northeast (Gillibrand et al., 1995), that specific area exhibits a lower salinity as opposed to the mouth of the loch (Bates et al., 2003). Notably, Loch Sunart was chosen as the control site due to the successful survival and thriving of spat transferred from Loch Eil by local mussel farmers. This practice, while potentially introducing genetic material from Loch Eil into Loch Sunart if the spat originated from different sources, highlighted the contrasting conditions between these two lochs.

iii. ***Hydrography***

Weather conditions such as wind and precipitation can influence the stratification and create a more estuary-like environment within the loch, despite of the tidal regime (CEFAS, 2014; Gillibrand et al., 1995). Comprehending the hydraulic effects such as the water movement and circulation patterns in Loch Eil and Loch Sunart is crucial for assessing the transportation and distribution of micronutrients, trace elements and other essential or non-essential substances.

Experimental design and observation of 2018/19 event

The experimental design employed in this study involved the strategic placement of lantern nets along grow-out lines in Loch Eil, with a control site established in Loch Sunart. With the anticipation to detect any spatial and temporal patterns in mortality dynamics and identify potential environmental drivers, the mortality rates were observed over time and compared between sites.

Lantern nets (Figure 2.1) provided protection from any predators within the loch, not only ensuring that any dead spat would have succumbed to whatever is causing the mass mortalities, but to also facilitate the quantification of mortalities of the stocked spat, similar to the experimental set up described in (Stirling and Okumuş, 1994). Based on the averaged weight of 100 spat, which was done on site and in triplicate using a portable balance (Ohaus Scoutpro), the nets were stocked with an estimated amount of 1000 spat. The spat was spread evenly onto the three central layers of the lantern nets, then strategically placed along four grow-out lines in Loch Eil. Each line contained five nets, placed evenly apart by approx. 50 metres. The nets were fastened to the grow-out ropes and submerged to a depth of 4 metres. The geographical locations of the study site and control site are depicted in Figure 1.7 in Chapter 1. The mussel farming site in Loch Sunart functioned as the control site, where only one grow-out line was available during the experiment. Therefore, lantern nets, stocked with Loch Eil spat, were fastened along the lines emulating similar placements of approximately 50 metres apart, however 5 out of the 10 nets were submerged to 4 metres, and the others to 1 metre depth. A more detailed view of the site locations as well as the net placements within the lochs is provided in Figure 2.2.

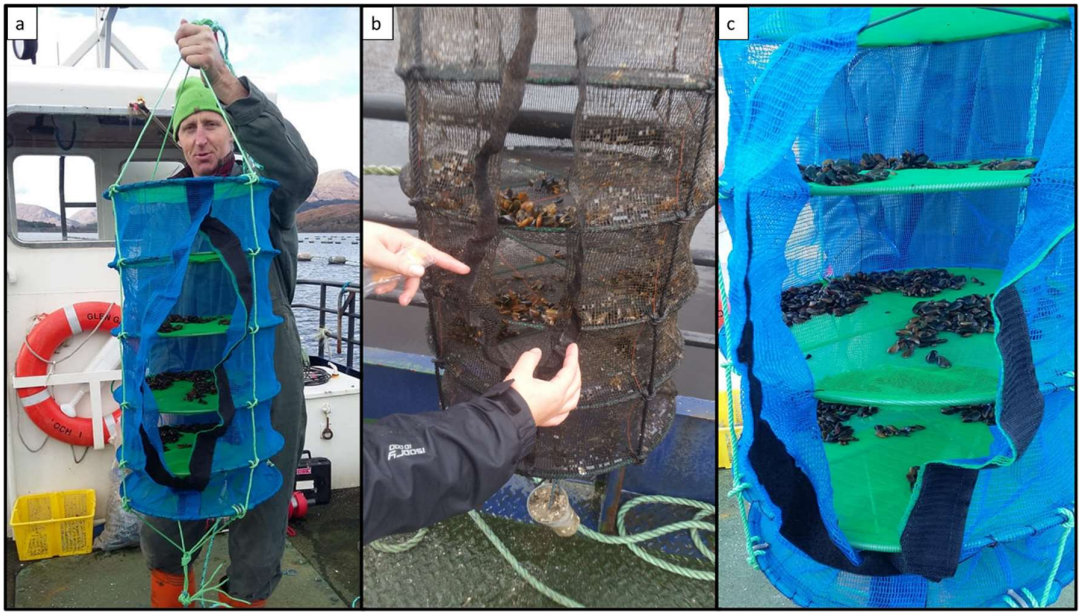

Figure 2.1: **Experimental Setup: Lantern Net Deployment in Loch Eil**. To quantify spat mortality rates, lantern nets were deployed in Loch Eil. The enclosed design of the nets provided a controlled environment, excluding predation as a factor and allowing for a more accurate assessment of mortality events observed by local mussel farmers.

The removal and counting of the dead individuals occurred during every time point, which were fixed on a fortnightly basis, apart from the last time point. The time points and the corresponding dates, including the amount of days post stocking the tanks, were as follows:

- Time point 0 2018/29/10 Day of stocking
- Time point 1 2018/12/11 14 d post stocking
- Time point 2 2018/29/11 30 d post stocking
- Time point 3 2018/12/12 44 d post stocking
- Time point 4 2019/16/01 79 d post stocking

On the last day of the observation (2019/16/01), the dead individuals were counted as well as the remaining survivors, after which the nets were removed from both Lochs.

During the observation period, moribund or live spat ($n = 18$ per net) and water samples were collected for analyses which are presented and discussed in Chapters 2 and 3. From May 2019 onwards, data loggers for salinity (HOBO Salt

Water Conductivity/Salinity Data Logger) and temperature (HOBO TidbiT v2 Water Temperature Data Logger) were introduced in Loch Eil to collect continuous in-situ measurements of the two environmental parameters, whilst temperature and salinity in Loch Sunart were still measured manually by probe.

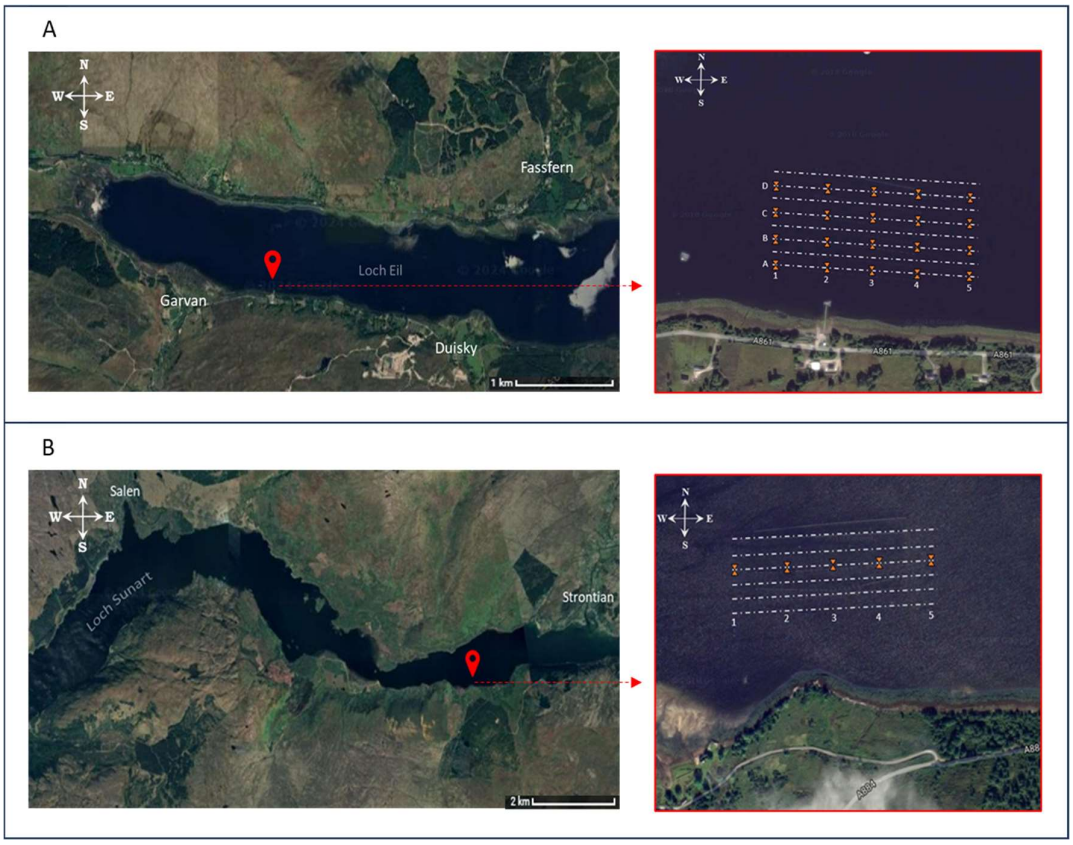

Figure 2.2: **Site locations and net placement within the mussel farms 2018.** The figure shows the site locations and placement of lantern nets within Loch Eil (A) and Loch Sunart (B). In the affected site within Loch Eil (A), the lines on which lantern nets were placed were labelled A – D, whereby line A is located nearest the shore on the southern shoreline near Garvan, and line D the furthest from the shore, yet not quite located in the centre of the of the loch. Numbers 1 – 5 were allocated to the lantern nets and their placement along the lines, forming a grid. Within Loch Eil (A), a total of 20 nets were placed at a depth of 4 m along four mussel lines approximately 50 m apart. Loch Sunart (B) functioned as the control site and was equipped with a total of 10 nets along one line only due to lack of space. However, half of the nets were placed at a depth of 4 m and the other half at 1 m.

Observation 2019/20 event

The following year, the start of the 2019 observation experiment was brought forward by a month with the anticipation of witnessing the actual start of the mortality event. The lantern nets were stocked identically to the previous year,

except the nets was reduced to six per loch and placed along the line into three stations (east, middle and west). Each station would serve as a set location for two lantern nets, one submerged at 2 metres and the other at 6 metres. The net placement within the mussel farms of each loch is depicted in Figure 2.2. After two months (time point 2, 2019), six additional nets were brought into Loch Sunart, three of which stocked with Loch Eil originating spat, and the other three with Loch Sunart originating spat, and suspended at 4 metres at each of the stations. As per the prior year, samples were collected, and the dead spat was counted and removed from the nets. The time points and the corresponding dates, including the amount of days post stocking the tanks, were as follows:

- Time point 0 2019/24/09 Day of stocking
- Time point 1 2019/16/10 14 d post stocking
- Time point 2 2019/25/11 30 d post stocking
- Time point 3 2019/09/12 44 d post stocking
- Time point 4 2020/23/01 79 d post stocking

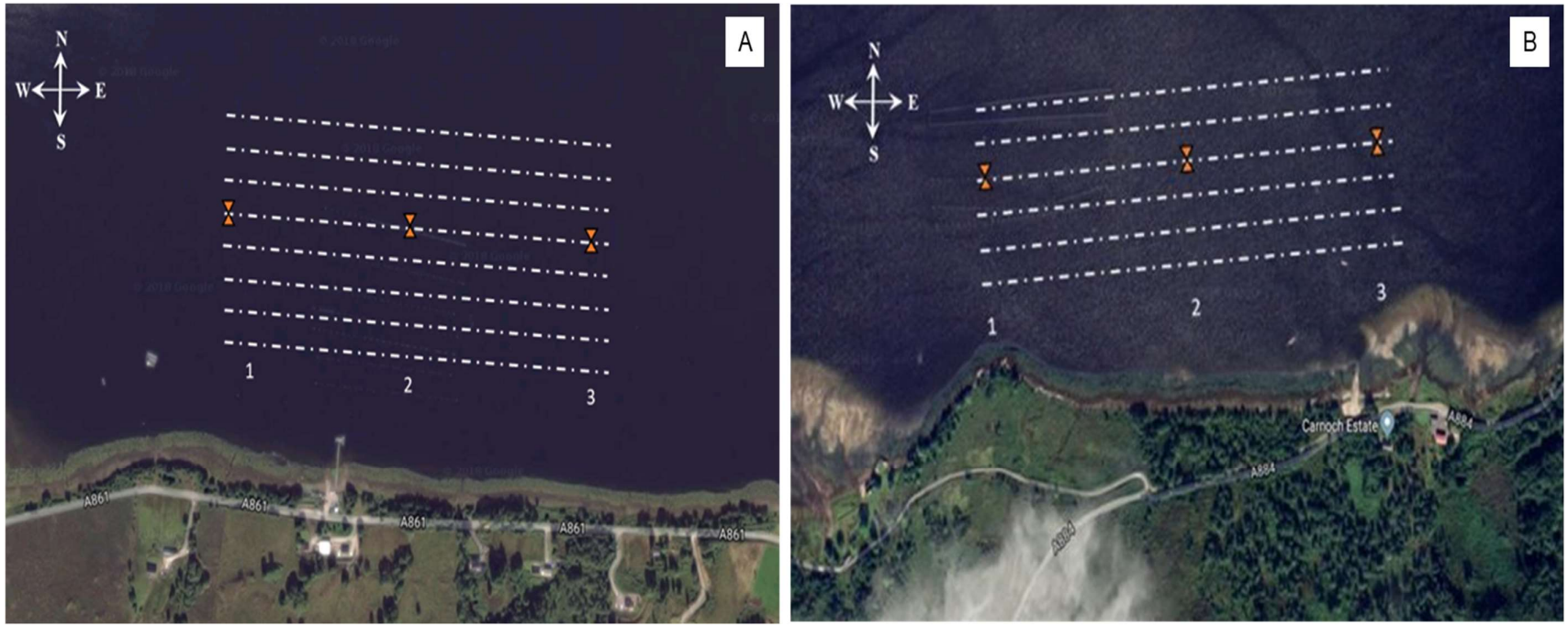

Figure 2.3: **Net placement within the mussel farms 2019.**The figure shows the placement of lantern nets within Loch Eil (A) and Loch Sunart (B). A total of six nets were placed along the most central line within the farms on both sites. Numbers 1 – 3 were allocated to the lantern nets and their placement along the line, whereby Number 1 represented the eastern nets, 2 the middle nets and 3 the western nets. Two nets were placed at each location; one net was submerged at a depth of 2 m, the other at 6 m.

An image analysis software (ImageJ Fiji), scripted with a customised module, was used to validate the mortality counts, which had originally been counted by hand. Splitting the shells collected during 2018 the observation period at the hinge and aligning the valves systematically on lightbox (Royal Sovereign Hancocks), enabled the mounted camera (Nikon J5 Model) to capture the valves from a high angle, providing images with sufficient resolution for the image analysis software.

Statistical Analysis

For the statistical analysis, a one-way analysis of variance (ANOVA) was conducted in Minitab 18 to compare the overall mortality rates between the two sites. Subsequently, within the affected site, one-way ANOVAs were performed to compare mortalities across each line for every time point, enabling a detailed examination of mortality trends. General linear models (GLM) were then employed to assess the total mortalities (sum of all lines, per time point) across the different time points, facilitating an understanding of temporal variations in mortality patterns. Additionally, comparisons were made between mortality rates among the lines within each time point, enhancing the granularity of the analysis and providing insights into localised mortality dynamics. P-values were considered significant when $p < 0.05$. The raw and transformed data tables for statistical analysis and for the graphs, can be found in Appendices 2.1 and 2.2.

2.3 Results

Interview findings and farmers perspectives

According to the mussel farmers, Loch Eil has water turnover time of approximately six weeks. Mussel growth patterns vary depending on their attachment method and mussels grown on pegged ropes tend to form clumps during thinning operations, while those grown on nets exhibited a more widespread attachment. First gonadal maturation in the mussels typically occurs in the first spring following settlement.

They reported that significant spat mortality events in Loch Eil began in the autumn of 2010. Prior to this, fish farming operations in the area ceased in 2005. Interestingly, the farmers observed that spat mortality was most pronounced at the site located nearest to the residential area. Further observations revealed that similar mortality events occurred in Loch Linnhe in 2011, and subsequently in both lochs annually since then.

i. ***Observed trends***

Their observations revealed several key trends. Mussel growth was observed to peak by the end of August, with spat settling directly on ropes exhibiting higher survival rates than those settling on top of adult mussels. Survival rates were highest immediately beneath the water surface and decreased with increasing depth. Despite these mortalities, no significant impact on the growth of surviving mussels was observed, and flesh quality remained unchanged. No known physiological changes were detected in the mussels around September/October. Spat settlement was observed to be very good on the collection ropes. Mussels typically reached approximately 20 mm in growth before significant mortality events began. Additionally, farmers observed an increase in barnacle overgrowth displacing dead mussels, which were described as quite smelly and friable, potentially indicating an ecological shift. An increase in starfish populations was observed, particularly at the tip of Loch Eil. Concerns were raised regarding elevated *E. coli* levels, potentially linked to increased runoff from new residential developments with large septic tanks. The arrival of skeleton shrimp (*Caprellidae*) before the onset of significant mortality events was also noted. Furthermore, an

increase in sea squirts (*Didemnum vexillum)* was observed, while no significant changes in algal populations or the presence of tubeworms.

ii. ***Factors hypothesised to contribute to spat mortality***

Through discussions with the mussel farmers, a range of potential factors contributing to the observed spat mortalities were considered. These included potential impacts from increased forestry activity, leading to increased runoff and potential pH shifts in the loch. Concerns were raised regarding the potential impact of increased nutrient runoff from new residential developments and associated septic tank systems, potentially leading to elevated *E. coli* levels. Other potential factors discussed included predation by eider ducks and starfish, and the potential impact of nanoparticles, although specific sources and impacts were not clearly identified.

iii. ***Farmers initiatives***

Farmers have undertaken several initiatives to investigate the causes of spat mortality. These efforts included multiple attempts to introduce spat from different sources to the site, all of which resulted in high mortality rates. In contrast, spat transferred from Loch Eil to Loch Sunart exhibited high survival rates, suggesting a site-specific issue within Loch Eil. Regular monitoring for harmful algal blooms (HABs) has been conducted, although farmers expressed concerns about the adequacy of the current screening methods, which primarily involve superficial sampling with a bucket near the residential area. While plankton samples are regularly checked for feed availability, no significant changes have been observed, except for a particularly poor year for mussel growth in 2017.

The mussel spat was observed over a 3 – month period and the cumulative percentage of mortalities from each time point is presented in Figure 2.4. The graph shows a difference in overall mortality between the two sites ($p =< 0.001$). Loch Sunart, the control site (orange), showed a consistently low mortality rate with a maximum of 0.9% by the end of the experiment. Loch Eil, however, appeared to exhibit a significantly higher mortality rate reaching 68.3% over the 3 months. Additionally, substantial differences were observed comparing the number of mortalities over each time point (T1 $p =< 0.001$; T2 $p =< 0.001$; T3 $p = 0.001$ and T4 $p = 0.007$). The plot shows a climb in the number of dead spat

reaching 25.2 % two weeks post stocking the nets. During the following fortnight, the mortality rate continues to rise reaching 57.9 % by time point 2. In the weeks thereafter, less dead spat was counted at T3 and T4 compared to the previous time points, yet the overall mortalities remained in an upward trend until the end of the experiment. The dead spat from each net were counted at every time point and the numbers were plotted in form of heat maps as shown in Figure 2.5. The quadrants are meant to represent the placement of lantern along nets along the lines within the mussel farm in Loch Eil. The numbers above the quadrants (1 – 4) refer to the time points, and the tiles within each of the quadrants represents the lantern nets (1 – 5) placed along the lines (A – D). This set up and visualisation of the mortality numbers assisted in attempting to identify pattern or a trajectory as to where the mortalities originate, however no obvious pattern was perceived after scrutinising the heatmaps.

In the first quadrant, nets D2, C3 and C4 (the darkest tiles) appeared to exhibit the highest number of mortalities during the first time point, each exceeding 450 deaths per net, 48.7 %, 32.5 and 41.8 % to be more precise. In quadrant 2, nets A2, A4 and D5 display the highest counts, adding to figures greater than 450 dead individuals (35.1 %, 32.4 % and 36.48 % respectively). As of time point 3, six weeks post stocking, the mortalities started to decrease, whereby the highest number of dead spat tallied to 157 (A3). Mortalities still occurred during time point 4, however considerably less dead spat were counted compared to earlier time points.

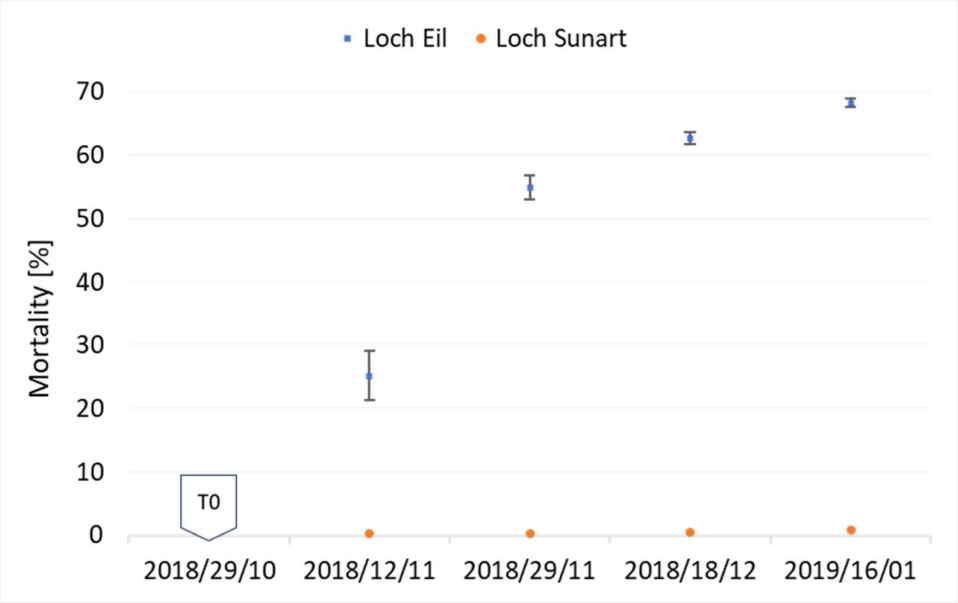

Figure 2.4: **Spat mortality during the 2018/19 observation period.** Spat mortality rates in the affected site Loch Eil (blue) and control site Loch Sunart (orange) over the 3-month observation period are compared. The graph shows the cumulative mortality rates of mussel spat over the observation period from time point 0 (T0 – 29/10/18) to time point 4 (T4 – 16/01/2019). Dead individuals were counted at each time point apart from T0. Samples collected for analysis ($n = 18$ per net, ergo $n = 360$ per time point) were subtracted from the cumulative mortality count. The total percentage was calculated based on the stocking densities at T0, which were determined during T4, after counting surviving spat. The error bars represent standard errors of the mean based on the mortality percentages at each timepoint. Individual variability between locations and nets is not depicted.

Anticipating observing a similar event the following year, there was an exceptionally large number of dead spat 3 weeks into the observation, in both Loch Eil and Loch Sunart (Figures 2.6 and 2.7). Approximately six weeks later, all lantern nets within Loch Eil were removed due to 100% mortality of the spat (Image of dead spat T2 LE 100%), which is why six additional nets were placed into the farm in Loch Sunart. By time point 3 (76 days post stocking) in Loch Sunart, the majority of the spat were found to be dead as well. However, the mortality observations from 2019/20 were deemed inconclusive as the majority of the lantern nets were infested with starfish, which most certainly would have had an impact on spat survival. Since the second mortality observation did not fulfil as a replicable event to the first experiment, the spat mortality data from 2019/20 was not viable and therefore not included in the results.

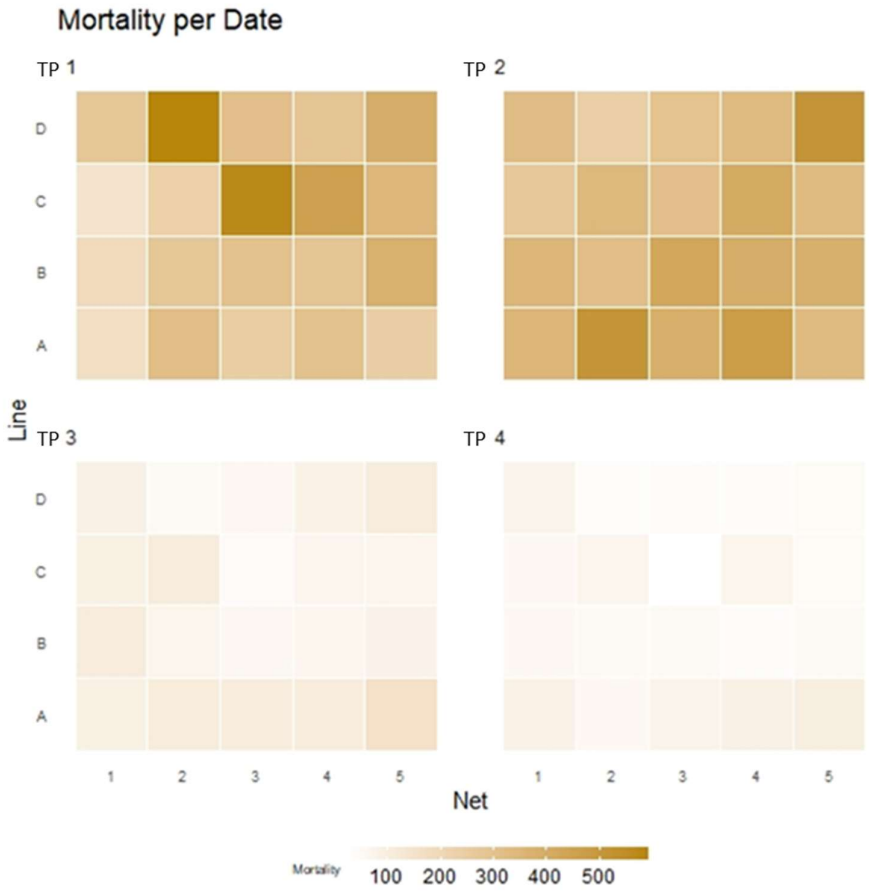

Figure 2.5: **Overview of mortality counts for each net at each time point in Loch Eil.** Each quadrant is labelled (1 – 4) and represents a time point. The columns labelled 1 – 5 represent the nets, and the rows A – D the lines. The colour intensity of the individual indicates the number of dead spat within the net tiles (least intense < 100 mortalities, most intense > 500 mortalities). Dead individuals were counted at each time point, and the samples collected for analysis were subtracted from the mortality count ($n = 18$ per net, ergo $n = 360$ per time point).

Figure 2.6: **Mass mortality in Loch Eil 2019/16/10**. The image shows the immense number of dead spat on the middle layers of the lantern nets within the first 3 weeks post stocking, in Loch Eil. Open valves indicate the dead animals.

Figure 2.7: **Mass mortality in Loch Sunart 2019/25/11**. The image shows the immense number of dead spat on the middle layers of the lantern nets within the first the first 2 months post stocking, in Loch Sunart. Open valves indicate the dead animals. The last image shows only one of multiple bags of collected dead spat.

2.4 Discussion and conclusions

The investigation into mortality dynamics of mussel spat Loch Eil delivered an understanding of what had previously been witnessed by the local farmers in the shellfish industry. Through diligent observational studies conducted over the 2018 and 2019 mortality events, this research and its experimental design aimed to quantify and understand patterns of mortality, thereby augmenting existing farmer observations with empirical data. The experimental setup, involving the strategic placement of lantern nets along grow-out lines in Loch Eil and the use of Loch Sunart as a control site, facilitated the comparison of mortality rates between sites and over time. Notably, the lantern nets served as a protective barrier against predators, ensuring that observed mortalities were primarily attributed to environmental factors, compromised water quality or the presence of pathogens rather than predation.

The results in this current study revealed significant differences in mortality rates between the two lochs, with Loch Eil exhibiting substantially higher mortality rates reaching up to 68.3 % throughout the observation period, whereas the mortality within Loch Sunart did not exceed 0.9 %. This variation emphasises the necessity of understanding the influence of environmental conditions and site-specific factors on spat development and survival (Stirling and Okumuş, 1994)

Moreover, the temporal variation in mortality rates within Loch Eil highlighted the dynamic nature of mortality events, with peaks observed at specific time points. The highest mortality rate occurred within the first two time points (Nov 12th and Nov 29th) of the observation in 2018, indicating that the experiment was set up too late to discover the origin and the actual beginning of the “mortality window” mentioned by Charles et al. (2020). However, the temporal variation could also be contributed to seasonal fluctuations and changes in environmental conditions (Capelle et al., 2021). Recognising that Loch Sunart is a different body of water, the fact that the two lochs are in geographic proximation to one another, and a high change the spat are of similar genetic stock, the spat in Loch Sunart would have also been subjected to similar factors, and yet the spat there appeared to be unaffected.

Furthermore, previous mass mortality events in European mussel populations, such as those observed in the Netherlands (Capelle et al., 2021) and France

(Charles et al., 2020), have highlighted the vulnerability of these bivalves to a complex interplay of environmental stressors and biological factors. These events often lack a single, clear cause, with researchers pointing to a combination of factors, such as high spawning activity, algal blooms, nutrient availability and changes in temperature and salinity. Moreover, significant research by Benabdelmouna et al. (2018), Fuentes et al. (1992) and Tremblay et al. (1998), among others, found that genetic factors, particularly the origin of mussel stocks, can significantly influence mortality rates.

Given the observed increase in spat mortality rates in Loch Eil and the potential for similar events to occur, a thorough understanding of the environmental conditions within the loch is crucial. This includes analysing factors such as water temperature, salinity, nutrient availability, and an understanding of the difference in hydrography of the lochs, which can influence the distribution and abundance of phytoplankton, a key food source for mussel larvae. Additionally, understanding the impact of weather patterns, including wind and precipitation events, which can influence water stratification and create more estuary-like conditions within the loch (CEFAS, 2014; Gillibrand et al., 1995) is essential for assessing the potential for environmental stressors to contribute to observed spat mortality rates.

This led to the research question that compared environmental parameters between the two lochs and is discussed in Chapter 2.

The analysis of mortality patterns, including the examination of mortalities across different lines and time points, suggests the complex and multifaceted nature of mortality events, despite the absence of clear spatial patterns in mortality distribution. The assumption of a myriad of environmental factors influencing the overall health of the affected spat is supported by several studies having conducted similar research in adult and juvenile bivalves (Bayne, 1965; Dailianis, 2010; Rayssac et al., 2010; Maar et al., 2015). Understanding the environmental conditions within each loch is crucial for assessing their potential influence on mussel health and survival. Variations in precipitation and temperature patterns between Loch Eil and Loch Sunart have implications for nutrient input and overall ecosystem dynamics. Monthly rainfall patterns exhibit seasonal variability, with higher averages observed during winter months and lower averages in summer. These climatic variations can significantly influence nutrient runoff, water

temperature, and overall water quality, all of which can impact mussel growth, survival, and reproduction.

Furthermore, the experiment was repeated the following year with the intent of attempting to witness the actual start of the “mortality window”, whilst gathering environmental data crucial for the environmental chapter. Therefore, the experiment was brought forward by a month.

The attempt to replicate the observation trial in 2019/20 was unsuccessful in terms of identifying the beginning of the mortality event, as the mortality within Loch Eil reached 100% within six weeks. Attempting to regroup and gather some data, additional lantern nets separately stocked with spat populations from Loch Eil and Loch Sunart, were placed into Loch Sunart to study the survival rate of the spat within the control site. Surprisingly, the majority of both spat populations within the nets in Loch Sunart were found dead by the following time point, most likely attributed to environmental stressors and infestation of starfish larvae, rendering the results for the 2019/20 observation inconclusive.

Upon consulting with the mussel farmers, the early onset and severity of the mortality could have also been very likely to be caused by sheer stress. The mussel farmers had stripped the spat from the collection ropes later in the season than usual, which would have already compromised the stress levels of the spat. The fact that there was only a two – week gap between the stripping of the spat and the start of the 2019 experiment and transferring the animals into the nets into different environments, could be a plausible explanation for the outcome of the event (Capelle et al., 2021).

In conclusion, the observational studies presented in this chapter contribute to our understanding of mortality dynamics in mussel spat and lay the groundwork for further investigations into the complex interactions between environmental factors, site-specific conditions, and spat survival. Moreover, the collection of samples for additional analyses highlights the interdisciplinary nature of aquaculture research, especially regarding the search of an unknown cause of mortality, thus setting the scene for comprehensive and integrative approaches to address the challenges in the investigation at hand.

2.5 Appendix

Appendix 2.1: **Mortality data during the 2018/19 observation period.** Lantern nets were stocked with spat and placed along the mussel lines within the farm sites of Loch Eil and Loch Sunart, which were then observed over a 3 – month period. Dead spat was counted and logged during each time point (T1 – T4). The data is presented as total counts, percentage, and cumulative percentage over the observation period. The percentage was calculated by dividing the total count over the stocking density, multiplied by factor 100. The cumulative data took the collected samples for analyses ($n = 18$ per net) into account. The total below the individual lines (A – D for Loch Eil, and only line A for Loch Sunart) represent the sum of the respective columns for the indicated line. The overall total represents the grand total at each time point, summing up the data within the respective columns. After T1, additional lantern nets stocked with spat were placed along line A in Loch Sunart at a shallower depth of 1 metre, which explains the lack of count and % during T1.

					T1		**T2**			**T3**			**T4**		
					12/11/2018		**29/11/2018**			**12/12/2018**			**16/01/2019**		
	Line	Net	Depth [m]	Stocked	Count	%	Count	%	Cumulative percentage	Count	%	Cumulative percentage	Count	%	Cumulative percentage
Loch Eil	A	1	4	1220	167	13.7	350	29.1	43.0	87	7.3	51.0	83	7.0	58.9
		2	4	1473	320	21.7	511	35.1	57.1	110	7.7	65.5	56	3.9	70.3
		3	4	1403	246	17.5	388	28.0	45.8	105	7.7	54.1	72	5.3	60.1
		4	4	1467	302	20.6	469	32.4	53.2	103	7.2	61.1	85	5.9	67.9
		5	4	1406	243	17.3	327	23.6	41.1	157	11.5	53.1	97	7.1	60.9
			Total	6969	1278	25.6	2045	40.9	48.3	562	8.2	57.2	393	5.7	63.9
	B	1	4	1155	186	16.1	350	30.8	47.1	107	9.6	57.5	56	5.0	63.5
		2	4	1062	278	26.2	314	30.1	56.7	71	6.9	64.6	52	5.1	70.9
		3	4	1219	302	24.8	423	35.2	60.4	59	5.0	66.3	51	4.3	71.7
		4	4	1181	285	24.1	394	33.9	58.4	64	5.6	64.9	36	3.1	69.1
		5	4	1292	380	29.4	383	30.1	59.9	76	6.1	66.8	48	3.8	71.6

Appendix 2.1 continued

					T1		T2			T3			T4		
					12/11/2018		29/11/2018			12/12/2018			16/01/2019		
	Line	Net	Depth [m]	Stocked	Count	%	Count	%	Cumulative percentage	Count	%	Cumulative percentage	Count	%	Cumulative percentage
Loch Eil	C	1	4	926	145	15.7	268	29.5	45.5	87	9.8	56.2	54	6.1	63.5
		2	4	1187	232	19.5	341	29.2	49.0	109	9.5	59.3	67	5.8	66.1
		3	4	1361	570	41.9	308	22.9	65.4	45	3.4	69.7	25	1.9	72.5
		4	4	1411	458	32.5	406	29.1	62.0	68	4.9	67.8	70	5.1	73.8
		5	4	1239	349	28.2	328	26.9	55.4	70	5.8	62.1	43	3.6	66.7
			Total	6124	1754	35.1	1651	33.0	56.4	379	6.3	63.6	259	4.3	69.1
	D	1	4	1132	277	24.5	321	28.8	53.7	85	7.8	62.3	72	6.6	70.0
		2	4	1212	590	48.7	239	20.0	69.4	46	3.9	74.4	32	2.7	78.3
		3	4	1115	308	27.6	297	27.1	55.2	58	5.4	61.4	36	3.3	65.9
		4	4	1189	285	24.0	332	28.4	52.7	80	6.9	60.5	39	3.4	64.8
		5	4	1416	392	27.7	510	36.5	64.5	110	8.0	73.3	44	3.2	77.5
			Total	6064	1852	37.0	1699	34.0	64.5	379	6.3	66.7	223	3.7	71.7

Appendix 2.1 continued

				T1 12/11/2018			T2 29/11/2018			T3 12/12/2018			T4 16/01/2019		
	Line	Net	Depth [m]	Stocked	Count	%	Count	%	Cumulative percentage	Count	%	Cumulative percentage	Count	%	Cumulative percentage
Loch Sunart	A	1	*1*	*1105*			4	0.4	0.4	3	0.3	0.7	2	0.2	0.9
			4	674	1	0.1	1	0.2	0.3	2	0.3	0.6	6	0.9	1.6
		2	*1*	*1264*			3	0.2	0.2	7	0.6	0.8	4	0.3	1.2
			4	786	3	0.4	0	0.0	0.4	12	1.6	2.0	8	1.0	3.1
		3	*1*	*1286*			3	0.2	0.2	1	0.1	0.3	7	0.6	0.9
			4	589	0	0.0	2	0.4	0.4	2	0.4	0.7	0	0.0	0.7
		4	*1*	*1262*			4	0.3	0.3	3	0.2	0.6	5	0.4	1.0
			4	734	3	0.4	1	0.1	0.6	0	0.0	0.6	5	0.7	1.3
		5	*1*	*1364*			0	0.0	0.0	0	0.0	0.0	6	0.4	0.5
			4	671	0	0.0	1	0.2	0.2	3	0.5	0.6	3	0.5	1.1
		Overall total		*9735* (3454)	7	0.2	19	0.2	0.3	33	0.0	0.5	46	0.5	0.9

Appendix 2.2: **Mortality observation 2018/19.** Lantern nets were stocked with spat and placed along lines (A – D) within the farm site in Loch Eil and observed over a 3-month period. Loch Sunart served as control site. Dead spat were counted and logged at each time point (TP), approximately every fortnight, during the observation period. One-way analysis of variance (ANOVA) was performed on the mortality data from Loch Eil, comparing mortalities across each line for each time point (in days [d] post stocking). General linear models (GLM) were used to investigate the effects of time point and line on total spat mortality within Loch Eil (Morts v TP). The GLM included both fixed factors (TP and line) and their interaction (TP V Line). An additional One-way ANOVA was conducted to compare overall mortality rates between Loch Eil and Loch Sunart. Transformed data are expressed as mean (± standard error of the mean). Tukey Method was used for post hoc examination. Different superscripts imply significant differences ($p < 0.05$).

					One-way ANOVA	GLM	
Line	A	B	C	D	Morts v Line	Morts v TP	TP v Line
Time point [d]							
14	0.44 (± 0.04)	0.51 (± 0.06)	0.55 (± 0.12)	0.58 (± 0.11)	0.11 ($F_{3, 16}$ = 2.4)	<0.001 ($F_{1, 8}$ = 248.48)	0.08 ($F_{3, 12}$ = 2.83)
30	0.57 (± 0.05)	0.60 (± 0.03)	0.55 (± 0.03)	0.55 (± 0.07)	0.35 ($F_{3, 16}$ = 1.17)	<0.001 ($F_{1, 8}$ = 249.79)	0.45 ($F_{3, 12}$ = 0.94)
44	0.08 (± 0.02)	0.06 (± 0.02)	0.06 (± 0.03)	0.06 (± 0.02)	0.46 ($F_{3, 16}$ = 0.9)	0.001 ($F_{1, 8}$ = 25.25)	0.38 ($F_{3, 12}$ = 1.12)
79	0.24 (± 0.03)	0.20 (± 0.02)	0.21 (± 0.05)	0.19 (± 0.04)	0.18 ($F_{3, 16}$ = 1.83)	0.007 ($F_{1, 8}$ = 12.8)	0.13 ($F_{3, 12}$ = 2.3)
			Loch Eil	Loch Sunart	One way-ANOVA		
			0.94 (± 0.06) ^{B}	0.11 (± 0.03) ^{A}	<0.001 ($F_{1, 28}$ = 1936)		

Chapter 3

Observing the environmental parameters of Loch Eil and Loch Sunart - Assessing the effects of temperature, salinity, heavy metals and food availability on the spat of Scottish farmed blue mussels (*Mytilus edulis*)

Abstract

This study investigated environmental factors influencing spat mortality in Scottish farmed blue mussels (*Mytilus edulis*) within Loch Eil and Loch Sunart. Key findings include: 1) Loch Eil exhibited greater salinity variability, potentially impacting spat physiology. 2) Higher lead concentrations were observed in mussels from Loch Sunart, although not likely the primary driver of observed mortality events in Loch Eil. 3) Loch Eil showed lower concentrations of essential elements (e.g., phosphorus, calcium). 4) Loch Eil displayed higher chlorophyll-a levels, indicating sufficient food availability. While these factors could potentially impact spat survival, the absence of observed mortality in Loch Sunart suggests that other factors, such as salinity fluctuations, nutrient availability, and potential metal contamination, likely interacting in complex ways, are primarily responsible for spat mortality events in Loch Eil. The findings underscore the importance of considering local environmental dynamics in assessing spat mortality risk and inform future management strategies for sustainable mussel aquaculture practices in Scottish coastal waters.

Keywords: spat mortality, *Mytilus edulis*, environmental parameters, Loch Eil, Loch Sunart, temperature, salinity, heavy metals, food availability

3.1 Introduction

The blue mussel (*Mytilus edulis*) is an adaptable species found inhabiting most coastal habitats in the northern hemisphere, ranging in various environmental conditions (Tillin & Mainwaring, 2024; Dales, 1979).

Scotland's waters provide a combination of environmental factors that form suitable conditions for different stages of their lifecycle. Estuaries, inlets and sheltered bays offer the most protection from adverse weather conditions but also provide good quality, nutrient rich and well exchanged sea water promoting healthy growth and reproduction (Connor et al., 2004).

Mussels feed by filtering water through their gills, ingesting plankton and detritus to obtain essential nutrients that are vital for their development. The growth and development of these bivalves is influenced by environmental conditions such as temperature, salinity, food availability and the water movement of the area (Maar et al., 2015). As much as 99% of mortality in larvae has been linked to the insufficiency of food availability in addition to being subjected to predation and environmental stressors (Dailianis, 2010).

Due to their feeding method, these organisms are often used as a reliable tool for observing aquatic environment, thus aiding in the assessment of the overall water quality and environmental health status (Dailianis, 2010). Consequently, during feeding, bivalves tend to take up any particles suspended within the environment, which include any non-nutritious organic or inorganic particles and potentially toxic elements (PTEs), as well as heavy metals. These particles tend to bioaccumulate within the various tissue types of the organism over time (Streit, 1998; Rodney et al., 2007).

Factors such as the metabolic rate of the mussels, concentration of the elements within the water as well as the subjected duration of exposure, can affect the uptake of material, thereby also influencing the concentration of accumulated particles within the organism. Furthermore, salinity and temperature play an important role regarding water chemistry, thus adding additional influential components on the subject of bioaccumulation (Mubiana & Blust, 2007; Baines & Fisher, 2008; Deruytter et al., 2015).

Chlorophyll-a (Chl-a) concentrations are indicative of primary production and food availability. Primary production occurs within the photic zone (Solórzano & Grantham, 1975) of the water column and is generally driven by phytoplankton, producing nutrients for aquatic organisms. Among other primary producers, phytoplankton acts as the main food source for most shellfish species (Page & Hubbard, 1987; Wong & Levinton, 2004). Within sea lochs, the phytoplankton production is mainly controlled by light, micronutrient supply and the stability of the water column (Malone et al., 2016), which occurs with freshwater input into the loch (Grantham, 1981). This creates a density stratification which reduces the mixing of surface waters with the deeper water (Solórzano & Grantham, 1975; Edwards et al., 1980; Grantham, 1981), allowing the phytoplankton to remain near the surface where light is most abundant. Generally, in order to replenish nutrients necessary for primary production, an interchange of stabilities generates mixing among the water columns, transporting nutrients back to the surface layers. In Loch Eil however, its atypical hydrography causes a deceleration in change of salinity within the surface waters, compared to other sea lochs. A rough estimate of seven days during some cycles had been suggested by Edwards et al., (1980) for the water columns in Loch Eil to properly mix (Pearson, 1971; Edwards et al., 1980; Grantham, 1981).

Particulate matter is described as particles that derive from detritus, living organisms and soil. It comprises both organic and inorganic material, but the main defining factor is that these particles are too small to naturally sink and therefore remain within suspended within the water column, yet they are also too large to dissolve to a homogenous solution. The colour and turbidity of water is largely due to the suspended particulate matter, which can have a negative impact on the aquatic environment as it inhibits light penetration which is necessary for the growth of fauna in aquatic ecosystems (Boyd, 2000).

Particulate organic matter (POM) is defined by organic matter which is made up of detritus, microbial residues as well as phytoplankton and zooplankton remains that are not bound to any minerals (Winogradow et al., 2019; Leuthold et al., 2023).

Although mussels, particularly *M. edulis*, can tolerate a wide range of temperatures (thermal tolerance window: -1 to 28°C) (Boroda et al., 2020), they exhibit optimal growth and reproduction within specific temperature ranges. Adult

M. edulis demonstrate highest growth rates between 10°C and 15°C (Westerbom et al., 2002; Rayssac et al., 2010; Boroda et al., 2020; Alter et al., 2024), while larvae exhibit optimal development at 17°C (Bayne, 1965; Rayssac et al., 2010). Larval development slows above 18°C and ceases above 25°C (Bayne, 1976; Rayssac et al., 2010).

The presence of *M. galloprovincilais* and *M. trossulus*, along with the mytilid complex hybrids, can influence the community's overall thermal response. *M. galloprovincilais* exhibits a thermal tolerance window of 10 to 32°C (Somero, 2011; Boroda et al., 2020) and optimal larval development at 20-25°C (Lazo & Pita, 2012), potentially expanding the community's temperature range. *M. trossulus*, originating in the North Pacific, is highly cold-tolerant (thermal tolerance window: -1 to 28°C; (Hofmann & Somero, 1995; Braby & Somero, 2006; Boroda et al., 2020) with optimal larval development at 10-20°C (Rayssac et al., 2010). Hybridization can further complicate these responses, potentially altering the thermal tolerances of offspring.

Temperature significantly influences the life cycle of mussels, particularly *M. edulis*. Low temperatures inhibit egg development, while rapid temperature changes can be lethal, especially for larvae (Bayne, 1976; Gleason et al., 2018; Boroda et al., 2020). Adult mussels exhibit greater cold tolerance than juveniles (Bourget, 1983) Research by Myint and Tyler (1982), found that in *M. edulis* from South Wales, oogenesis commences only above 18°C, thus highlighting the importance of temperature for reproductive success (Boroda et al., 2020). These findings emphasise the vulnerability of mussel populations to climate change, as fluctuating temperatures can disrupt their reproductive cycles and impact their survival (Thompson, 1984; Mičić et al., 2001; Sokolova et al., 2004; Kefaloyianni et al., 2005).

When the water temperatures rise, the metabolic rate is increased, whereby the energy demand for processes such as growth and reproduction become greater. Additionally, the temperature of the water plays a vital role in the reproductive timing of *M edulis*. Increased water temperatures can lead to an acceleration of reproductive events as the warmer water may be indicatory of the spawning seasons such as spring or late summer (Tillin and Mainwaring, 2016). Lastly,

extreme temperatures, especially heatwaves, can lead to heat stress in mussels. Additionally, the combination of increasing temperature and its effects on phytoplankton populations can result in inadequate supply of food and nutrients for juvenile spat, ultimately leading to mortalities as also it affects the physiological functions of the animal (Incze et al., 1980; Tillin and Mainwaring, 2016).

Apart from temperature, *Mytilus* spp. Are also known to endure a wide range of salinities within their habitat.

The three blue mussel species (*M. trossulus*, *M. edulis*, and *M. galloprovincialis*) exhibit varying salinity tolerances. *M. trossulus*, originating in the North Pacific, is likely most tolerant of low salinity conditions, while *M. galloprovincialis*, from the Mediterranean, is expected to be more tolerant of higher salinities McDonald & Koehn, 1988; McDonald et al., 1991; Seed, 1992; Seed & Suchanek, 1992; Hilbish et al., 2000; Braby & Somero, 2006).

Settling in areas such as intertidal areas and estuaries, the salinity tolerance range for *M. edulis* is from 10 – 35 parts per thousand (ppt), whereas in more sublittoral environments, where the salinity is more stable, their tolerance can range from 30 – 35 ppt (Connor et al., 2004; Landes et al., 2015).

Research in Denmark has observed the native population and concluded that the growth rates varied with fluctuating salinity, where the mussels exposed to 'average' salinities (25.7 – 29.5 ppt) appeared to show better growth rates as opposed to those exposed to an unstable salinity over time which averaged to 20.5 ppt (Landes et al., 2015). The ability of *Mytilus* species to cope with varying salinities is a product of speciation, where geographic isolation and subsequent adaptation to different salinity environments have resulted in distinct physiological and genetic traits (Wenne et al., 2022).

Several studies observed reduced growth rates of *M. edulis* in at lower salinities, most likely as a result of physiological stress (Westerbom et al., 2002; Wing & Leichter, 2011; Maar et al., 2015). Additionally, other research has suggested that mussels exposed to salinities below 8 ppt not only impacts their development, generally resulting in stunted or dwarfed shape, but could also lead to mortality (Kautsky, 1982; Vuorinen et al., 2002; Hawkins et al., 2013; Maar et al., 2015).

According to Grantham (1981), Loch Eil takes in only minimal volumes of freshwater from the surrounding rivers and burns, yet the largest amounts derive from sources within the adjacent Loch Linnhe, entering Loch Eil via flood tides through The Narrows (Johnston & Topping, 1972). The inflow of freshwater results in a partly distributed brackish layer, mainly at a depth between 10 -15 metres. Additionally, as opposed to exhibiting a density stratification within the loch from the incoming freshwater, the strong tidal movements from the eastern end result in a pycnocline that is rather mixed. However, during the rainy season (mostly over the winter months) freshwater input is increased, creating a more stable water column, where further mixing through other external factors such as wind is limited to the surface layer of the loch.

The surrounding area of Loch Eil is characterised by a diverse landscape, featuring residential areas such as Fassfern, Corribeg and Kinlocheil on one side, and predominantly crofting communities on the other, providing a blend of habitation and traditional agricultural practices that could potentially introduce harmful substances and potentially toxic elements into the aquatic environment (Baxter et al., 2011; Griffith, 2017).

The presence of elements such as cadmium, vanadium, chromium, cobalt, nickel, copper, zinc and selenium within the loch can be attributed to industrial discharges, processes and other industrial activities (Renault, 2015) such as metal plating (Artiola et al., 2019). Arsenic is an element that is very abundant within the earth's crust, creating a natural source within the aquatic environment (Rodney et al., 2007). However, arsenic can also stem from anthropogenic sources such as sewage treatment facilities and agricultural run-off (Rodney et al., 2007), but also from industrial processes such as smelting, and has also been found in some wood-preservatives according to (Eisler, 1988).

Sodium, magnesium, copper, zinc, and calcium are essential for mussel development (Wang et al., 2009; McDougall et al., 2022) and are naturally present in Loch Eil. Calcium, the most critical element (Gosling, 2003d; Yarra et al., 2021), is primarily used for building the strong, protective shells. Calcium deficiency can lead to weak shells, making the mussels more vulnerable to predators and environmental stressors. Magnesium (Weiss et al., 2002; Huang & Zhang, 2022) also contributes to the formation and maintenance of the shell.

Sodium (Lin, Yeh & Lee, 2016; Zhao et al., 2020) is essential for maintaining proper osmotic balance within the mussel's body and helps regulate fluid and electrolyte levels. Copper and zinc (Zhao et al., 2020) are biologically essential trace elements that act as cofactors for various enzymes involved in vital metabolic processes, including growth and development.

Copper specifically is an essential micronutrient which is readily abundant in our environment and plays a role in the metabolism and growth of not only aquatic life forms, but all living organisms (Schroeder et al., 1966; Carbonell and Tarazona, 1994; Nordberg et al., 2007; Rodney et al., 2007). Excessively elevated levels of these elements potentially derive from road salt run-off (Granato, Church & Stone, 1995; Corsi et al., 2010; Hintz & Relyea, 2017; Bogart, Azizishirazi & Pyle, 2019), natural weathering processes (Dixon-Anderson & Science, 2021), and also agricultural run-off (Dickerson, Hubert & Bergman, 1996; Bogart, Azizishirazi and Pyle, 2019; Oduor, Cristina and Costa, 2023). In overly high concentrations, even the essential elements such as copper and zinc can become problematic for bivalves (Deruytter et al., 2015) especially in the early developing stages, including the trochophore, veliger, and pediveliger larvae (Bayne, 1964, 1965). These early life stages are particularly vulnerable (McDougall et al., 2019; McDougall et al., 2022) due to rapid growth, development of physiological systems, limited detoxification mechanisms, and a high surface area-to-volume ratio (Hall, Moffett & Gracey, 2020). Table 3.1 outlines key findings from the literature on the effects of PTEs on *Mytilus* spp., highlighting the sensitivity of early life stages to various metals, including copper, cadmium, zinc, mercury, and lead, and outlining key findings such as EC50 values and observed impacts on their development (Tyler-Walters et al., 2022).

According to a selective review of literature on the effect copper on a range of wildlife including mammals, birds, fish and terrestrial and aquatic invertebrates, the highest accumulation of the element was largely found in the tissues of bivalve molluscs, primarily in oysters and cephalopods (Eisler, 1998; Rodney et al., 2007). Research by (Nadella et al., 2009a) studied the toxicity of cadmium, copper, nickel and zinc on what has been described as “the most sensitive life-stage of *M. trossolus*”, presumably the planktonic larval stage, and has found that out of the four mentioned elements, copper has shown to be the most toxic. This observation was also confirmed by (Schroeder et al., 1966; Betzer & Yevich,

1975), however, the total concentration and the speciation of the element are major factors that determine the bioavailability and the toxicity to aquatic life forms (Hung, Meng & Chuang, 1992).

The elements lead and vanadium found within the loch could, like the majority of these potentially toxic elements, be a result of urban runoff, industrial discharge but can also arise from fossil fuel combustion and the use of leaded petrol (Rodney et al., 2007; Milik & Pasela, 2018). The fossil fuel combustion theory makes sense in the fact that Loch Eil is also surrounded by paved roads for residential, agriculture, industrial and recreational traffic.

Several studies stated that even low concentrations of potentially toxic elements (PTEs) in seawater have caused metal toxicity resulting in larval mortalities in green-lipped mussels (*Perna canaliculus*), a bivalve mollusc endemic to New Zealand (McDougall et al., 2019; McDougall, Kihara, et al., 2020; McDougall, Vignier, et al., 2020; McDougall et al., 2022). However, the study also suggests that the *Perna* larvae exhibit a higher vulnerability to the low concentrations of PTEs compared with larvae of the *Mytilus galloprovincialis.* (Purbonegoro & Hindarti, 2019; Zitoun et al., 2019; Cledon et al., 2021). Due to their tolerance not only to a wide range of salinity and temperature, but also to increased concentrations of a broad spectrum of heavy metals, *Mytilus spp.* are commonly used for ecotoxicology assays (Zitoun et al., 2019; McDougall et al., 2022). However, elevated concentrations of certain trace elements, particularly heavy metals such as arsenic and cadmium, can pose toxicity risks to aquatic organisms including juvenile bivalves & (Wang et al., 2009; Moreira et al., 2018; McDougall et al., 2022).

According to research, cadmium tends to be found in low concentrations in aquatic systems, where the concentration in sea water is seemingly lower (0.02 $\mu g\ L^{-1}$) than the total dissolved cadmium measured in freshwater (0.5 $\mu g\ L^{-1}$) (Pan et al., 2010; McGeer et al., 2011). However, despite the low concentrations, the element has been found in tissues of marine and estuarine fin- and shellfish (Rodney et al., 2007). The exposure to elevated (non-natural) concentrations of arsenic and cadmium to the aquatic environment is incredibly dependent on the speciation of the element, the form and its valence state (Rodney et al., 2007; McGeer et al., 2011). Research by Vallee et al. (1960) and Penrose & Woolson (1974) suggest that inorganic forms of arsenic are considered to be more toxic

than the organic forms of the element, and trivalent arsenic compounds more toxic than pentavalent arsenic compounds (Rodney et al., 2007). An assessment by the Scottish government assigned Environmental Quality Standards (EQS) for the trace metals cadmium, copper, lead, zinc and mercury in marine waters (Table 3.3). Additionally, they have provided trace-element concentration guidelines within mussels, as per the international OSPAR assessment criteria (Table 3.4).

This chapter examines a number of environmental parameters that could potentially affect the environment in Loch Eil, with a focus on identifying factors that may trigger a negative response in mussel spat, ultimately leading to mortality. The parameters analysed include salinity and temperature as well as the water quality comprising of chlorophyll (Chl-a), particulate organic matter (POM), total particulate matter (TPM), heavy metal and potentially toxic elements (PTEs). The results will be compared between the locations (depicted in Figure 2.1 in Chapter 2) and previous research, aiming to establish any significant differences that could possibly lead to a clue as to what the mussel spat in Loch Eil are succumbing to.

Table 3.1: **Summary and key findings from literature** in (Tyler-Walters et al., 2022) on the effects of different PTEs on Mytilus spp. and development

Literature	**Research**	**PTE**	**Key Findings**
Balbi et al., (2018)	Cadmium effects on *Mytilus galloprovincialis* embryos and larvae.	Cadmium	Significant decrease in normal D-larvae; abnormalities in trochophore and pre-veliger stages.
Beiras and Albentosa, (2004)	Cadmium, mercury, zinc toxicity, and lead inhibitory effects on *Mytilus galloprovincialis* embryos.	Cadmium	48-hour EC50 of 1925 µg/L Cd; LOEC of 500 µg/L Cd; increased abnormal larvae.
		Mercury	48-hour EC50 of 2 µg/L Hg.
		Zinc	48-hour EC50 of 160-320 µg/L Zn; increased abnormal larvae.
		Lead	48-hour EC50 values: 221 µg/L Pb
Beiras and His, (1995)	Mercury toxicity on *Mytilus galloprovincialis* embryos and larvae.	Mercury	Embryos more sensitive than larvae; D-shaped larvae most sensitive stage.
Chalkiadaki, Dassenakis and Lydakis-Simantiris, (2014)	Nickel toxicity on *Mytilus galloprovincialis* adults.	Nickel	No mortalities observed at 20 mg/L Ni after 20 days.
DeForest and Schlekat, (2013)	Nickel toxicity on *Mytilus galloprovincialis* larvae.	Nickel	EC10 values between 228-350 µg/L Ni; no clear influence of DOC or salinity on toxicity.
Domouhtsidou & Dimitriadis (2000)	Long-term lead toxicity on *Mytilus galloprovincialis.*	Lead	48.5% mortality at 100 µg/L Pb during 98-day exposure.
Freitas et al., (2017)	Lead toxicity on *Mytilus galloprovincialis* at various temperatures and salinities.	Lead	No mortalities observed at 50 µg/L Pb for 28 days.
Hrs-Brenko, Claus and Bubic, (1977)	Lead toxicity on *Mytilus galloprovincialis* embryos at different salinities and temperatures.	Lead	Inhibited embryonic development with increasing lead concentrations; mortality increased after 96 hours of exposure.
Lussier et al., (1999)	Lead toxicity on *Mytilus* spp. larvae.	Lead	48-hour EC50 values: 476 µg/L Pb

Table 3.1 continued

Martin et al., (1981)	Toxicities of As, Cr, Hg, Pb to *Mytilus edulis* embryos, and Ni and Se toxicity on *Mytilus edulis* larvae	Arsenic	48-hour EC50 for abnormal development >3 mg/L As.
		Chromium	48-hour EC50 for abnormal development 4469 µg/L Cr.
		Mercury	48-hour EC50 of 5.8 µg/L Hg.
		Lead	48-hour EC50 values: 476 µg/L Pb
		Nickel	48-hour EC50 of 891 µg/L Ni.
		Selenium	48-hour EC50 for abnormal development >10 mg/L Se.
Micallef and Tyler, (1990)	Selenium toxicity on *Mytilus edulis* adults.	Selenium	No mortality at 50 µg/L Se; reduced filtration rates observed.
Miramand and Ünsal, (1978)	Vanadium toxicity on *Mytilus galloprovincialis*.	Vanadium	50% mortality within 9 days at 6500 µg/L V.
Morgan, Mitchell and Chapman, (1986)	Manganese toxicity on *Mytilus* larvae.	Manganese	100% mortality at 560 mg/L Mn; EC50 of 30 mg/L Mn; 100% abnormal development at 320 mg/L Mn.
Nadella et al., (2009b)	Zinc and nickel toxicity on *Mytilus trossulus* embryos and larvae.	Zinc	48-hour EC50 of 150 µg/L Zn; EC20 of 99 µg/L Zn; abnormal larval development.
		Nickel	48-hour EC50 of 150 µg/L Ni; EC20 of 82 µg/L Ni; abnormal larval development.
Pavičić et al., (1994)	Cadmium, zinc, and mercury toxicity on *Mytilus galloprovincialis* larvae.	Cadmium	Cadmium caused significant growth decreases at >2200 µg/L; increased abnormal veliger larvae.
		Zinc	Increased abnormal veliger larvae with increasing zinc concentrations; 48-hour EC50 of 145 µg/L Zn.
		Mercury	Mercury most toxic of the three metals tested (Cd, Zn, Hg); increased abnormal veliger larvae; 48-hour EC50 of 3.5 µg/L Hg.

Table 3.1 continued

Prato and Biandolino, (2007)	Cadmium and mercury toxicity on *Mytilus galloprovincialis* embryos.	Cadmium	48-hour EC50 of 21 µg/L Cd; LOEC of 6.25 µg/L Cd; significant effects on larval development at low concentrations.
		Mercury	Significant effects at lowest tested concentration (0.4 µg/L Hg); 48-hour EC50 of 1 µg/L Hg.
Strømgren, (1982)	Nickel effects on *Mytilus edulis* behaviour and growth.	Nickel	No significant effects observed at 200 µg/L Ni.
Talbot, Magee and Hussain, (1976)	Lead toxicity on *Mytilus edulis* adults.	Lead	Lethality dependent on exposure duration and concentration.
Vlahogianni and Valavanidis, (2007)	Short-term lead toxicity on *Mytilus galloprovincialis*.	Lead	24-hour LC50 of 4500 µg/L Pb; no mortality at 150 µg/L Pb for 10 days.
Yaroslavtseva and Sergeeva, (2007)	Copper toxicity on *Mytilus* embryo/larvae.	Copper	100% developmental abnormalities at 10 µg/L Cu after 48-hour exposure.

3.2 Materials and methods

Sample collection and preparation

From a designated point within the sites, temperature, salinity and water samples for mineral and trace element analysis were taken at 2 and 6 metres depth using a Van Dorn sampler and transported in 100 ml fix pots for further processing. Sampling dates were labelled as time points, with T0 marking the beginning and T4 as the end of the observation period (Table 3.2).

Table 3.2: **Dates of time points during the mortality events of 2018 – 19 and 2019 – 20.** The observation period was divided into time points, with T0 marking the start date when the nets were filled with spat and introduced to Loch Eil and Loch Sunart. Each subsequent sampling date was assigned a corresponding time point.

Year	**T0**	**T1**	**T2**	**T3**	**T4**
18/19	29.10.18	12.11.18	29.11.18	12.12.18	16.01.19
19/20	24.09.19	16.10.19	25.11.19	09.12.19	23.01.20

Water samples for all water quality analyses were collected from May 2019 until November 2019, from the same point within the sites at 1, 2, 4 and 6 metres depth. 1 litre plastic bottles were rinsed with water from the loch prior to filling with actual sample. The samples for chlorophyll a, total particulate matter (TPM), particulate organic matter (POM) were collected in triplicate from 2 and 6 metres and stored overnight at 4 °C.

Analysing Minerals and trace elements including heavy metals and TPM

The samples were analysed for total minerals and trace elements by inductively-coupled-plasma mass spectrometry (Thermo Scientific iCap RQ ICP-MS) to detect any potentially toxic substances or indicators thereof. The results were then compared between sites and to the legal limits, also referred to Environmental Quality Standards (EQS) in Scotland (Table 3.3) and the OSPAR assessment criteria in mussels (Table 3.4). The water samples were diluted to 1:5 with MilliQ water and acidified to 2% with nitric acid within 24 hours of collection prior to being analysed via ICP-MS. To determine whether any heavy metals have been taken up by the mussels and bioaccumulated within their

tissue, 45 adult (estimated age 1.5+ years) individuals were collected from Loch Eil and Loch Sunart for analysis. The tissue samples were removed from the mussels and prepared by the method described in (Sprague et al., 2020) prior to ICP-MS analysis. The ICP-MS instrument utilised these specific isotopes of cadmium (^{111}Cd), arsenic (^{75}As), lead (^{208}Pb), mercury (^{202}Hg), sodium (^{23}Na), Magnesium (^{24}Mg), phosphorus (^{31}P), potassium (^{39}K), calcium (^{44}Ca), vanadium (^{51}V), chromium (^{52}Cr), manganese (^{55}Mn), iron (^{56}Fe), cobalt (^{59}Co), nickel (^{60}Ni), copper (^{63}Cu), zinc (^{66}Zn) and selenium (^{78}Se). It then calculated the concentrations for the total element, based on expected isotope ratios.

Total particulate matter and particulate organic matter samples were all prepared using glass fibre filters (Whatman filters GF/F 0.45µm) and weighed following the method described in Hawkins et al., (2013) prior to the analysis.

Table 3.3: **Environmental Quality Standards (EQS) for dissolved trace metals in marine waters.** The EQS is set below the level of concentration upon which toxic traits are displayed in sensitive organisms, and therefore considered safe. Source: Water Research Centre, UK (Baxter et al., 2011)

	Annual average EQS µg L^{-1}
Cadmium (Cd)	0.2
Copper (Cu)	5
Lead (Pb)	7.2
Zinc (Zn)	40
Mercury (Hg)	0.05

Table 3.4: **OSPAR assessment criteria in mussels**. Values are presented as µg kg^{-1} dry weight. Concentrations below the Background Assessment Concentration (BAC) are considered to be low, concentrates greater than the BAC but below the Environmental Assessment Criteria (EAC) are of concern, and concentrations above the EAC are considered to be toxic and may result to harmful effects in aquatic life forms (Baxter et al., 2011).

	>BAC	BAC - EAC	<EAC
		µg kg^{-1} dry weight	
Cd	<1940	1940	5000
Pb	<1520	1520 - 7500	7500
Hg	<140	140 - 2500	2500

Water Quality

Using a filtering apparatus, the samples were filtered one litre at a time through the prepared glass fibre filters. The filters were rinsed with 10 ml of 0.5 M ammonium formate and distilled water, dried in the oven at 60°C for 48 hours, and incinerated in a muffle furnace at 450°C for 4 hours. Filters were weighed once after oven drying, and the Total Particulate Matter (TPM) and Particulate Organic Matter (POM) samples were re-weighed after combustion.

Chlorophyll a

Determining the content of chlorophyll a (Chl-a), which is the main type of chlorophyll found in green algae, and particulate organic matter (POM) helps analyse and quantify food availability within the water samples.

Samples were filtered one litre at a time through glass fibre filters (Whatman filters GF/F 0.45 µm) which were then folded in half, placed in aluminium foil and stored in a −20°C freezer overnight. The frozen samples were analysed by acetone extraction described in literature by (Golterman, R.S. Clymo and Ohnstad, 1978; Riemann and Ernst, 1982; HMSO, 1986). The absorption of the samples was read at 663 nm and 750 nm on the spectrophotometer (UVIKON 860, Kontron Instruments) after it had been calibrated with an acetone based blank and control sample.

Comparing environmental parameters in both lochs

Salinity (g kg^{-1}, ppt, ‰) and temperature (°C) were compared between both lochs with the purpose of identifying any severe variances between the environmental parameters; thus, potentially narrowing down the scope of the mortality investigation.

From May 2019 onwards, data loggers for salinity (HOBO U24-002-C) and temperature (HOBO UTBI-001) were calibrated to record measurements once an hour for the duration of the sampling period prior to being deployed in Loch Eil. The data from the loggers was extracted using specific attachments (HOBO U-DTW-1) and HOBOware software on a fortnightly basis, whereas the temperature and salinity in Loch Sunart were still measured manually by probe. The data loggers were transferred from Loch Eil to Loch Sunart as of November 2019. The

extracted data from the loggers was calibrated to in situ salinity and temperature values measured during pre-deployment and post retrieval. The information was then converted to desired units and processed into a useable format using the HOBOware software. The collected and structured data was compiled into graphs created in MS Excel.

Precipitation data was sourced from the World Weather for Water Data Service (W3S). Loch Eil and Loch Sunart were the selected regions and climate data (precipitation and max/min temperature) was provided for the years 2008 – 2019. Climate data past March 2023 were not available. The W3S application sourced the historical precipitation data from IMERG: Integrated Multi-satellite Retrievals for Global Precipitation Measurement (GPM), an algorithm which combines information from the GPM satellite constellation, whereas the historical temperature data were sourced from the Climate Prediction Center of Physical Sciences Laboratory (NOAA Physical Sciences Laboratory).

Statistical analyses

Statistical analyses were performed using Minitab 18. The data are presented as mean (± standard deviation, SD). The outliers were identified and trimmed via a Grubb's Test to ensure a consistent data set limiting errors (e.g. sample processing) that could impact the validity of the results. Analysis of variance (ANOVA) was used to determine any statistically significant difference of TPM, POM, Chl-a and potentially toxic elements (PTEs) between sites, or the different time points. A general linear model (GLM) was applied to the trace element data in mussel tissue to determine significance in site, timepoint and the interaction of the two, however, ANOVA was also used to examine any statistical significance between PTEs and site, and PTEs and time point. The Tukey Method was used for *post hoc* examination. In all analyses, $p < 0.05$ was considered significantly different.

3.3 Results

Heavy metals and Total particulate matter

Table 3.5 displays the results of elements detected in the water samples with statistical significances. Although samples were analysed for mercury (^{202}Hg), cadmium (^{111}Cd) and arsenic (^{75}As), the results for these elements did not show any statistically significant differences (Appendix 3.1). Table 3.6 displays the results for the selected elements detected in the mussel tissues of Loch Eil and showed statistically significant differences between the sites and time points.

Comparing the analysed elements from the populations of both sites, the mussel tissues of from Loch Sunart measured higher concentrations in contrast to those from Loch Eil, especially lead showed a significantly higher concentration ($p < 0.001$) than that of the samples from Loch Eil. Additionally, certain trace elements such as phosphorus ($p < 0.001$) and calcium ($p < 0.001$) have roughly a third the concentration within the tissues from Loch Eil as opposed to the control site.

Since mortalities have been believed to be more severe with increased depth, the heavy metal and trace element analysis on the water samples were performed on those collected from 1 and 4 metres in Loch Eil (Appendix 3.4). The concentration of trace elements in all water samples, apart from cadmium, were well below the environmental quality standard (EQS) set by SEPA (2022) for freshwater bodies in Scotland. When analysing the data over the sampling dates, lead ($p < 0.027$) and iron ($p < 0.03$) show significant differences in concentrations across the time points (Table 3.5). There were no significant differences observed between the concentration of the trace elements between the different depths.

Table 3.5: **Heavy metal analysis of Loch Eil water.** Water samples from 1 and 4 metres depth were collected from the same location within Loch Eil during each time point (n=3 per timepoint) over a 3 – month period. The samples were analysed for total minerals, trace elements and potentially toxic elements (PTEs) by inductively-coupled-plasma mass spectrometry (ICP-MS). LoD – limit of detection is the lowest detectable quantity from a blank with a specific a certain confidence level within the analysis. Statistical analyses were performed using Minitab 18. The data are presented as mean (± standard deviation, SD). Analysis of variance (ANOVA) was used to determine any statistically significant between the different time points and to examine any statistical significance between PTEs and site, and PTEs and time point. Tukey Method was used for post hoc examination. Different superscript letters imply significant differences ($p < 0.05$).

TP: Date	**Pb (ppb)**	**Fe (ppb)**
T0: 29.10.18	0.17 (± 0.07) ^{A,B}	14.12 (± 2.78) ^{A,B}
T1: 12.11.18	0.15 (± 0.04) ^{A,B}	30.01 (± 2.41) ^{A,B}
T2: 29.11.18	0.03 (± 0.01) ^{B}	36.51 (± 11.1) ^{A}
T3: 12.12.18	0.07 (± 0.06) ^{A,B}	10.18 (± 1.74) ^{B}
LoD	0.002	0.071
T4*: 16.01.19	0.25 (± 0.02) ^{A}	**
LoD*	0.005	**
[1]EQS	7.2	
ANOVA P-value	0.027 ($F_{4, 5}$= 7.06)	0.03 ($F_{3, 4}$= 9)

Samples were analysed on a different day, the LoD is the corresponding LoD for that analysis on the day

**No data, samples were not analysed for Fe due to separate run on ICP-MS

[1]The Environmental Quality Standards (EQS) set for water bodies in Scotland are standards outlining the maximum permissible concentrations for various pollutants, including heavy metals (Baxter, 2011)

Table 3.6: **Mean concentrations of trace elements including heavy metals in mussel tissue** (mg Kg^{-1} dry weight) from Loch Eil and Loch Sunart ($n = 45$ per site) collected on 13/10/2020. The samples were analysed for total minerals, trace elements and potentially toxic elements (PTEs) by inductively-coupled-plasma mass spectrometry (ICP-MS). LoD – limit of detection is the lowest detectable quantity from a blank with a specific a certain confidence level within the analysis. The samples from Loch Sunart were analysed on a different day, therefore the LoD corresponding to the Loch Sunart samples is noted as LoD*. Data are presented as mean mg Kg^{-1} dry weight (ppm) (± standard deviation, SD). Analysis of variance (ANOVA) was used to determine any statistically significant difference of potentially toxic elements (PTEs) between the sites. Different superscript letters imply significant differences ($p < 0.05$). Tukey Method was used for post hoc examination. Variation in degrees of freedom (F) indicate the removal of outliers and results $< LoD$.

	Loch Eil		Loch Sunart			One-way ANOVA
		LoD		LoD	LoD*	
As	6.2 (± 0.6)	0.010	9.0 (± 2.8)	0.010	0.005	<0.001 ($F_{1, 27}$= 15.9)
Pb	0.5 (± 0.1)	0.010	3.7 (± 2.4)	0.010	0.814	<0.001 ($F_{1, 27}$= 65.9)
Mg	1085.7 (± 216)	0.380	1884.6 (± 489)	0.380	0.250	<0.001 ($F_{1, 28}$= 33.5)
P	3539.3 (± 321)	2.610	9432.0 (± 4457)	2.610	2.282	<0.001 ($F_{1, 28}$= 26.1)
Ca	737.7 (± 223)	4.300	1985.1 (± 1499)	4.300	4.136	<0.001 ($F_{1, 27}$= 62.5)
V	0.3 (± 0.1)	0.000	0.6 (± 0.2)	0.000	0.001	<0.001 ($F_{1, 28}$= 15.5)
Cr	0.6 (± 0.2)	0.010	0.4 (± 0.1)	0.010	0.008	<0.001 ($F_{1, 28}$= 16.7)
Co	0.7 (± 0.1)	0.020	0.2 (± 0.1)	0.020	0.016	<0.001 ($F_{1, 22}$= 80.0)
Cu	6.2 (± 1.1)	0.010	4.9 (± 1.0)	0.010	0.011	<0.001 ($F_{1, 28}$= 11.2)
Zn	54.7 (± 19)	0.160	80.8 (± 31)	0.160	0.182	0.01 ($F_{1, 28}$= 7.9)
Se	1.9 (± 0.4)	0.020	2.6 (± 0.7)	0.020	0.008	<0.001 ($F_{1, 28}$= 12.0)

*Samples from within the Loch Sunart sample set were analysed on a different day

The total particulate matter (TPM) concentration in both lochs is shown in Figure 3.1 and all samples from the 2019-05-02 stand out with the highest TPM concentration within the sampling period. The first measurement taken on the 2019-05-02, the sample from 2 metres in Loch Eil (Figure 3.1a) measured approximately double the amount of TPM than that found in the sample collected at 6 metres. Throughout the rest of the sampling period, the measured TPM values between the two depths remain within the range of ± 0.6 mg L^{-1} of one another. Similar to the trend in Loch Eil, Loch Sunart samples also measure similar concentrations as of the 2019-16-05 onwards (Figure 3.1b), differing in a maximum of ± 0.6 mg L^{-1} between the two depths. Other observable significant differences are in the samples from the 2019-19-09 and the 2019-25-11 in Figure 3.1b and 3.1c.

Only the first sampling date shows a peak concentration of TPM in Loch Sunart, however, opposite to that of Loch Eil, the higher concertation measured 6.6 mg L^{-1} at 6 metres in comparison to 5.4 mg L^{-1} in the samples from 2 metres. The concentrations then decrease to 0.7 – 2.8 mg L^{-1} in both lochs over the remaining months, whereas Loch Eil shows to exhibit higher TPM concentrations than Loch Sunart as of the end of May. Due to an inadequate sample size, statistical analysis for comparing TPM concentration between depths was not feasible, therefore no meaningful differences were observed.

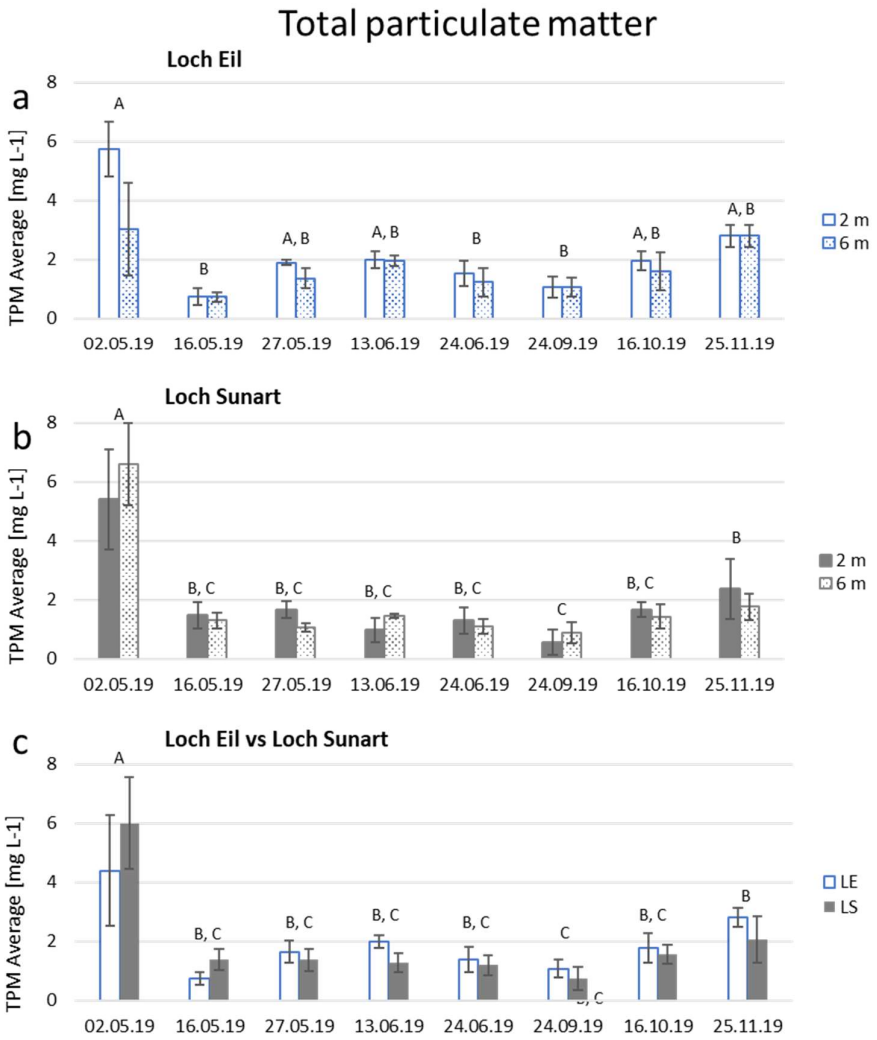

Figure 3.1: **Total particulate matter concentration (mg L^{-1}) in Loch Eil and Loch Sunart** over a 6-month period from May 2019 to November 2019. Graphs a and b show water samples from 2 meters and 6 meters depth from Loch Eil and Loch Sunart respectively and display the average values of TPM measured across analytical replicates ($\boldsymbol{n = 3}$). Graph c displays a comparison of the TPM concentration in both lochs, in which the measurements from the two different depths were combined for each time point ($\boldsymbol{n = 6}$). The data are presented as mean ± standard deviation. Analysis of variance (ANOVA) was used to determine any statistically significant difference in TPM concentration between time points and between sites. Different letters imply significant pairwise differences ($\boldsymbol{p < 0.05}$).

The water quality including primary production and food availability is described by presence and concentrations of Chl-a (Figure 3.2) and particulate organic matter (Figure 3.3); these parameters were compared between the sites and between the depths of 6 m and 2 m within the lochs. Figure 3.2a shows the measured Chl-a at the different depths in Loch Eil. In late September, the surface

water measured double the concentration of what was measured at 6 metres: 2.9 µg L^{-1} at 2 metres and 1.4 µg L^{-1} at 6 metres. In November however, samples from 2 metres measured 1.0 µg L^{-1} compared to the higher concentration of 2.7 µg L^{-1} measured in those collected at 6 metres.

Significant differences ($p = 0.001$) in Chl-a concentration are seen in samples from the 27th of May, 24th of June and the 16th of October. Similarly in Figure 3.2b, samples from the 27th of May and the 25th of November showed a significant difference in Chl-a concentration within Loch Sunart ($p = 0.015$). where the surface water contained higher Chl-a concentrations compared to the samples collected from 6 metres. When comparing the Chl-a values between Loch Eil and Loch Sunart samples (Figure 3.2c), there have been significant differences observed across the time points ($p < 0.01$), between sites ($p < 0.001$) and the interaction of the TP vs site ($p < 0.01$).

The Chl-a values between the sites showed that at the beginning of May, during the pre-summer period, the concentration of Chl-a was higher in Loch Sunart than in Loch Eil, albeit it only by 0.5 – 1 µg L^{-1}. However, a fortnight later, the Chl-a concentration peaked at just over 5 µg L^{-1} in Loch Eil, whereas the Chl-a concentration in Loch Sunart did not shift much beyond 3.5 µg L^{-1}. The Chl-a trend in Loch Eil continued until September and was observed again in December, however decreased significantly to a concentration of <0.5 µg L^{-1} in November. Chl-a in Loch Sunart however remained between 1 and 2 µg L^{-1} apart from December where values dropped to <0.5 µg L^{-1}.

Figure 3.2 clearly shows that Chl-a values are predominantly higher in Loch Eil as to those measured in Loch Sunart, which can also be observed comparing Figures 3.2a and 3.2b. Due to an inadequate sample size ($n = 2$), statistical analysis for comparing Chl-a concentrations between depths was not feasible, therefore no meaningful differences were observed. Although a slight trend of POM concentrations over the time points is noticeable in Figures 3.3a and 3.3b, the values in Loch Eil do not significantly differ to those of Loch Sunart.

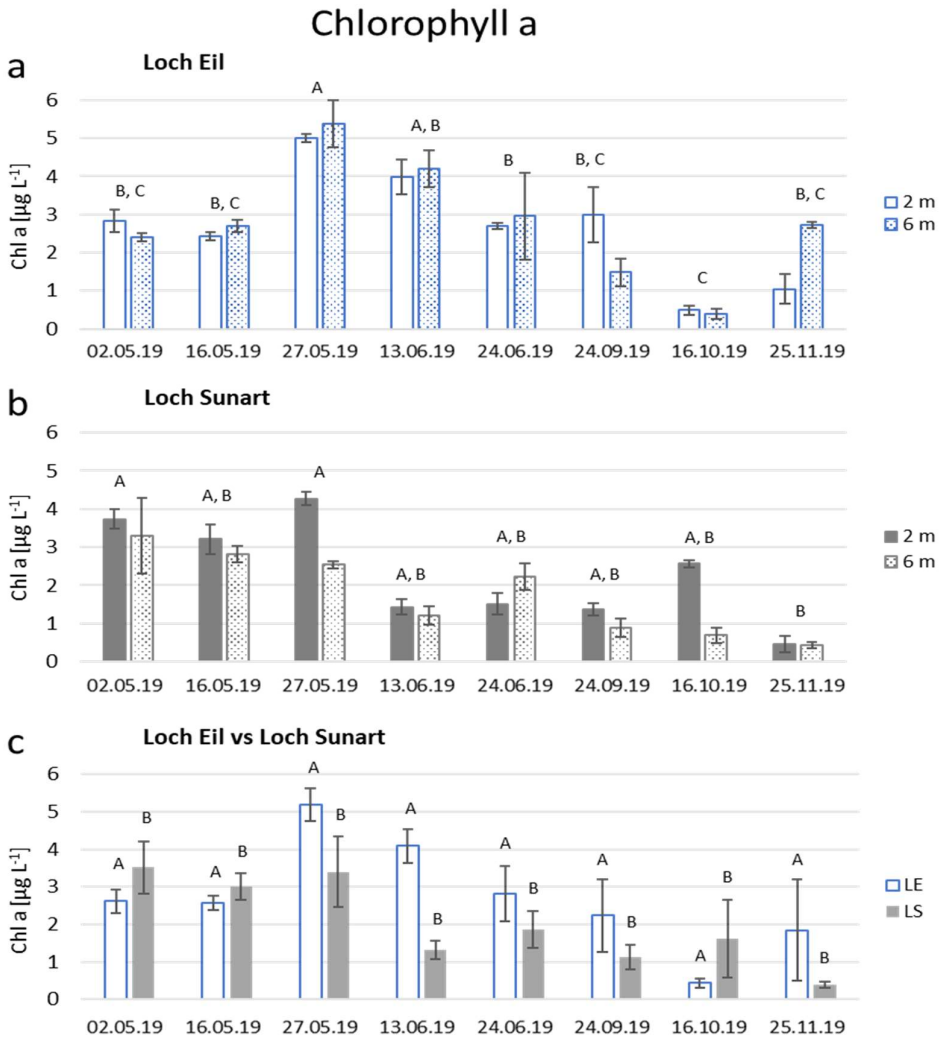

Figure 3.2: **Chlorophyll a concentration (µg L^{-1}) in Loch Eil and Loch Sunart** over a 6-month period. The chlorophyll a (Chl-a) concentration was analysed in water samples from both lochs over a 6-month period from May 2019 to November 2019. Graphs a and b show the values from 2 meters and 6 meters depth from Loch Eil and Loch Sunart respectively and display the average values of Chl-a measured across replicates ($\boldsymbol{n = 3}$). Whereas graph c displays a comparison of the Chl-a concentration in both lochs, in which the measurements from the two different depths were combined for each time point ($\boldsymbol{n = 6}$). Statistical analyses were performed using Minitab 18. The data are presented as mean (± standard deviation, SD). Analysis of variance (ANOVA) was used to determine any statistically significant difference of Chl-a concentration between the time points and the site. Different letters imply significant pairwise differences ($\boldsymbol{p < 0.05}$).

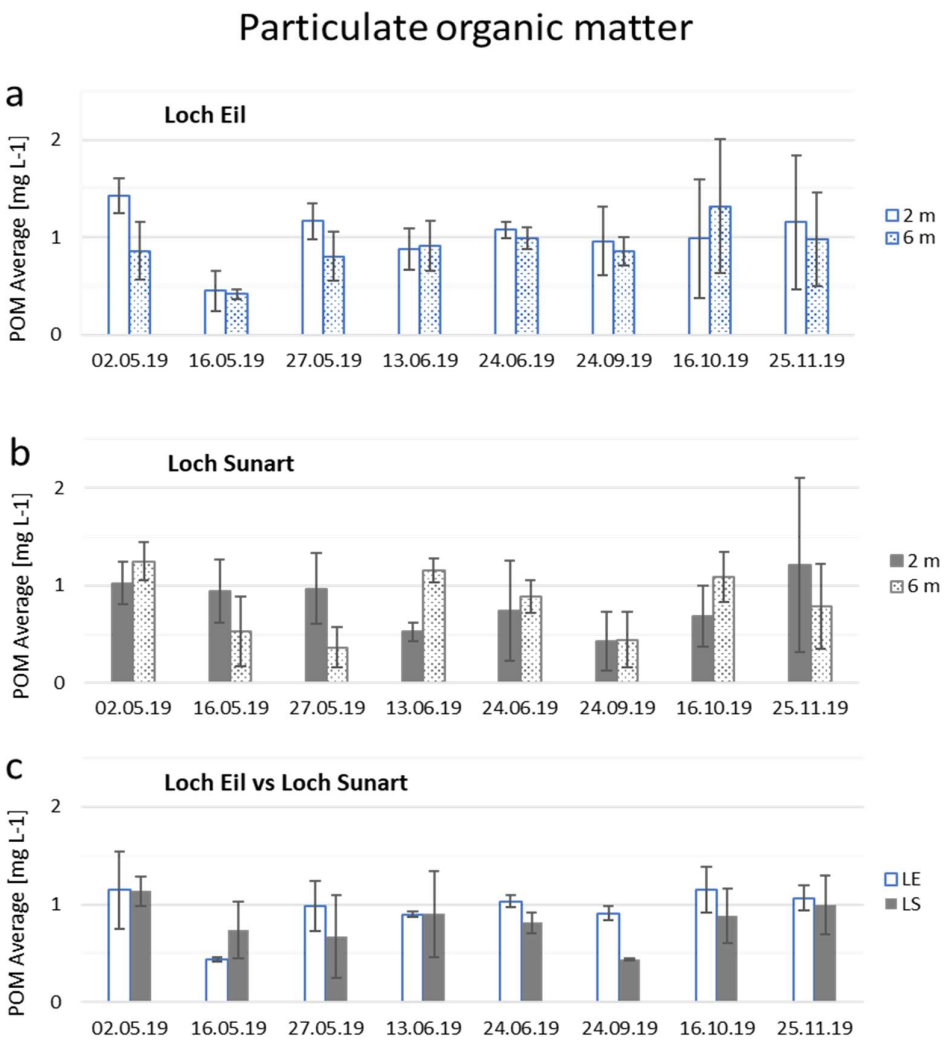

Figure 3.3: **Particulate organic matter (µg L^{-1}) in Loch Eil and Loch Sunart** over a 6-month period. The particulate organic matter (POM) concentration was analysed in water samples from both lochs over a 6-month period from May 2019 to November 2019. Graphs a and b show the values from 2 meters and 6 meters depth from Loch Eil and Loch Sunart respectively and display the average values of POM measured across replicates ($\boldsymbol{n = 3}$). Whereas graph c displays a comparison of the POM concentration in both lochs, in which the measurements from the two different depths were combined for each time point ($\boldsymbol{n = 6}$). Statistical analyses were performed using Minitab 18. The data are presented as mean (± standard deviation, SD). Analysis of variance (ANOVA) was used to determine any statistically significant difference of POM concentration between the time points and the site.

In Loch Eil (Figure 3.3a), POM concentrations were generally higher in shallower depths. In Loch Sunart (Figure 3.3b), a peak in POM concentration was observed at the beginning of May, with higher values at the deeper sampling depth. However, from late May onwards, POM concentrations were predominantly

higher in shallower depths in Loch Eil. Subsequently, a shift occurred, with higher concentrations observed in deeper waters. Despite variations in particulate matter (POM) concentrations between depths and lochs (Figure 3.3c), the statistical analysis showed no significant differences of measured POM. Due to an inadequate sample size, statistical analysis for comparing POM concentrations between depths was not feasible, therefore no meaningful differences were observed.

Comparing logged temperature and salinity data between both lochs

The salinity and temperature data loggers collected information in Loch Eil from May 2019 until November 2019, and Loch Sunart from November 2019 to October 2020. Figure 3.4 shows the salinity values (ppt, ‰) from Loch Eil (orange) and the values from Loch Sunart (purple). Although the salinity was measured a year apart, this graph shows the daily fluctuations over the same months throughout the logging periods.

It is apparent that the salinity fluctuates more in Loch Eil than it does in Loch Sunart, however the area of interest is emphasised within the figure. During August, the values in Loch Eil appear to rise from 31 ppt to nearly 37 ppt over a period of two weeks, then drop significantly by approximately 15 ppt, from ~37 ppt mid-month to ~20 ppt towards the end of the month. The salinity profile of Loch Sunart shows fluctuations by approximately 11 ppt throughout the logging period, where the highest of salinity measured ~31 ppt in mid-May of 2020 and the lowest at ~20 ppt two months later. Overall, comparing both salinity profiles, Loch Eil shows a notable shift in salinity as opposed to Sunart.

Figure 3.5 shows the salinity profile of Loch Eil (orange) over the logging period in 2019 with the addition of the daily precipitation over the same period (blue). The higher peaks of daily precipitation appear to occur once every month, whereby September experienced the highest amount of 64 mm, followed by a wet spell in June with 58 mm. August, however, shows to have a higher total rainfall with approximately 234 mm compared to the previous four months, despite displaying lower peaks.

The total rainfall over the previous years is visualised in heatmaps in Figure 3.6. Considering that the Lochs are within geographical proximity to one another, the climate data does not differ significantly between the two locations (Loch Eil:

Latitude: 58.85° N, Longitude: 5.30° W and Loch Sunart Latitude: 56.65° N, Longitude: 5.65° W) especially from June onwards in every year.

The climate data served to validate the significant salinity drop in Loch Eil and to distinguish any long-term trends. Heat maps in Figure 3.6 depict precipitation patterns over the past decade, revealing individual rainfall levels for Loch Eil and Loch Sunart. While both lochs share similarities in environmental conditions due to geographic proximity, Loch Eil experienced higher average monthly rainfall ranging from 200 mm to 500 mm, with notable peaks in winter months. Conversely, Loch Sunart displayed slightly lower average monthly rainfall, typically ranging from 150 mm to 400 mm, with similar winter peaks.

The temperature data illustrated in Figure 3.7 shows variations in monthly and yearly temperature readings for both Loch Eil and Loch Sunart. Average monthly temperatures in Loch Eil ranged from 5°C to 15°C, with warmer temperatures occurring in the summer months of June, July, and August. In contrast, Loch Sunart experienced slightly cooler temperatures, with average monthly readings ranging from 3°C to 13°C during the same period. Raw data pertaining to annual precipitation and temperature can be found in Appendices 3.5 – 3.8.

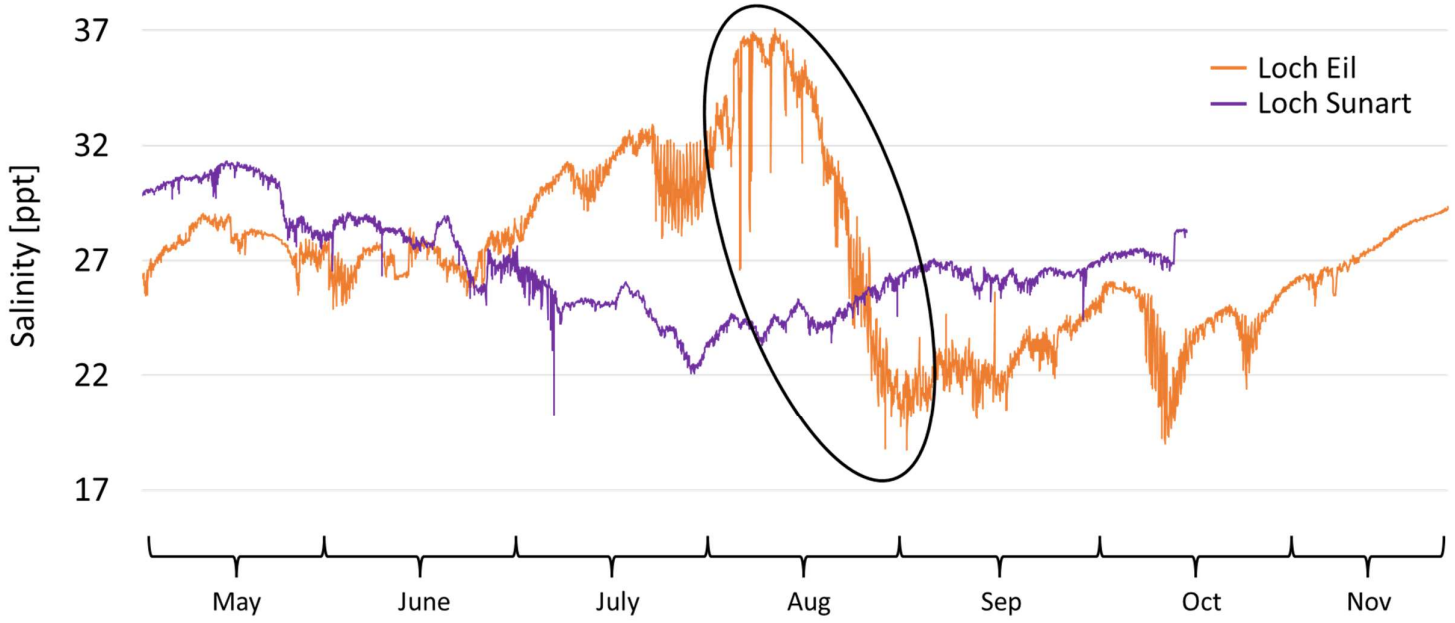

Figure 3.4: **Salinity profiles from Loch Eil and Loch Sunart.** From May 2019 to November 2019, a salinity data logger (HOBO U24-002-C) recorded hourly measurements in Loch Eil. Then from November 2019, hourly salinity measurements were recorded in Loch Sunart until October 2020. The salinity measurements were processed and transformed to salinity profiles. The graph shows an overlap of salinity profiles from Loch Eil (orange) and Loch Sunart (purple). The salinity values are presented in parts per thousand (ppt).

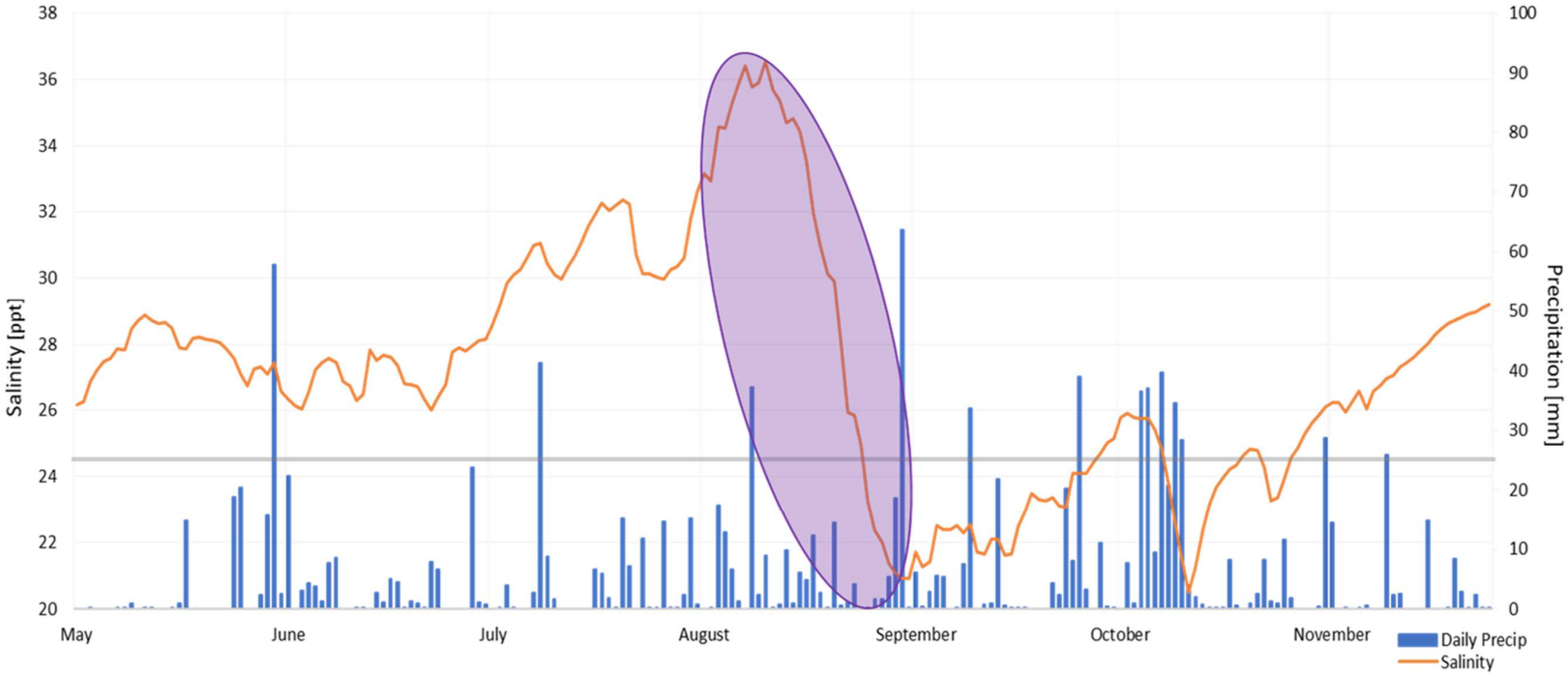

Figure 3.5: **Salinity and precipitation data from Loch Eil in 2019.** From May 2019 to November 2019, a salinity data logger (HOBO U24-002-C) recorded hourly measurements in Loch Eil. The salinity data was processed and transformed into a salinity profile (orange) and overlayed with the average daily precipitation data (blue) and the average monthly precipitation (grey) from Loch Eil in 2019 over the mentioned period. The circled area of the graph shows area of interest – the rapid decrease in salinity between August and September 2019.

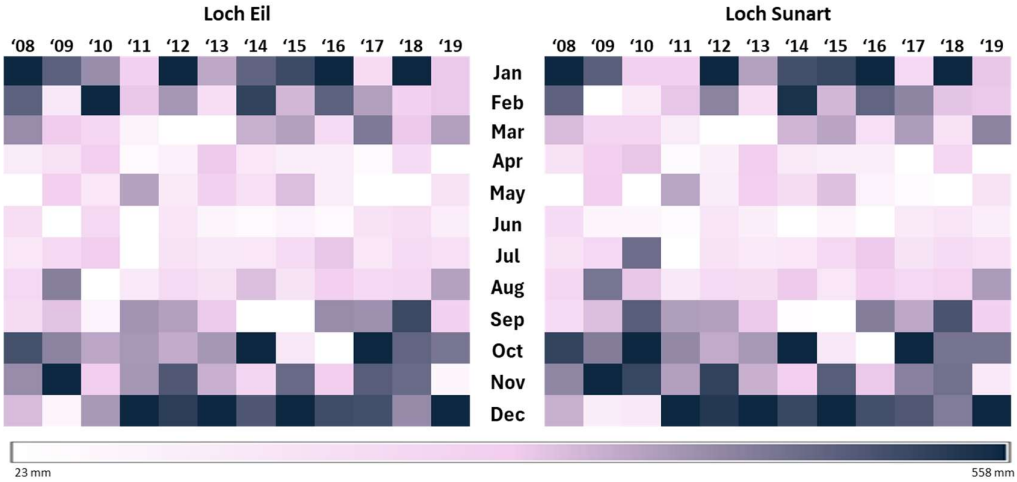

Figure 3.6: **Total yearly precipitation in Loch Eil and Loch Sunart from 2008 – 2019.** Precipitation data was sourced from the World Weather for Water Data Service (W3S) for Loch Eil (Latitude: 58.85° N, Longitude: 5.30° W) and Loch Sunart (Latitude: 56.65° N, Longitude: 5.65° W) from 2008 – 2019. The colour key denotes the minimum value of 23 mm and the maximum value of 558 mm total precipitation. Raw data of the precipitation values for all heatmaps can be found in the appendix.

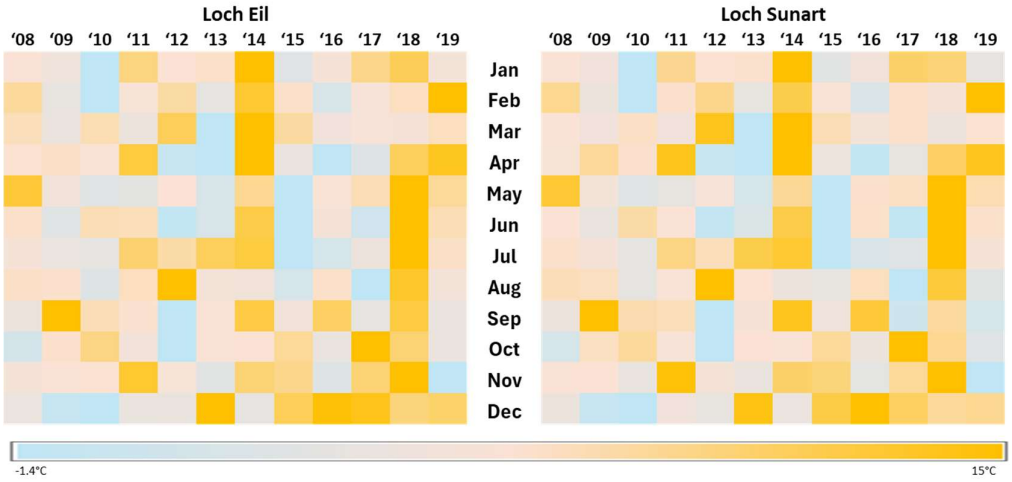

Figure 3.7: **Average yearly air-temperature around Loch Eil and Loch Sunart from 2008 – 2019**. Temperature data was sourced from the World Weather for Water Data Service (W3S) for Loch Eil (Latitude: 58.85° N, Longitude: 5.30° W) and Loch Sunart (Latitude: 56.65° N, Longitude: 5.65° W) from 2008 – 2019. The colour key denotes the minimum recorded temperature of −1.4°C and a maximum of 15°C. Temperature values for all heatmaps can be found in the appendix.

3.4 Discussion and conclusions

Having compared all the analysed trace elements from both water and tissue samples to literature and environmental standards, the concentrations within both lochs remain well beneath the established threshold. However, some studies have confirmed that certain trace elements can speciate under various environmental conditions, including changes in salinity and pH, and therefore convert to more harmful substances (Rainbow, 1985; McLusky, Bryant & Campbell, 1986; Kumar et al., 2015; Cledon et al., 2021). Further investigation with continuous monitoring of environmental parameters and more frequent sampling could be useful for a more detailed outcome.

The relatively low sample sizes (n-values) for water quality parameters were primarily a result of logistical and financial constraints. The significant travel time involved in reaching the sampling site, typically requiring 6-7 hours for a round trip, coupled with the time-consuming nature of water quality analyses in the laboratory, presented logistical challenges. Furthermore, budgetary limitations restricted the frequency of the sampling dates. Additionally, sampling dates had to be adjusted to avoid severe weather conditions, which could have impacted water quality and sampling safety, and accommodate the availability of the farmers who provided access to the site locations. The combination of these constraints resulted in a reduced sampling frequency and ultimately a limited dataset.

The comparative analysis of heavy metals, water quality parameters, and climate data between Loch Eil and Loch Sunart provides valuable insights into the environmental factors influencing mussel spat mortality in these regions.

The analysis of heavy metal concentrations in mussel tissues revealed significant differences between Loch Eil and Loch Sunart. The results showed that all analysed elements were found in higher concentrations within the mussel tissue collected from Loch Sunart compared to Loch Eil. In particularly lead concentrations were significantly elevated within the tissue samples from Loch Sunart (0.5 ± 0.1 Loch Eil vs 3.7 ± 2.4 Loch Sunart). High concentrations of lead could potentially cause implications for the health of the mussels, as a

consequence of the known toxicity of the element (Nelson, Miller & Calabrese, 1988; McDougall et al., 2022). However, as there have not been any mortality of mussel spat observed in Loch Sunart, the assumption of lead toxicity in the spat can be ruled out. In addition, lower concentrations of essential trace elements such as phosphorus and calcium were observed in Loch Eil (P: 3539.3 ± 321 Eil vs 9432.0 ± 4457 Sunart, Ca: 737.7 ± 223 Eil vs 1985.1 ± 1499 Sunart), potentially affecting mussel growth and development (Buer et al., 2020).

Reviewing the results regarding the total particulate matter concentrations, both lochs showed fluctuations over time, with the occasional yet notable peaks observed on specific sampling dates (May and November). Despite the similarities in TPM concentrations between the two depths within each loch, the higher concentrations observed in Loch Eil towards the end of May suggest localised variations in particulate matter dynamics that may influence mussel spat health (Boyd, 2000; Dailianis, 2010)

Chlorophyll-a (Chl-a) concentrations, indicative of primary production and food availability, exhibited significant differences between Loch Eil and Loch Sunart. While both lochs displayed fluctuations in Chl-a concentrations over time, Loch Eil consistently showed higher values compared to Loch Sunart, particularly during the summer months. Variations in Chl-a concentrations may influence mussel spat survival and growth, as they reflect differences in phytoplankton abundance and nutrient availability (Dailianis, 2010). As indicated by Schalles (2006), Chlorophyll-a concentrations in coastal and estuarine waters range from 0.01 to 1000 μg L^{-1}, depending on the time of year.

Considering Loch Eil exhibited higher concentrations compared to Loch Sunart, the lack of food availability does not seem to be a matter of concern as to what is causing the spat mortality events.

Salinity and temperature profiles between Loch Eil and Loch Sunart revealed distinct patterns. Loch Eil exhibited greater fluctuations in salinity compared to Loch Sunart, particularly during the summer months, which possibly may have impacted mussel spat physiology and osmoregulation (Landes et al., 2015)

In Loch Eil, salinity exhibited notable variability throughout the monitoring period, with daily fluctuations often exceeding those observed in Loch Sunart. This

variability could be attributed to several factors, including tidal influences, freshwater inputs, and local weather conditions (Edwards et al., 1980). The abrupt shifts in salinity observed in Loch Eil, particularly during August, highlight the dynamic nature of this water body and the potential influence of episodic events such as heavy rainfall or freshwater discharge from surrounding catchment areas (Edwards et al., 1980; SEPA, 2011; Hintz & Relyea, 2017). Conversely, the salinity profile of Loch Sunart demonstrated relatively more stable conditions, with fluctuations of smaller magnitude compared to Loch Eil. The limited variability in salinity suggests that Loch Sunart may be less influenced by external factors such as freshwater inputs or tidal fluctuations, leading to a more homogeneous aquatic environment (Gillibrand et al., 1995; CEFAS, 2014). The correlation between daily precipitation and salinity fluctuations in Loch Eil highlights the significant impact of rainfall on freshwater inputs and subsequent salinity levels (Edwards et al., 1980; Grantham, 1981). Despite lower peak precipitation events in August, higher total rainfall suggests prolonged rainfall may have a pronounced effect on salinity dynamics. Heatmaps comparing total rainfall between Loch Eil and Loch Sunart indicate similar climatic conditions, supporting comparable meteorological influences despite their geographic proximity. Differences in precipitation and temperature patterns between the two regions have implications for nutrient input and ecosystem dynamics. Monthly rainfall patterns vary throughout the year, with higher averages in winter months and lower averages in summer. Total annual rainfall ranged from 1854.0 mm to 3059.0 mm, with Loch Eil averaging 2416 mm annually compared to 2607 mm in Loch Sunart. Variability in annual rainfall totals suggests fluctuations from year to year. Overall, Loch Eil tends to receive slightly less average rainfall and marginally cooler temperatures compared to Loch Sunart, possibly due to geographical location and elevation differences.

During periods of heavy rainfall in Loch Eil, such as in the winter months, freshwater runoff from nearby areas, including croft lands, urban developments, and even small-scale industries, flows into the loch (Granato et al., 1995; Milik & Pasela, 2018; Pourmozaffar et al., 2020). This influx of freshwater can decrease the salinity levels in the water column, affecting the balance of ions and nutrients critical for the health of marine organisms, including mussel spat. Runoff from these industrial sites located along the shores of Loch Eil, may contain

contaminants such as heavy metals from various processes. Following a rainfall event, these pollutants can be washed into the loch, where they may accumulate in sediments or become dissolved in the water column, potentially posing risks to mussel spat and other aquatic life (Grantham, 1981; Pourmozaffar et al., 2020).
Variability in rainfall amounts has been observed across different years, suggesting the influence of climatic factors or atmospheric phenomena on precipitation patterns. Some years, such as 2011, 2015, and 2017, experienced higher-than-average rainfall totals, possibly due to specific weather events or atmospheric conditions. In contrast, years such as 2010 and 2018 recorded below-average rainfall totals, indicating periods of drier conditions or altered atmospheric circulation patterns. Overall, the data underlines the dynamic nature of rainfall patterns in Loch Eil, with fluctuations observed both within and across years.
While certain months consistently exhibit higher or lower rainfall amounts, there is no clear linear trend observed in the data, however, applying statistical analyses such as a general liner model or even regression modelling could aid in identifying any significant patterns or long-term trends within the rainfall data.
In summary, the rainfall patterns observed in Loch Eil can influence salinity Evaluating the results among other research has led to the assumption that all results apart from the salinity drop within Loch Eil individually don’t appear to have a detrimental impact on the mussel spat. dynamics, trace element circulation, and potential toxicity risks for mussel spat survival. Understanding the interactions between rainfall, water chemistry, and biological responses is essential for evaluating ecosystem health.
The observed differences in environmental parameters between Loch Eil and Loch Sunart suggest potential drivers of mussel spat mortality (Rayssac et al., 2010; Pourmozaffar et al., 2020). The presence of heavy metals (Renault, 2015), even in low concentrations, fluctuations in particulate matter dynamics (Boyd, 2000; Gokul et al., 2023), variations in water quality parameters (Lynch et al., 2014), and distinct climate patterns (Brenko & Calabrese, 1969; Mubiana & Blust, 2007) may collectively influence the health and survival of mussel spat in these regions.
In conclusion, the comparative analysis of environmental parameters between Loch Eil and Loch Sunart has been insightful and provided valuable information

regarding the dynamics of each loch. Analysis revealed key differences between the lochs. Loch Sunart showed higher lead concentrations in mussels, though mortality suggests this is not the primary driver of spat deaths. Lower essential elements (e.g., phosphorus, calcium) were observed in Loch Eil. Loch Eil exhibited greater salinity fluctuations, potentially impacting spat physiology. Higher chlorophyll-a levels in Loch Eil indicate sufficient food availability. While heavy metals, nutrient availability, and salinity fluctuations may influence spat survival, it remains difficult to determine a cause when there are multiple factors imminent, which all could have an influence on one another, creating variable environments and consequently having an impact on the mussel spat (Capelle et al., 2021; Charles et al., 2020).

3.5 **Appendix**

Appendix 3.1: **Trace metal analysis of Loch Eil water.** Water samples from 1 and 4 metres depth were collected from the same location within Loch Eil during each time point ($\boldsymbol{n = 3}$ per timepoint) over a 3 – month period. The samples were analysed for total minerals, trace elements and potentially toxic elements (PTEs) by inductively-coupled-plasma mass spectrometry (ICP-MS). LoD – limit of detection is the lowest detectable quantity from a blank with a specific a certain confidence level within the analysis. The data are presented as mean ppb (parts per billion) (± standard deviation, SD). Analysis of variance (ANOVA) was used to determine any statistically significant between the different time points and to examine any statistical significance between PTEs and site, and PTEs and time point. Tukey Method was used for post hoc examination. Different superscript letters imply significant pairwise differences ($p < 0.05$).

Element (ppb)	T0	T1	T2	T3	T4*	*Depth*	One-way ANOVA
Pb	0.17 (± 0.07) $^{A, B}$	0.15 (± 0.04) $^{A, B}$	0.03 (± 0.01) ^{B}	0.07 (± 0.06) $^{A, B}$	0.25 (± 0.02) ^{B}		0.03 ($F_{4, 5}$ = 7.06)
1 m						*0.15 (± 0.09)*	*0.56 ($F_{1, 8}$ = 0.38)*
4 m						*0.12 (± 0.09)*	
Hg	0.49 (± 0.00)	< LoD	< LoD	< LoD	0.05 (± 0.00)		**
1 m						*0.27 (± 0.31)*	**
4 m						*0.05 (± 0.00)*	
Cd	1.19 (± 1.16)	0.28 (± 0.00)	0.01 (± 0.00)	< LoD	0.74 (± 0.08)		0.33 ($F_{3, 4}$ = 1.58)
1 m						*0.78 (± 0.88)*	*0.38 ($F_{1, 6}$ = 0.91)*
4 m						*0.34 (± 0.28)*	
As	1.02 (± 0.05)	0.95 (± 0.19)	1.20 (± 0.04)	1.17 (± 0.04)	1.21 (± 0.08)		0.13($F_{4, 5}$ = 3.02)
1 m						*1.09 (± 0.19)*	*0.78 ($F_{1, 8}$ = 0.08)*
4 m						*1.12 (± 0.05)*	
Fe	14.12 (± 2.78) $^{A, B}$	30.1 (± 2.41) $^{A, B}$	36.51 (± 11.14) ^{A}	10.18 (± 1.74) ^{B}	*		0.03 ($F_{3, 4}$ = 9)
1 m						*24.92 (± 16.03)*	*0.66 ($F_{1, 6}$ = 0.22)*
4 m						*20.49 (± 9.66)*	

Appendix 3.1 continued

Element (ppb)	T0	T1	T2	T3	T4*	*Depth*	One-way ANOVA
Co	0.04 (± 0.00) [A]	0.04 (± 0.00) [A]	0.01 (± 0.01) [B]	< LoD	*		0.00 ($F_{2, 3}$ = 54.76)
1 m						*0.03 (± 0.02)*	*0.87 ($F_{1, 4}$ = 0.03)*
4 m						*0.03 (± 0.02)*	
Cu	1.42 (± 0.67)	1.01 (± 0.17)	< LoD	< LoD	*		**
1 m						*1.39 (± 0.71)*	**
4 m						*1.03 (± 0.13)*	
Zn	2.86 (± 1.11)	3.00 (± 0.07)	2.01 (± 0.17)	1.11	*		0.50 ($F_{3, 4}$ = 0.93)
1 m						*2.91 (± 0.76)*	*0.20 ($F_{1, 5}$ = 2.15)*
4 m						*2.03 (± 0.80)*	

*Samples collected during T4 were analysed on a different date, therefore all data pertaining to that specific ICP-MS were marked.

** These samples were not analysed for Fe, Co, Cu, Zn due to separate run on ICP-MS.

Appendix 3.2: **Trace elements analysis of mussel tissue from Loch Eil and Loch Sunart.** The table displays the concentrations of trace elements including heavy metals in mussel tissue (mg kg^{-1} dry weight) from Loch Eil and Loch Sunart ($n = 15$ per site) collected on 13/10/2020. The samples were analysed in analytical triplicates for total minerals, trace elements and potentially toxic elements (PTEs) by inductively-coupled-plasma mass spectrometry (ICP-MS). LoD – limit of detection is the lowest detectable quantity from a blank with a specific and certain confidence level within the analysis. The data are presented as mean (± standard deviation). Analysis of variance (ANOVA) was used to determine any statistically significant difference in potentially toxic elements (PTEs) between sites. Different letters imply significant pairwise differences ($p < 0.05$).

(ppb)	Loch Eil	LoD	Loch Sunart	LoD	LoD*	One-way ANOVA
Cd	0.3 (± 0.1)	0.006	0.3 (± 0.1)	0.010	0.008	0.00 ($F_{1, 27}$= 0.29)
As	6.2 (± 0.6) ^{B}	0.006	9.0 (± 2.8) ^{A}	0.010	0.005	0.00 ($F_{1, 27}$= 15.94)
Pb	<LoD ^{B}	0.804	3.7 (± 2.4) ^{A}	0.010	0.814	0.00 ($F_{1, 27}$= 65.94)
Hg	0.1 (± 0.1)	0.009	0.2 (± 0.2)	0.000	0.005	0.06 ($F_{1, 28}$= 3.92)
Na	11571.2 (± 4233)	0.542	13396.0 (± 4377)	1.430	0.628	0.26 ($F_{1, 28}$= 1.35)
Mg	1085.7 (± 216) ^{B}	0.213	1884.6 (± 489) ^{A}	0.380	0.250	0.00 ($F_{1, 28}$= 33.49)
P	3539.3 (± 321) ^{B}	2.147	9432.0 (± 4457) ^{A}	2.610	2.282	0.00 ($F_{1, 28}$= 26.08)
K	5862.2 (± 1560)	1.002	6052.6 (± 1426)	2.990	1.311	0.73 ($F_{1, 28}$= 0.12)
Ca	737.7 (± 223) ^{B}	3.215	1985.1 (± 1499) ^{A}	4.300	4.136	0.00 ($F_{1, 27}$= 62.54)
V	0.3 (± 0.1) ^{B}	0.005	0.6 (± 0.2) ^{A}	0.000	0.001	0.00 ($F_{1, 28}$= 15.54)
Cr	0.6 (± 0.2) ^{B}	0.007	0.4 (± 0.1) ^{A}	0.010	0.008	0.00 ($F_{1, 28}$= 16.71)
Mn	8.6 (± 2.7)	0.010	6.7 (± 3 .1)	0.020	0.012	0.09 ($F_{1, 28}$= 3.19)
Fe	117.3 (± 46)	0.026	108.4 (± 38)	0.110	0.035	0.57 ($F_{1, 28}$= 0.33)
Co	0.7 (± 0.1) ^{B}	0.014	0.2 (± 0.1) ^{A}	0.020	0.016	0.00 ($F_{1, 22}$= 79.95)
Ni	0.5 (± 0.1)	0.012	0.6 (± 0.3)	0.030	0.017	0.40 ($F_{1, 28}$= 0.62)
Cu	6.2 (± 1.1) ^{B}	0.009	4.9 (± 1.0) ^{A}	0.010	0.011	0.00 ($F_{1, 28}$= 11.19)
Zn	54.7 (± 19) ^{B}	0.145	80.8 (± 31) ^{A}	0.160	0.182	0.01 ($F_{1, 28}$= 7.89)
Se	1.9 (± 0.4) ^{B}	0.005	2.6 (± 0.7) ^{A}	0.020	0.008	0.00 ($F_{1, 28}$= 12.04)

Samples were analysed on a different day, the LoD is the corresponding LoD for that analysis on the day

Appendix 3.3: **Limit of detections (LoD) and R^2 (coefficient of determination) values of the ICP-MS analytes from the water and mussel tissue samples collected from Loch Eil and Loch Sunart.** The LoD is the lowest concentration of an analyte that can be reliably detected by an analytical method. R^2 is a statistical measure that indicates how well a regression line fits the data points. Lower LoD and higher R^2 indicate better method sensitivity and accuracy.

	Tissue samples						Water samples			
	Loch Eil		Loch sunart				Loch Eil			
(ppb)	LoD	R^2	LoD	R^2	LoD*	R^2*	LoD	R^2	LoD*	R^2*
Cd	0.006	0.9999	0.010	0.9999	0.008	1.000	0.009	0.9985	0.007	0.9966
As	0.006	0.9997	0.010	0.9998	0.005	0.9999	0.007	0.9983	0.002	0.9948
Pb	0.804	0.9880	0.010	0.9971	0.814	0.9987	0.002	0.9963	0.005	0.9982
Hg	0.009	0.9980	0.000	0.9999	0.005	0.9999	0.006	0.9985	0.007	0.9991
Na	0.542	0.9999	1.430	0.9991	0.628	0.9983	-	-	-	-
Mg	0.213	0.9997	0.380	0.9983	0.250	0.9993	-	-	-	-
P	2.147	0.9989	2.610	0.9980	2.282	0.9994	-	-	-	-
K	1.002	0.9999	2.990	0.9984	1.311	0.9997	-	-	-	-
Ca	3.215	0.9987	4.300	0.9993	4.136	0.9992	-	-	-	-
V	0.005	0.9993	0.000	0.9994	0.001	0.9998	-	-	-	-
Cr	0.007	0.9999	0.010	0.9999	0.008	0.9996	-	-	-	-
Mn	0.010	0.9993	0.020	0.9998	0.012	0.9999	-	-	-	-
Fe	0.026	0.9980	0.110	0.9990	0.035	1.0000	0.071	0.9959	-	-
Co	0.014	0.9997	0.020	0.9999	0.016	0.9998	0.004	0.9982	-	-
Ni	0.012	0.9993	0.030	0.9999	0.017	0.9999	-	-	-	-
Cu	0.009	0.9999	0.010	0.9999	0.011	0.9998	0.010	0.9972	-	-
Zn	0.145	0.9980	0.160	0.9998	0.182	0.9992	0.566	0.9968	-	-
Se	0.005	0.9993	0.020	0.9999	0.008	0.9394	-	-	-	-

Samples were analysed on a different day, the LoD is the corresponding LoD for that analysis on the day

Appendix 3.4: **Water quality analysis of water samples collected from Loch Eil and Loch Sunart from May to November in 2019.** Total particulate matter (TPM), chlorophyll a (Chl-a) and particulate organic matter (POM) concentrations were analysed in water samples ($n = 3$), collected from 2 and 6 metres depth from both lochs over a 6-month period, from May to November in 2019. Statistical analyses were performed using Minitab 18. One-way analysis of variance (ANOVA) (3.4 -A) and a GLM (3.4-B) were used to determine any statistically significant differences of the separate TPM, Chl-a and POM concentrations between the time points, sites and the interaction of time points and sites. All data expressed are as Mean (± SD); TPM and POM as mg L^{-1} and Chl-a as µg L^{-1}. Tukey Method was used for post hoc examination. Different superscripts imply significant pairwise differences ($p < 0.05$), whereby capital superscripts indicate significant differences between the sites, and lowercase superscripts the significant difference between the time points (TP: T0 – T7).

A

		T0	T1	T2	T3	T4	T5	T6	T7		One-way ANOVA
	Site									*Depth*	P-value
TPM [mg L^{-1}]	**LE**	4.38 (± 1.91) [a]	0.73 (± 0.00) [b]	1.63 (± 0.38) [a, b]	1.98 (± 0.03) [a, b]	1.38 (± 0.21) [b]	1.07 (± 0.26) [b]	1.78 (± 0.26) [a, b]	2.80 (± 0.00) [a, b]		0.014 ($F_{7,8}$ = 5.48)
	2 m									*2.22 (± 1.55)*	*0.441 ($F_{1,14}$ = 0.63)*
	6 m									*1.72 (± 0.82)*	
	LS	5.19 (± 1.53) [a]	1.06 (± 0.38) [b, c]	1.50 (± 0.36) [b, c]	1.60 (± 0.48) [b, c]	1.29 (± 0.18) [b, c]	0.90 (± 0.24) [c]	1.67 (± 0.22) [b, c]	2.43 (± 0.49) [b]		0.00 ($F_{7,24}$ = 19.21)
	2 m									*2.07 (± 1.48)*	*0.653 ($F_{1,30}$ = 0.21)*
	6 m									*1.84 (± 1.42)*	
Chl-a [µg L^{-1}]	**LE**	2.62 (± 0.31) [b, c]	2.57 (± 0.18) [b, c]	5.19 (± 0.26) [a]	4.09 (± 0.15) [a, b]	2.82 (± 0.18) [b]	2.24 (± 1.07) [b, c]	0.44 (± 0.07) [c]	1.88 (± 1.19) [b, c]		0.001 ($F_{7,8}$ = 11.55) [A]
	2 m									*2.69 (± 1.45)*	*0.905 ($F_{1,14}$ = 0.01)*
	6 m									*2.78 (± 1.53)*	
	LS	3.52 (± 0.31) [a]	3.01 (± 0.28) [a, b]	3.40 (± 1.22) [a]	1.32 (± 0.16) [a, b]	1.87 (± 0.51) [a, b]	1.13 (± 0.35) [a, b]	1.62 (± 1.33) [a, b]	0.44 (-± 0.03) [b]		0.015 ($F_{7,8}$ = 5.39) [B]
	2 m									*2.32 (± 1.33)*	*0.373 ($F_{1,14}$ = 0.85)*
	6 m									*1.76 (± 1.09)*	
POM [mg L^{-1}]	**LE**	1.15 (± 0.40)	0.44 (± 0.02)	0.99 (± 0.25)	0.90 (± 0.03)	1.03 (± 0.06)	0.91 (± 0.07)	1.15 (± 0.24)	1.07 (± 0.12)		0.088 ($F_{7,8}$ = 2.77)
	2 m									*1.01 (± 0.28)*	*0.383 ($F_{1,14}$ = 0.81)*
	6 m									*0.89 (± 0.25)*	0.088 ($F_{7,8}$ = 2.77)
	LS	1.14 (± 0.16)	0.74 (± 0.29)	0.67 (± 0.42)	0.84 (± 0.44)	0.82 (± 0.11)	0.44 (-± 0.01)	0.89 (± 0.28)	1.00 (± 0.30)		0.452 ($F_{7,8}$ = 1.08)
	2 m									*0.82 (± 0.27)*	*0.982 ($F_{1,14}$ = 0)*
	6 m									*0.81 (± 0.34)*	0.452 ($F_{7,8}$ = 1.08)

B

	GLM P-value		
	TP	Site	TP v Site
TPM [mg L^{-1}]	0.000 (f 7, 16 = 23.64)	0.886 (f 1, 16 = 0.02)	0.131 (f 7, 16 = 1.93)
Chl-a [µg L^{-1}]	0.008 (f 1, 16 = 9.16)	0.000 (f 7, 16 = 11.27)	0.005 (f 7, 16 = 4.7)
POM [mg L^{-1}]	0.078 (f 7, 16 = 2.31)	0.128 (f 7, 16 = 2.57)	0.520 (f 7, 16 = 0.91)

Appendix 3.5: **Raw data of the total yearly precipitation in Loch Eil 2008 – 2019.** Precipitation data was sourced from the World Weather for Water Data Service (W3S) for Loch Eil (Latitude: 58.85° N, Longitude: 5.30° W) from 2008 – 2019. Data was extracted as daily measurements (mm precipitation) and summed up and averaged per month and year.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total/year	Average/year
2008	449.1	358.3	292.1	93.2	26.3	135.0	109.3	155.7	138.9	384.9	291.9	217.4	2652.2	408.0
2009	322.3	131.1	182.4	142.7	177.2	85.4	157.2	281.4	194.4	276.4	393.8	105.9	2450.2	377.0
2010	245.0	419.2	112.4	129.5	78.3	107.3	132.6	22.9	49.8	200.9	132.1	224.1	1854.0	285.2
2011	255.3	274.0	142.0	119.0	332.7	101.7	98.8	171.0	353.3	346.8	348.6	515.9	3059.0	470.6
2012	347.8	236.6	44.6	84.9	104.7	117.3	126.9	146.0	227.7	216.5	300.1	326.0	2279.1	350.6
2013	248.7	121.4	45.8	165.5	149.2	69.1	97.6	116.1	166.3	288.9	230.1	553.0	2251.7	346.4
2014	364.1	415.3	246.5	121.0	142.1	57.9	119.6	224.0	35.8	455.9	173.3	383.2	2738.8	421.4
2015	484.6	256.5	306.2	111.2	244.1	100.7	170.3	145.3	66.0	131.8	420.9	558.0	2995.6	460.9
2016	350.9	289.6	150.8	116.4	117.6	94.2	182.1	172.8	244.7	83.7	174.2	312.4	2289.4	352.2
2017	155.7	203.9	222.1	91.1	80.8	139.1	132.1	156.2	210.5	262.8	236.0	243.2	2133.5	328.2
2018	315.8	134.9	144.0	112.4	38.8	108.2	113.8	121.4	280.6	251.0	244.8	211.2	2076.9	319.5
2019	186.9	186.2	236.6	62.7	134.1	106.0	139.1	234.1	173.7	286.8	84.0	381.1	2211.1	340.2
Total/month	3726.1	3027.1	2125.7	1349.5	1625.8	1222.0	1579.5	1947.0	2141.5	3186.3	3029.8	4031.3		
Average/month	310.5	252.3	177.1	112.5	135.5	101.8	131.6	162.3	178.5	265.5	252.5	335.9		

Appendix 3.6: **Raw data of the total yearly precipitation in Loch Sunart 2008 – 2019.** Precipitation data was sourced from the World Weather for Water Data Service (W3S) for Loch Sunart (Latitude: 56.65° N, Longitude: 5.65° W) from 2008 – 2019. Data was extracted as daily measurements (mm precipitation) and summed up and averaged per month and year.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total/year	Average/year
2008	445.9	358.4	224.4	126.8	32.0	151.1	129.5	165.3	149.9	405.0	303.5	242.5	2734.5	420.7
2009	348.5	71.3	169.1	183.1	183.9	90.8	161.9	316.5	207.4	304.0	429.7	115.7	2581.9	397.2
2010	137.9	84.9	129.5	147.8	38.4	57.6	210.7	148.4	221.6	259.7	238.4	89.0	1763.8	271.3
2011	270.6	298.9	173.0	111.8	357.6	109.0	94.0	182.5	368.8	405.1	365.5	573.4	3310.4	509.3
2012	364.1	271.6	51.1	97.5	108.9	129.9	136.7	158.0	242.0	230.8	336.9	347.6	2474.9	380.8
2013	287.8	135.2	41.4	180.5	177.0	88.9	104.8	137.9	189.7	309.3	251.4	573.5	2477.5	381.1
2014	413.6	459.1	260.0	132.3	172.7	61.5	145.2	227.0	71.1	473.3	216.0	426.2	3057.8	470.4
2015	509.1	278.0	315.9	125.5	258.6	108.6	200.8	155.7	74.8	149.5	461.4	570.2	3208.0	493.5
2016	362.6	302.8	165.6	129.3	125.3	96.8	203.0	196.0	277.6	95.9	204.7	324.3	2483.8	382.1
2017	188.3	251.4	239.1	93.3	102.5	144.8	158.0	187.3	232.3	304.5	254.0	277.3	2432.8	374.3
2018	354.0	164.9	111.5	135.7	55.1	109.0	127.6	141.8	301.4	262.7	267.0	253.5	2284.2	351.4
2019	207.3	203.8	294.8	63.6	140.0	110.5	151.7	263.0	189.4	313.5	124.8	412.0	2474.5	380.7
Total/month	3889.7	2880.3	2175.4	1527.1	1752.0	1258.5	1824.0	2279.3	2525.9	3513.5	3453.2	4205.1		
Average/month	324.1	240.0	181.3	127.3	146.0	104.9	152.0	189.9	210.5	292.8	287.8	350.4		

Appendix 3.7: **Raw data of the average temperature around Loch Eil 2008 – 2019**. Temperature data was sourced from the World Weather for Water Data Service (W3S) for Loch Eil (Latitude: 58.85° N, Longitude: 5.30° W) from 2008 – 2019. Data was extracted as daily measurements (°C) and averaged per month.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2008	1.3	3.1	4.3	5.6	11.4	10.7	11.1	10.6	8.7	5.1	3.1	1.4
2009	0.9	1.0	2.6	5.8	7.1	9.4	10.7	10.5	10.9	7.5	3.2	-0.7
2010	-1.0	-1.4	4.4	5.5	6.1	11.1	10.4	9.8	9.4	8.7	3.2	-1.1
2011	2.6	2.1	2.7	8.1	6.3	11.0	13.3	10.7	9.2	6.9	6.0	1.3
2012	1.3	3.0	5.5	2.5	7.7	8.3	12.2	13.9	7.2	4.0	3.1	1.3
2013	1.5	1.0	-1.3	1.8	5.6	9.1	13.6	10.3	9.1	7.3	2.4	4.3
2014	4.4	4.7	6.6	9.2	9.3	13.1	14.2	10.2	10.4	7.4	4.9	1.0
2015	0.3	2.4	4.6	4.5	4.2	8.1	8.7	9.6	8.8	8.3	4.4	3.4
2016	1.1	0.1	3.1	1.9	7.6	10.4	9.6	10.6	10.1	6.4	2.2	4.2
2017	2.4	2.1	3.7	3.8	8.4	8.8	10.8	9.2	8.5	10.8	4.8	4.1
2018	3.2	2.6	3.4	7.6	12.7	14.4	15.4	13.2	10.4	8.9	6.8	3.1
2019	1.1	5.4	4.3	8.6	9.1	11.1	11.6	10.3	8.6	6.4	1.2	3.3
Average	1.6	2.2	3.7	5.4	8.0	10.5	11.8	10.7	9.3	7.3	3.8	2.1

Appendix 3.8: **Raw data of the average temperature around Loch Sunart 2008 – 2019.** Temperature data was sourced from the World Weather for Water Data Service (W3S) for Loch Sunart (Latitude: 56.65° N, Longitude: 5.65° W) from 2008 – 2019. Data was extracted as daily measurements (°C) and averaged per month.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2008	3.0	4.5	5.0	6.4	12.0	11.4	12.5	12.0	10.2	6.8	4.9	2.9
2009	2.7	2.9	4.4	7.4	8.4	10.8	12.1	11.9	11.8	9.0	4.9	0.7
2010	0.6	0.3	5.2	6.6	7.5	12.1	11.5	11.1	10.8	9.4	4.1	0.3
2011	3.7	3.8	4.4	9.2	7.8	11.4	13.6	11.6	10.7	8.6	7.6	3.1
2012	3.1	4.6	7.0	4.0	8.6	9.6	12.7	14.3	8.9	5.6	4.7	2.6
2013	3.1	2.6	0.7	3.5	7.0	10.4	14.4	11.7	10.4	8.8	4.2	5.6
2014	5.2	5.3	7.3	9.6	10.0	13.4	14.8	11.3	11.7	8.7	6.3	2.8
2015	2.1	3.6	5.4	5.9	5.9	9.4	10.2	11.1	10.2	9.5	6.1	5.1
2016	2.7	1.9	4.6	3.7	8.9	11.6	11.1	11.9	11.5	8.0	3.9	5.8
2017	4.1	3.8	5.2	5.5	9.2	9.5	11.3	10.0	9.3	11.4	5.7	4.9
2018	4.0	3.4	4.2	8.1	13.0	14.6	15.6	13.5	10.9	9.6	7.7	4.3
2019	2.2	6.2	5.1	9.2	9.5	11.5	12.2	10.9	9.5	7.3	2.3	4.4
Average	3.0	3.6	4.9	6.6	9.0	11.3	12.7	11.8	10.5	8.6	5.2	3.5

Chapter 4

Investigating the Effects of environmental parameters on spat mortality in Scottish Blue mussels (*Mytilus edulis*): A controlled in situ experiment in Loch Eil

Abstract

This study investigated the spat mortality in Loch Eil through controlled experiments, exposing spat to various environmental parameters, specifically to the impact of different dilutions of Loch Eil water, changes in salinity, and exposure to UV radiation on the survival of spat. Results revealed significant variations in spat mortality across different treatments. The controlled in situ experiments challenged initial hypotheses. Artificial seawater controls exhibited higher mortality than expected in the Loch Eil dilution series, as opposed to the mortalities exceeding 40% in treatments containing 10%, 25% and 50% Loch Eil water. Salinity experiments revealed a non-linear relationship, with higher salinity leading to increased spat mortality. Despite documented wide-ranging salinity tolerances of blue mussels, the study underscored the importance of salinity fluctuations on spat survival, with mortality rates varying between 17% and 41% in different experimental conditions. Surprisingly, higher salinity control treatments (31.8 ppt) exhibited mortality rates of 41%, contrasting with lower rates in lower salinity conditions, where mortality was 17%. UV treatment experiments yielded mixed results, suggesting potential benefits but inconclusive evidence for a pathogen-related cause of mortality. Cohabitation experiments with spat from Loch Sunart highlighted differential mortality rates compared to the spat population from Loch Eil, emphasising the possible influence of genetic variety on spat response to environmental stressors. The unexpected outcomes of these experiments shed light on the complex interplay of environmental, physiological, and microbial factors influencing spat survival in Loch Eil. The precise causes of mass mortality events in Loch Eil remain elusive, highlighting the challenges in addressing this significant issue in mussel aquaculture.

4.1 Introduction

The sustainability of aquaculture practices is intrinsically linked to our understanding of the complex interactions between farmed organisms and their surrounding environment. In the context of blue mussel (*Mytilus edulis*) aquaculture, the health and survival of mussel spat, particularly in the early stages of development (Petton et al., 2013), are critical determinants of overall production success. Spat mortality, influenced by various environmental parameters, poses significant challenges to the industry, demanding comprehensive investigations to understand the potential factors that lead to mass mortalities of mussel spat (Lynch et al., 2014; Pourmozaffar et al., 2020).

The method of exposing organisms to a dilution series, where the concentration of a substance is systematically reduced, has been widely employed in toxicological studies (Jha et al., 2005; Rajagopal et al., 2004), which warranted the thought of subjecting the spat to a dilution series of Loch Eil water to gather some valuable insight as to whether the mortalities are caused by a factor distinct to that particular body of water. Exposing the spat to various treatments in form of dilutions may possibly lead to a perceptible dose-dependent response, narrowing down the considerable reasons of recurring mortalities. Additionally, since the spat population in Loch Sunart seem unaffected by what has been occurring in Loch Eil, despite the occasional transfer of Loch Eil spat ropes into the mussel farms of Loch Sunart, the spat and environmental parameters of this loch acted as controls throughout most of the investigations and analyses in studies from previous chapters. This study focuses on exposing Loch Eil spat to an array of environmental parameters in controlled in situ experiments. Building upon previous research on spat mortality in mussels, this experimental chapter aims to investigate the effects of water dilution, salinity variations, and UV exposure on spat survival in Loch Eil.

Based on previous findings and anecdotal evidence from individuals within the shellfish sector in Scotland, Loch Eil has been exhibiting mortality events for over a decade. Chapter 2 delved into the environmental parameters of Loch Eil, analysing water quality, potentially toxic elements (PTEs) such as heavy metals (Rodney et al., 2007), salinity, and chlorophyll concentrations. Building upon

these findings, the next step was to conduct controlled in situ experiments to further investigate the potential causes of mass mortalities.

Given the observed differences in salinity between the two lochs (Chapter 2), it was hypothesised that salinity could be a contributing factor to spat mortality. To test this hypothesis, spat were exposed to two salinity levels in controlled experiments. Studies have shown that *M. edulis* can survive in salinities ranging from 10 – 35 parts per thousand (ppt) in various environments such as estuaries, intertidal and sublittoral areas (Connor et al., 2004; Landes et al., 2015; Maar et al., 2015). However, in the early ontogenetic stages, *M. edulis* may be less tolerant to low salinity stress compared to later stages (Qiu et al., 2002; van der Gaag et al., 2016).

Spat exposed to treated water were expected to exhibit improved survival rates compared to those exposed to untreated water. This would pose an assumption with a high probability that the mass mortalities in Loch Eil are being driven by a pathogenic causative agent. To broaden the scope of the study and elucidate the response of Loch Eil spat, Loch Sunart spat were also subjected to the treatments, allowing for comparisons between populations. If both spat populations were negatively impacted by UV-treated water, this would strengthen the hypothesis of a pathogen-related issue within the loch. Conversely, if both populations experienced high mortality rates, it might suggest the presence of a toxic compound resistant to UV treatment. However, if only one population exhibited significantly higher mortality in UV-treated water, genetic variability could be a contributing factor to differential susceptibility (Tremblay et al., 1998). Exposing Loch Eil spat to untreated Loch Eil water would serve as a negative control, given the regular occurrence of mortalities in this loch. Similarly, Loch Sunart spat, when exposed to untreated Loch Eil water, were expected to exhibit similar mortality rates, supporting the hypothesis of harmful pathogens within the loch. However, if one population displayed significantly higher survival rates in untreated water, this could indicate compromised immune function or genetic differences in susceptibility between the two populations.

4.2 Materials and methods

Experimental design

The goal of this experiment was to expose the spat originating from Loch Eil to different environmental parameters and monitoring their response. The experiments included a dilution series of the water from Loch Eil water mixed with artificial sea water (ASW), a salinity challenge comprising of two different salinities, and lastly, subjecting Loch Eil and Loch Sunart spat to UV-treated and untreated water deriving straight from the loch itself. The experimental design was constructed to mimic natural conditions, while regulating environmental variables and controlling factors that could influence the experiments and can be seen in Figure 4.1.

An on-site structure with electrical supply, located just a few metres from the pontoon, provided protection from any weather conditions throughout the trial. A trough (Length: 200 cm x Width: 120 cm x Depth: 12 cm) was placed on top of several wooden pallets until it functioned as an even, stable water bath for the experimental tanks, which all measured 10 litres in capacity. Tanks were equipped with air stones and silicone tubing connected to aquarium air pumps (Hidom HD-603), ensured adequate oxygenation and water circulation, where one air pump supplied two tanks. To keep the silicone tubes within the designated tanks, the ends were weighed down with stainless steel hex nuts.

tanks were filled up with the corresponding treatment seven days prior to the introduction of spat to inspect for any flaws in the experimental design. The spat were introduced to the corresponding tanks and left to acclimatise for 4 days prior to the first sampling date. By this point, the presence of faecal and pseudofaecal deposits within the tanks suggested that the spat had acclimatised to the experimental treatments. (Mizuta & Wikfors, 2019). All tanks were stocked with a roughly estimated amount of approximately 50 individuals, based on a weighed sample set, as the priority was to avoid inflicting any excessive stress on the animals (Table 4.1). The raw data containing the individual weights and replicates can be found in Appendix 4.1.

Table 4.1: **Spat weights for stocking trial tanks.** Replicates (A – B) of 50 spat from Loch Eil and Loch Sunart were individually weighed on a portable balance (Ohaus Scoutpro) on 14/10/2021. The recorded weights (wt) of the individual spat can be found in Appendix 4.1. The anticipated average weight per spat was calculated by averaging the total weight of 50 spat per replicate ($n = 50$) across all replicates (A – B, $n = 3$).

	Loch Eil			Loch Sunart		
Replicate	A	B	C	A	B	C
Total wt	66.77	56.00	30.26	37.86	22.52	27.30
Average wt per replicate	1.34	1.12	0.61	0.76	0.45	0.55
Sum of replicates		3.06			1.75	
Average of replicates		1.02			0.58	
Anticipated wt for $n = 50$		**51.01**			**29.23**	

The total amount of spat within each tank was eventually counted on the last day of the trial, and the number of individuals removed for analyses were added on for an accurate representation of the stocking density. Loch Eil originating spat was used for the dilution and salinity trials, whereas a combination of estimated equal amounts of Loch Eil and Loch Sunart spat populations cohabited the tanks in the UV challenge. Daily checks of the trial tanks included temperature and salinity measurements with an EcoSense® probe (EC300A) and the removal of dead spat.

Apart from spat in tanks 28 – 30, all were fed with Algae Shellfish Diet 1800 (Reed Mariculture, Campbell, CA) consisting of four inactivated algae (*Isochrysis*, *Pavlova*, *Thalassiosira weissflogii*, and *Tetraselmis*). According to the technical data sheet, a concentration of 0.7 ml Shellfish Diet per gram live weight of spat should be administered per day. After consulting with the farmers on site, the concentration was adjusted to 120 ml shellfish diet per 51 g live weight of spat (2.4 ml diet per gram live weight of spat) and administered as per the instructions on the technical data sheet. With the intent of mimicking the natural food availability during October in Loch Eil and maintaining optimal water chemistry throughout the trial period, the feeding frequency was set to a four-day regime. The adjusted feed concentration was estimated using chlorophyll-a concentrations, measured from water samples taken in October 2019 from Loch Eil (0.39 – 0.49 µg L^{-1}, Figure 3.2: Chlorophyll a concentration (µg L^{-1}) in Loch Eil and Loch Sunart, in Chapter 3), as a proxy. To avoid further added stress to the spat, the thorough examination and removal of dead spat took place once a week and never on the same day as feeding or water quality testing.

The observation period monitoring the response of the spat to each of the experiments took place over a 3-week period where sampling occurred 4-, 10- and 22-days post stocking. On the last day of the experimental trial all the individuals within the tanks were thoroughly examined and the last of the dead spat was removed. The remaining live spat was counted to provide the precise stocking density of each tank. All treatments were performed in triplicate to assure a suitable sample set for the data quality assessment and statistical analysis.

Tests for water quality parameters were conducted to ensure a stable, well oxygenated, and suitable environment for an overall standardised experiment, for which the metadata can be found in results Table 4.3. A Tropic Marin Professional Lab Test Kit Set (Tropical Marine Centre) was used and included tests for phosphate, ammonia and ammonium (NH_3 and NH_4), nitrate and nitrite. As soon as the water chemistry within any tank displayed unsuitable levels of the tested parameters, the water was replenished until appropriate levels were measured. Given the classification of Loch Eil as a Class A water body, a formal sterility check was not performed. All additional metadata pertaining to water quality and maintenance are shown in Appendices 4.2 – 4.4. Individual tank numbers, corresponding treatments including stocking density and mortality numbers can be found in Appendix 4.5.

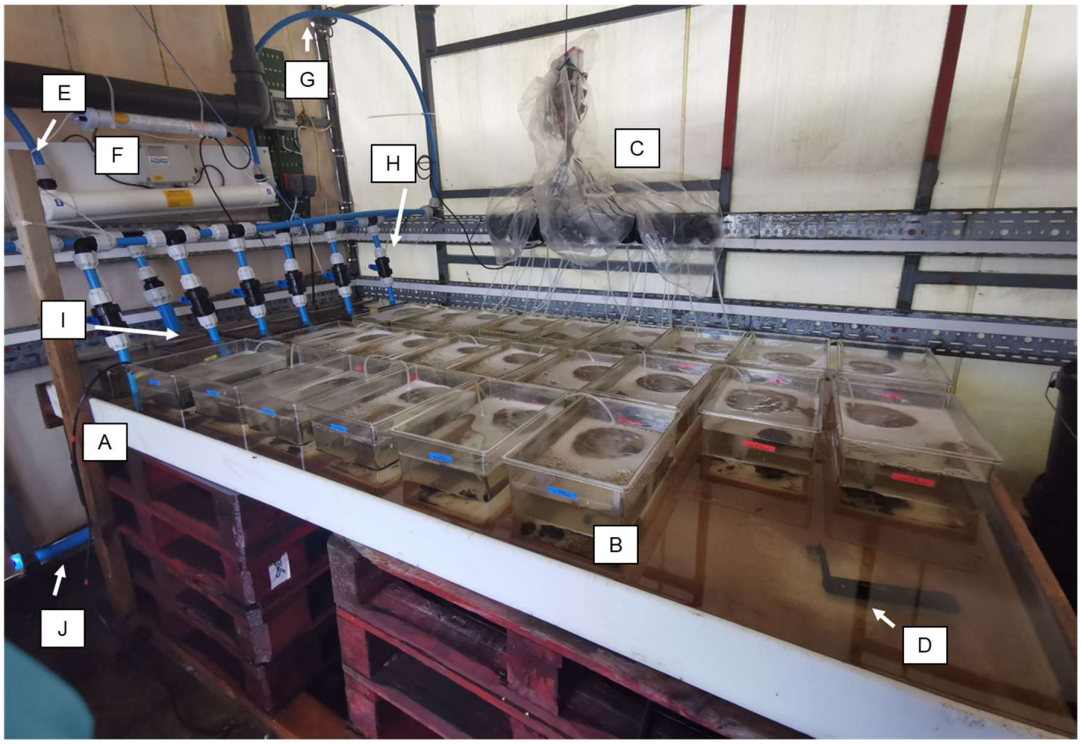

Figure 4.1: **Experimental set up of the tanks for the individual trials.** Inside an on-site structure, a modified trough acted as a water bath (A) for the trial tanks (B), enabling a stable and consistent temperature over the duration of the experiment. Aquarium pumps (C) were covered to protect from moisture. Data loggers (D) were placed in the trough to record temperature and salinity for the duration of the trial. Water was directly pumped from Loch Eil (E) and split, half of which directed through the UV steriliser (F) filling the designated tanks (I) with UV treated water (G). The untreated water filled the other assigned tanks (H). Water flow was controlled with valves and taps. Excess water was led away from the workspace by an overflow pipe (J).

Loch Eil dilution challenge

Because Loch Eil has a history of exhibiting mortality events of spat, exposing the animals to a series of diluted Loch Eil water could provide some insight as to whether the mortalities are caused by something that is distinct to this particular body of water. Exposing the spat to various treatments in form of dilutions may possibly lead to a perceptible dose-dependent response, narrowing down the considerable reasons of recurring mortalities.

A total of 18 tanks were filled with ca. 10 litres with six different concentrations of Loch Eil water (Table 4.2). Tanks 1 – 3 were filled with water obtained directly from Loch Eil; tanks 4 – 6 received an even mixture of Loch Eil water and artificial sea water, which was prepared with Tropical Marine salt and degassed fresh water from the mains on site. The dilutions were prepared representing the same

salinity as to the in situ measurements from Loch Eil at that given time (25 parts per thousand, ‰). Tanks 7 – 9, 10 – 12, 13 – 15 and 16 – 18 were filled with a ratio (Loch Eil water: ASW) of 1:4, 1:10, 1:100 and 1:1000 respectively, creating an overall dilution series encompassing 100%, 50%, 25% 10%, 1% and lastly 0.1% Loch Eil water. The salinity treatment functioned as a control to this experiment as the spat were exposed to 100% ASW only.

Table 4.2: **Dilution series for the dilution challenge.** The trial was performed in triplicate, whereby each tank trio (4 – 6, 7 – 9, 10 – 12, 13 – 15, 16 – 18) was filled with a dilution made up of water from Loch Eil and artificial sea water (ASW). Tank trio 1 – 3 was filled with Loch Eil water only and acted as the positive test control. The dilution percentages refer to the quantity of Loch Eil water within the trial tanks. Salinity for each tank was adjusted to the in-situ salinity of Loch Eil. The volume is presented in ml.

Tanks	**1 - 3**	**4 - 6**	**7 - 9**	**10 - 12**	**13 - 15**	**16 - 18**
Dilution	100%	50%	25%	10%	1%	0.1%
Vol Loch Eil [ml]	10,000	5,000	2,500	1,000	100	10
Vol ASW [ml]	-	5,000	7,500	9,000	9,900	9,990

Salinity challenge

During the investigation of environmental parameters in Chapter 3, significant differences in salinity were observed. Therefore, conducting an experiment subjecting Loch Eil spat to two extremes of the recorded salinities from Loch Eil and Loch Sunart would establish the preference and tolerance limit of the spat. Each of the salinities was prepared in 30 litre buckets ensuring a sufficient and well-mixed solution fitting for the triplicated experiment.

Six tanks (19 – 24) were filled with two different salinities. Tanks 19 – 24 were filled with ASW mixed to 18.1 parts per thousand (ppt), which represented the lowest salinity recorded in Loch Eil over a 2-year period, whereas tanks 22 – 24 were filled ASW made up to 31.8 ppt; which referred to the highest salinity recorded in Loch Sunart. Given the lack of historical data on the effects of salinity on Loch Sunart spat, this experiment served as a preliminary investigation to assess the potential impact of salinity on spat survival. Without a well-established control group, the results from this experiment were exploratory, providing insights into the potential role of salinity in mortality events.

UV treated cohabitation challenges

By exposing Loch Eil spat to UV-treated Loch Eil water, any viruses present in the water would be inactivated. In contrast, if there is a causative agent present in untreated Loch Eil water, spat exposed to this water were expected to experience mortality. Spat originating from the control site, Loch Sunart, did not only function as the control in this experiment but assumed to also provide some insight, depending on the spat's response to the different treatments.

Using a soldering iron, small holes (ca. 1 cm in diameter) were pierced through one side, roughly 5 cm from the rim, of 6 tanks (25 – 30). These holes functioned as an overflow without risking flushing any mussel spat from the tanks. The drainage from tanks 25 – 30 filled the water trough, keeping the other trial tanks in place, but also facilitating a steady overall temperature within all the tanks throughout the trial period. The individual tanks were separated by modified pieces of durable plastic and covered with fine mesh netting to fit firmly into the centre of the tanks, providing a barrier between the two populations. Water was directly pumped from the loch through 100 m of 50 mm diameter polyethylene pipe. A tee joint was attached on the receiving side of the pipe to divide the waterflow through a different set of polyethylene pipes, one of which went straight to the taps supplying tanks 25 – 27, whereas the other pipe diverted the water through a commercial UV steriliser equipped with a 55W UV tube (P1 55W Lamp; 50-62WATTS 110-240V 50-60Hz) before reaching the taps supplying tanks 26-30. The submersible pump was strategically placed at a depth of eight metres in Loch Eil, approximately 10 metres away from the shore, to ensure a stable supply of Loch Eil water with consistent salinity levels, rather than freshly mixed water from the surface area. Additionally, placing the pump at that depth facilitated an allowable scope of any fluctuations in water intake due to spring tides.

According to the mussel farmers, high spring tide usually measures a static pressure head of three metres, in which case the pump will supply 12.5 m^3 h^{-1}, whereas low spring tide measures 7 metres static head, resulting to the pump supplying 8.5 m^3 h^{-1}. By accurately calculating the appropriate flow rate necessary for the UV-challenge experiment, the pump had significant reserve as the flow was regulated with the valves that were installed at every end of the pipes supplying the tanks.

The flowrate was manually regulated by taps that were installed at every inlet pipe for the tanks, this ensured a steady flow when tidal or weather conditions fluctuated, and to generate an appropriate flow through the UV steriliser, attaining an adequate UV dosage of the water. The flow rate had to equally create some circulation within the tanks but with the intent of avoiding any disturbances that could cause further stress to the spat. To achieve this, the friction head of the PE pipes was calculated and read along the pump performance curve (Figure 5.2). The mussel farmers recommended a 10-fold flow per hour, per tank, which calculated to a flow of 100 L h^{-1} for one 10-litre tank, and to supply six 10 litre tanks, the total required flow was 600 L h^{-1}. The friction head at this flow measured 0.025 m, which was considered almost negligible. The recommended UV fluence for small scale systems by (CEFAS, n.d.) showed 25W for a flow of 2.5 L h^{-1}, whereby our system supplied 3 tanks at a total flow rate of 300 L h^{-1} (or 5 L min^{-1}) via a new 55W lamp.

Feeding did not occur for the animals in tanks 28 – 30, as these were supplied with a continuous flow-through of Loch Eil water pumped directly from the loch. The spat in tanks 25 – 27, subjected to the UV-treated water, were fed 120 ml Algae Shellfish Diet (2.4 ml per gram live weight) every 4 days, as most UV-sterilisers kill phyto- and zooplankton. A more detailed visual description of the UV-treatment set up is illustrated in Figure 4.3.

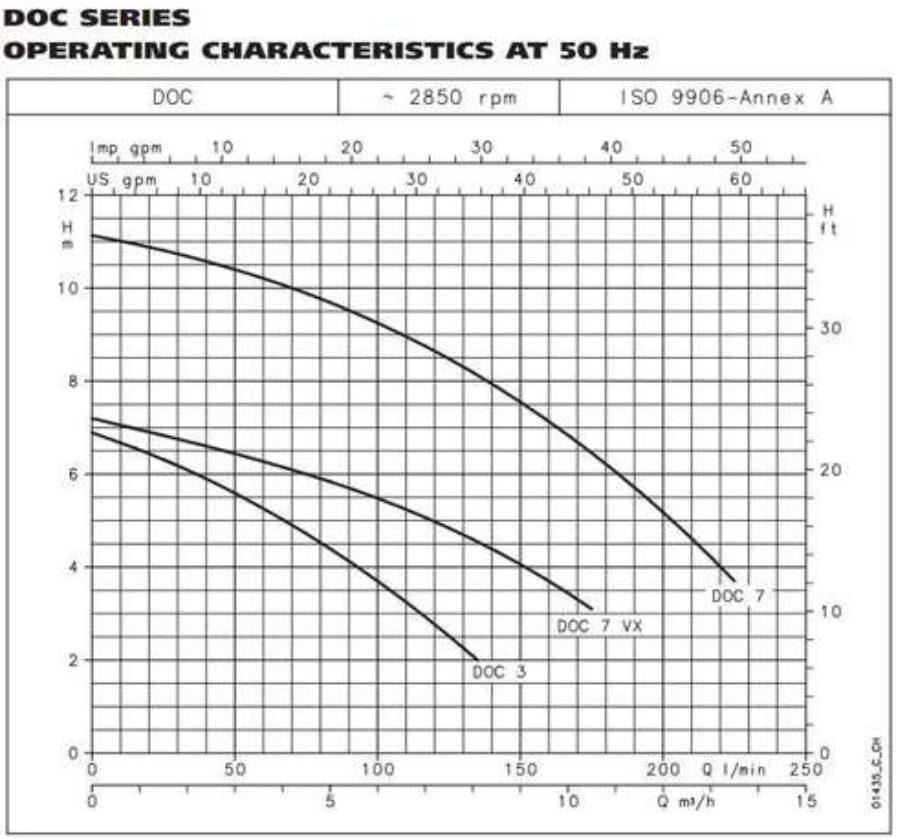

Figure 4.2: **DOC pump performance curve**. The pump performance curve for DOC 7 model was essential during the set-up of the equipment. This allowed for optimal pump performance and generating a steady and consistent flow rate, suitable for the trial.

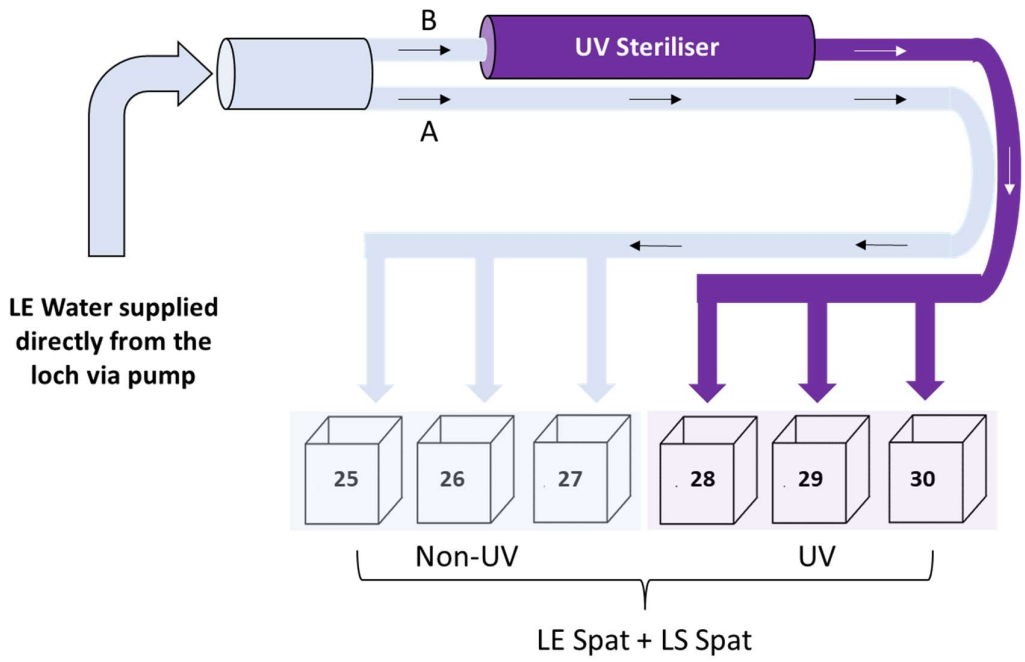

Figure 4.3: **Experimental set up of the UV treatment challenge**. Water pumped directly from Loch Eil was split into two pipes, pipe A led to tanks 25 – 27, supplying the tank trio with non-UV treated water. Pipe B led the pumped water through a UV steriliser (P1 55W) before filling tanks 28 – 30. Taps were adjusted to a steady, continuous flow rate of 0.6 m^3 h^{-1} (10 L min^{-1}) to each of the tanks. Tanks 28 – 30 were fed 120 ml Algae Shellfish Diet 1800 (Reed Mariculture – Inactivated *Isochrysis*, *Pavlova*, *Thalassiorira weissflogii* and *Tetraselmis)* every 4 days. The trial was performed in triplicate. All trial tanks accommodated a cohabitation of Loch Eil and Loch Sunart originating spat.

Statistical Analyses

To address the need for standardising the dataset and facilitating the detection of linear relationships among variables, the raw counts of spat mortalities were firstly transformed into proportions by dividing the count of dead spat from each treatment by the corresponding stocking density. After converting the counts into proportions, the inverse sine of the square root was calculated of those proportions with the purpose of normalising, stabilising the variance, and therefore ensuring a robust statistical inference in the proportional dataset, suitable for parametric analyses. One-way ANOVA tests were performed to identify significant associations between mortalities and experimental treatments, while general linear models were applied to examine temporal patterns in spat mortalities across different timepoints and treatments and the interactions between the two factors. The Tukey Method was used for *post hoc* examination

in all analyses. The calculated p-values were considered significantly different when $p < 0.05$.

4.3 Results

Loch Eil dilution challenge

The results from the Loch Eil dilution challenge are depicted in Figure 4.4. The total mortality from each concentration, including two controls, over the experimental trial is illustrated in Figure 4.4 a, whereas Figure 4.4 b shows the percentage of mortalities from each treatment at the given time points (number of days post stocking), and Figure 4.4 c shows the cumulated percentage values of the mortalities over the trial. The counted dead individuals over each time point and from every dilution can be found in Appendix 4.5**,** as well as the statistical analyses in Appendix 4.6.

During the first time point (4 d) the spat mortalities in all dilution trial tanks, apart from one control, measured below 10%. The pure Loch Eil water represents the natural environment in which the mortalities are occurring real time whereas the 100% ASW treatments from the salinity challenge function as the controls.

Figure 4.4 a clearly shows a significant variation in between one of the controls (100% ASW, 18.7 ppt, blue) and the different dilutions of Loch Eil water ($p < 0.001$). However, the second control, which is 100% ASW at 31.8 ppt (orange) exhibited higher mortality than the 100%, 1% and 0.1% Loch Eil water dilutions, yet slightly less than 50%, 25% and 10% Loch Eil water dilutions. By week 2 the mortalities have increased by approx. 50% in all tanks apart from dilutions 25%, 1% and 31.8 ppt control. In Figure 4.4 b, the mortalities of each treatment at 4 d, 10 d, and 22 d post stocking show a significant increase over time, whereby the highest percentage occurred during the last time point ($p < 0.001$). The mortality counts after the 4- and 10-day observation period show no notable differences between the time points nor between the individual dilutions. As of day 22, there is a significant difference between the treatments ($p < 0.001$), control 18.7 ppt ($p < 0.001$) and control 31.8 ppt ($p = 0.001$).

Figure 4.4 c shows the cumulated percentage of mortalities of each treatment (no controls). The observed mortalities of each of the time points are visualised in

stacked bars for the individual dilution, representing the total mortality percentage over the entire experimental period. By the end of week 3, the highest spat mortality was observed in the 25% Loch Eil dilution with just under 40%, closely followed by treatments 50% and 10%, exhibiting roughly 36% mortality. The mortalities in the controls have increased over the trial yet remain below the values of the other dilutions.

Salinity challenge

The results of the salinity challenge are shown in Figure 4.5, in which Loch Eil spat was exposed to the lowest recorded salinity in Loch Eil (18.7 ppt – blue) and the highest measured salinity in Loch Sunart (31.8 ppt – orange).

There is a difference in mortalities when comparing the two different salinity treatments over the entire experimental trial ($p = 0.03$) (Figure 4.5 a). The spat exposed to the lower salinity reached an overall mortality of 17%, whereas a mortality of 41% was observed in the higher salinity treatment. Over the individual time points (Figure 4.5 b), noticeable differences between the treatments have been observed 4 d ($p = 0.007$) and 22 d ($p = 0.2$) post stocking, however only near significant differences were observed between the mortality and the time points ($p = 0.1$). Spat mortality remained under 10% within the lower salinity over the entire period, whereas the higher salinity exhibited just over 10% after 4 and 10 days, and nearly 23% after 22 d post stocking. The severe increase of mortalities within the 31.8 ppt salinity treatment is emphasised in Figure 4.5 c. The mortalities of the individual time points are visualised as stacked bars for each treatment, expressing the total mortality of each treatment over the experimental period.

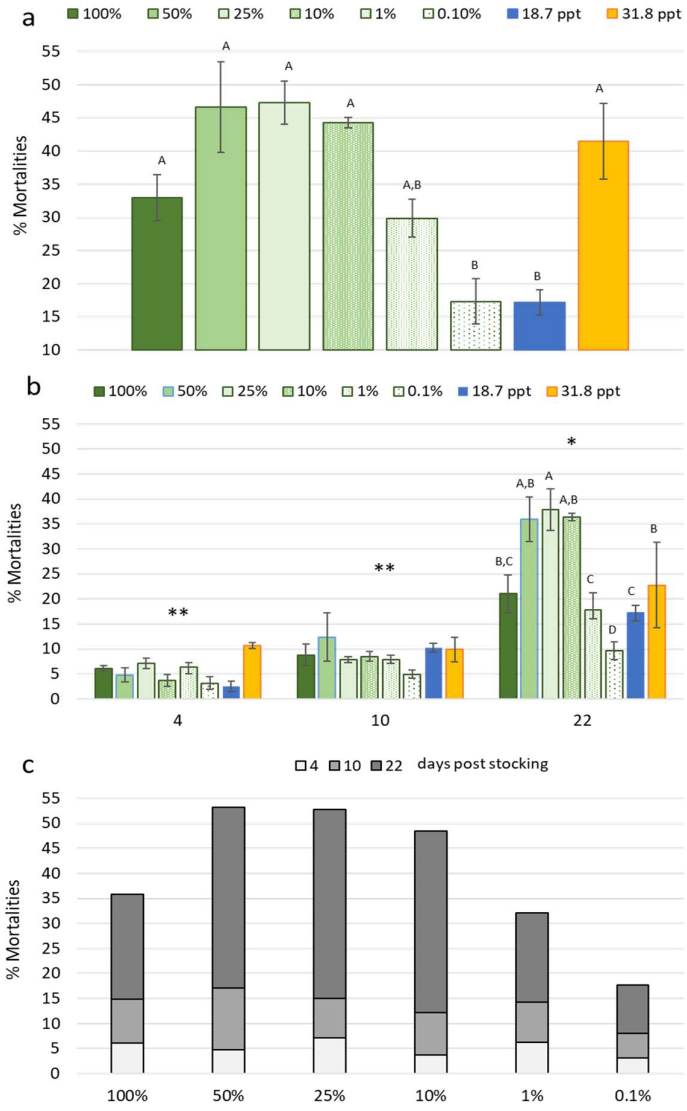

Figure 4.4: **Dose dependent response of Loch Eil dilution challenge.** Loch Eil spat were exposed to 100% -, 50% -, 25% -, 10% -, 1% - and 0.1% - dilutions of Loch Eil water mixed with artificial seawater (ASW). Dead spat were counted and removed from tanks weekly. Percentage mortality was calculated based on the stocking densities of the tanks and averaged by the number of replicates ($n = 3$). Salinity trial tanks with 100% ASW (18.7 ppt – blue, 31.8 ppt – orange) acted as controls for this experiment. Panel a shows the mortality percentage in each treatment over the trial period. Panel b shows the mortality percentage in each treatment at different time points. Panel c shows a stacked representation of panel b, whereby mortality percentages from each time point are stacked on the corresponding treatment. Error bars in panels a and b report the standard error of the mean. Tukey Method was used for post hoc examination. Different letters imply significant pairwise differences ($p < 0.05$) between the various dilutions. Asterisks (* vs **) imply significant differences ($p < 0.05$) between time points (4-, 10-, 22 days post stocking). No controls were used in panel c as the aim of this experiment was to observe any dose-dependent response within the different dilutions of Loch Eil water.

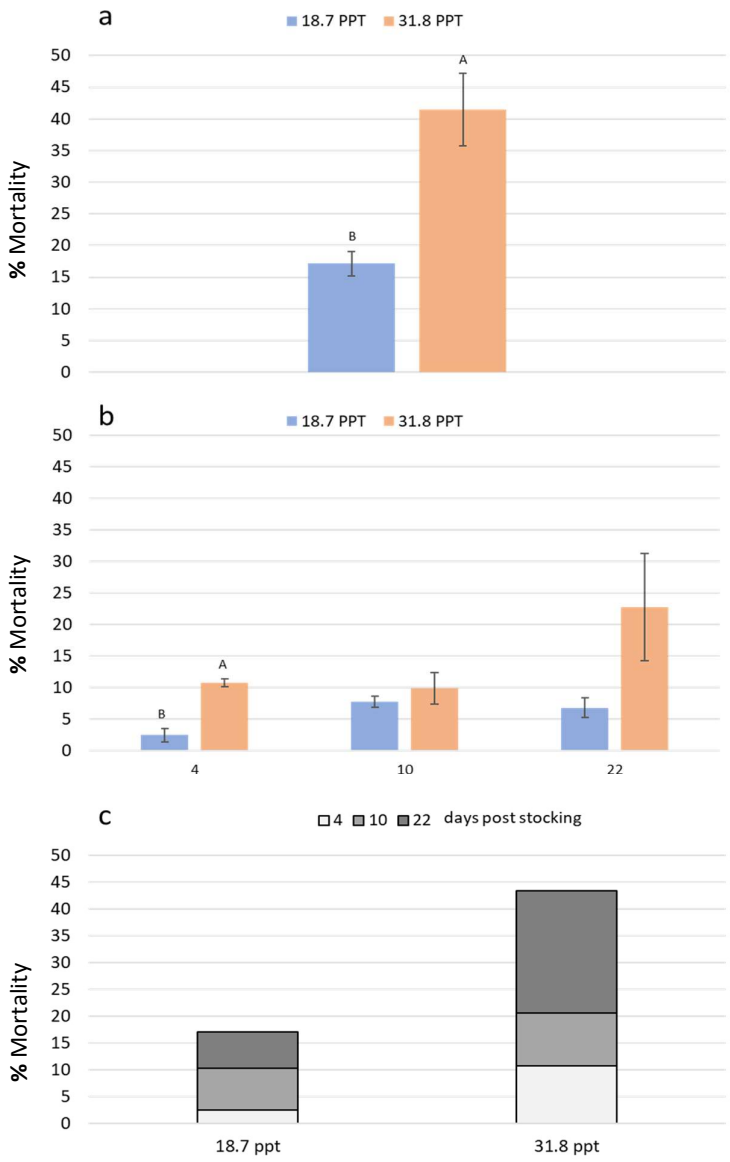

Figure 4.5: **Salinity tolerance of Loch Eil spat.** Loch Eil spat were exposed to two distinct salinities in ASW, 18.7 ppt (blue – lowest recorded salinity in Loch Eil) and 31.8 ppt (orange – highest recorded salinity in Loch Sunart). Percentage mortality was calculated based on the stocking densities of the tanks and averaged by the number of replicates ($n = 3$). Panel a shows the mortality percentage in each treatment over the trial period. Panel b shows the mortality percentage in each treatment at different time points. Panel c shows a stacked representation of panel b, whereby mortality percentages from each time point are stacked on the corresponding salinity. Error bars in panels a and b report the standard error of the mean. Tukey Method was used for post hoc examination. Different letters imply significant pairwise differences ($p < 0.05$) between the two salinities.

UV vs non-UV treatment and cohabitation challenge

Each of the treatment tanks was stocked with Loch Eil and Loch Sunart spat. The comparison of the cohabitants within the treatments is depicted in Figure 4.6. No mortalities were observed in any treatments during the first time point (4 d), Both populations appear to remain below the 10% mortality percentile within the two treatments, displayed in Figure 4.6 a and Figure 4.6 b, by 10 d post stocking. However, mortality rate of the Loch Sunart spat population increases substantially by the second time point, nearly reaching 60% mortality. Contrasting to spat originating from Loch Eil, exhibiting a mortality of 14% (Figure 4.6 a), which displays obvious differences between the mortalities of the cohorts during the UV-treatment ($\mathrm{p} = 0.003$) and the two time points ($\mathrm{p} = 0.02$). A similar trend has been detected in the mortalities exposed to the untreated water (Figure 4.6 - b). Despite an increase in mortalities by 22 d post stocking and no notable changes in the mortalities of the separate cohabitants, a clear difference was observed between the time points within the non-UV treatment ($\mathrm{p} = 0.02$).

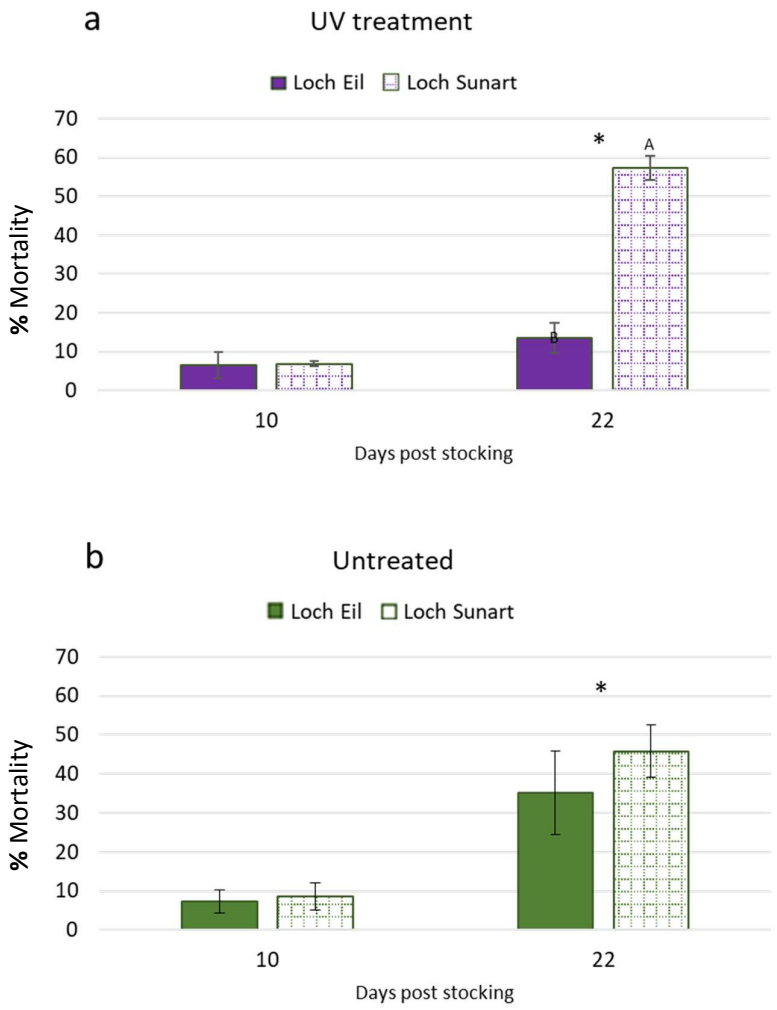

Figure 4.6: **Mortality observation in UV/non-UV trial.** A comparison of mortalities from the different spat populations within treatments. The error bars express the standard error of the mean. Different letters imply significant differences ($\boldsymbol{p < 0.05}$) between the populations at the given time point, whereas the asterisks imply significant differences ($\boldsymbol{p < 0.05}$) between the time points (10- and 22-days post stocking).

.

Water quality, maintenance and feeding regime

A strict water quality management system was maintained throughout the experiment, assessing oxygen levels, ammonia, nitrite, and nitrate on a weekly basis, for which the metadata can be found in Table 4.3. Salinity limits were self-established for the Loch Eil dilution series (24.4 ppt – 25.4 ppt) and the salinity challenge (18.7 $\pm$ 0.5 ppt and 31.8 $\pm$ 0.5 ppt). Tables with temperature and salinity data for the individual experiments are shown in Appendices 4.2 and 4.3. Tanks that indicated suboptimal salinity or water chemistry and required replenishing are shown in Appendix 4.4.

Table 4.3: **Summarised metadata of the experimental trial 2021.**

Daily checks included temperature and salinity measurements. Water quality measurements including PO_4, NH_3/NH_4, NO_3 and NO_2 were performed weekly. Data is provided for each tank (1 – 30) and is expressed as mean (± standard deviation, SD), whereby daily temperature and salinity measurements and the weekly WQ measurements were averaged per tank. Temperature values are presented in °C, salinity in parts per thousand (ppt) and the water quality data in mg L^{-1}. Fields without SD, indicate and SD of 0.

Tank	Treatment	Temp °C	Salinity ppt	WQ [mg L^{-1}]			
				PO_4	NH_3/NH_4	NO_3	NO_2
			Accuracy of test	*0.05*	*0.03*	*0.5*	*0.002*
1		11.8 (± 1)	25.2 (± 0.3)	0.02 (± 0.01)	0.02	0.04 (± 0.05)	0
2	100% Loch Eil	11.8 (± 1)	25 (± 0.4)	0.02 (± 0.01)	0.02	0.15 (± 0.13)	0.01 (± 0.01)
3		11.7 (± 1.1)	24.9 (± 0.5)	0.03 (± 0.05)	0.03	0.15 (± 0.23)	0.01 (± 0.01)
4		11.5 (± 1.2)	25 (± 0.4)	0.01 (± 0.01)	0.02	0.06 (± 0.05)	0
5	50% Loch Eil	11.5 (± 1.2)	25.2 (± 0.3)	0.03 (± 0.01)	0.03 (± 0.02)	0.18 (± 0.13)	0.01 (± 0.01)
6		11.5 (± 1.2)	25.1 (± 0.2)	0.02 (± 0.01)	0.02	0.25 (± 0.21)	0.01 (± 0.01)
7		11.5 (± 1.2)	24.9 (± 0.4)	0.03 (± 0.02)	0.04 (± 0.02)	0.19 (± 0.23)	0.05 (± 0.1)
8	25% Loch Eil	11.5 (± 1.3)	25 (± 0.2)	0.02 (± 0.01)	0.02	0.6 (± 0.05)	0.01 (± 0.01)
9		11.5 (± 1.3)	25.1 (± 0.4)	0.01 (± 0.01)	0.02	0.15 (± 0.23)	0
10		11.9 (± 1.3)	25.1 (± 0.4)	0.02	0.02	0.05 (± 0.04)	0
11	10% Loch Eil	11.9 (± 1.3)	25.1 (± 0.4)	0.02 (± 0.01)	0.03 (± 0.02)	0.05 (± 0.04)	0.01 (± 0.01)
12		11.7 (± 1.4)	25.2 (± 0.3)	0.02 (± 0.01)	0.02	0.09 (± 0.09)	0
13		11.6 (± 1.4)	25 (± 0.4)	0.04 (± 0.04)	0.03 (± 0.02)	0.18 (± 0.22)	0.01 (± 0.01)
14	1% Loch Eil	11.6 (± 1.4)	25.2 (± 0.3)	0.02 (± 0.01)	0.03 (± 0.02)	0.16 (± 0.23)	0.01 (± 0.01)
15		11.6 (± 1.4)	25.1 (± 0.3)	0.02 (± 0.01)	0.03 (± 0.02)	0.25 (± 0.29)	0.6 (± 0.1)
16		11.6 (± 1.4)	25.1 (± 0.3)	0.01	0.02	0.05 (± 0.04)	0
17	0.1% Loch Eil	11.6 (± 1.4)	25.1 (± 0.3)	0.02 (± 0.01)	0.02	0.16 (± 0.23)	0.03 (± 0.05)
18		11.6 (± 1.4)	25.1 (± 0.4)	0.02 (± 0.01)	0.02	0.18 (± 0.22)	0.01 (± 0.01)
19		11.8 (± 1.3)	19.1 (± 0.3)	0.02 (± 0.01)	0.02	0.02 (± 0.05)	0.01 (± 0.01)
20	18.7 ppt	11.7 (± 1.3)	19.1 (± 0.3)	0.02 (± 0.02)	0.02 (± 0.02)	0.02 (± 0.04)	0.02 (± 0.01)
21		11.6 (± 1.3)	19.1 (± 0.2)	0.03 (± 0.01)	0.03 (± 0.02)	0.02 (± 0.14)	0.02 (± 0.05)
22		11.6 (± 1.4)	32 (± 0.5)	0.01 (± 0.01)	0.02	0.02 (± 0.03)	0.02 (± 0.01)
23	31.8 ppt	11.5 (± 1.4)	32 (± 0.3)	0.03 (± 0.02)	0.02	0.09 (± 0.09)	0.02 (± 0.01)
24		11.5 (± 1.4)	31.9 (± 0.4)	0.02 (± 0.04)	0.02 (± 0.02)	0.02 (± 0.13)	0.02 (± 0.05)
25		12.4 (± 0.9)	22.9 (± 3.7)	0.01 (± 0)	0.02 (± 0)	0.04 (± 0.03)	0
26	UV	12.4 (± 0.9)	22.9 (± 3.7)	0.01 (± 0)	0.02 (± 0)	0.04 (± 0.03)	0
27		12.4 (± 0.9)	22.9 (± 3.7)	0.01 (± 0)	0.02 (± 0)	0.04 (± 0.03)	0
28		12.4 (± 0.9)	22.9 (± 3.7)	0.01 (± 0)	0.02 (± 0)	0.04 (± 0.03)	0
29	Non-UV	12.4 (± 0.9)	22.9 (± 3.6)	0.01 (± 0)	0.02 (± 0)	0.04 (± 0.03)	0
30		12.4 (± 0.9)	22.9 (± 3.7)	0.01 (± 0)	0.02 (± 0)	0.04 (± 0.03)	0

4.4 Discussion and conclusions

This study aimed to investigate the response of Loch Eil spat to various environmental parameters through controlled in situ experiments. The experimental setup involved exposing spat to different dilutions of Loch Eil water, varying salinities, and subjecting them to UV-treated and untreated water. The findings provide valuable insights into the factors influencing spat mortality, and by integrating the outcome from this experiment with observations from previous chapters, we anticipate discovering the driving causes that lead to the reoccurring mortality events in Loch Eil.

Loch Eil dilution series

Anticipating an observable dose-dependent response, spat was exposed to a dilution series of Loch Eil water. The dilution challenge revealed significant variations in spat mortality across different treatments, indicating that dilutions with 25%, 50% and 10% Loch Eil water content exhibited the highest spat mortalities of over 40%. Mortality rates within those treatments increased over time, with higher mortalities observed in tanks containing higher concentrations of Loch Eil water.

Interestingly, the ASW control with the higher salinity exhibited higher mortality than expected, highlighting the complex interplay between salinity and mortality. The cumulative mortality percentages underscore the importance of considering long-term effects on spat health (Qiu et al., 2002; van der Gaag et al., 2016)

The Loch Eil dilution challenge provided some insights into the susceptibility of mussel spat to variations in water salinity. The experiment revealed significant differences in spat mortality between treatments, with the pure Loch Eil water and artificial seawater controls exhibiting contrasting outcomes. Notably, mortalities increased over time across all dilution treatments, highlighting the progressive nature of any present stressors influencing spat health. The observed mortalities draw attention to the vulnerability of mussel spat to environmental fluctuations.

Salinity challenge

The salinity challenge demonstrated a clear difference in mortality rates between the low and high salinity treatments. Spat exposed to the salinity concentration of

18.7 ppt exhibited lower mortality rates showing a 17% total mortality, compared to those exposed to higher salinity of 31.8 ppt, where 41% of the spat died by the end of the trial. Initially, this result came as a surprise as the higher salinity treatment was expected to show higher spat survival rates during the experiment, for two reasons. First, the mass mortality events were localised and limited to Loch Eil, and Loch Linnhe many years ago. Secondly, prior to the start of the general investigation of spat mortality, the mussel farmers had transferred populated spat ropes from Loch Eil to Loch Sunart, in which case the spat survived and thrived. The significant increase in mortalities over time, particularly in the higher salinity treatment, emphasises the impact of salinity fluctuations on spat survival (van der Gaag et al., 2016; Westerbom et al., 2002; Wing & Leichter, 2011). The fact that the spat succumbed in the higher salinity could have been influenced by a variety of factors and circumstances. One assumption is that the immune response of the spat was already compromised prior to stocking the trial tanks, and the exposure to an additional stressor, in this case an increased salinity of nearly 7 ppt. Therefore, it would appear that the sudden exposure of Loch Eil spat to the higher salinity treatment could have “shocked” the already weakened spat, thus leading to fatalities. Additionally, farmers would have relocated spat from Loch Eil to Loch Sunart during a different time of the year, rather than in the winter months, such as November, when this experiment took place. Furthermore, despite having meticulously prepared the ASW, the medium itself is designed for recreating marine environments. It could have therefore lacked certain essential components necessary for spat survival in a modified environment.

Overall, the salinity challenge shed light on the impact of salinity levels on spat mortality, with contrasting outcomes observed between treatments. During each time point, the spat exposed to lower salinity conditions exhibited lower overall mortality rates compared to those subjected to higher salinity levels. Despite the documented wide range of salinity tolerances of the blue mussel, this finding accentuates the importance of the effects of salinity on the endurance of mussel spat (van der Gaag et al., 2016; Wing & Leichter, 2011). The experiment also revealed temporal variations in mortality rates, denoting the dynamic nature of environmental stressors and their influence on spat health over time (Lynch et al., 2014; Rayssac et al., 2010).

UV exposure cohabitation challenge

The cohabitation challenge in UV vs. non-UV treatment explored the effects of UV treatments on spat mortality from Loch Eil and Loch Sunart populations. During the research conducted for Chapter 4, bacteriological analyses have revealed the presence of *Vibrio* spp., a known mass mortality causing pathogen among shellfish including oysters and mussels (Paillard et al., 2004; Zannella et al., 2017).

The addition and observation of Loch Sunart population within the treatments aided in the understanding of the spat response from Loch Eil, therefore spat from Loch Sunart were simultaneously subjected to the treatments, in cohabitation with the population from Loch Eil. The cohabitation with spat from Loch Sunart resulted in differential mortality rates compared to Loch Eil spat, highlighting the importance of population diversity, genetics, and long-term exposure to various environmental factors. Cohabitation experiments revealed differences in mortality rates between spat originating from Loch Eil and Loch Sunart, highlighting the influence of genetic factors on spat response to environmental stressors (Lynch et al., 2014). Exposing Loch Eil spat to untreated Loch Eil water functioned as a negative control, as mortalities regularly occur within this body of water. By subjecting Loch Sunart spat to the untreated water, we anticipated a similar response as to that of Loch Eil, which ultimately is fatality, based on the assumption of harmful pathogens in the loch. However, the results show no defined outcome complementing the assumptions stated in the introduction. There is a possibility that the immune response of the spat may have already been compromised and were therefore more susceptible to the addition of environmental stressors. Furthermore, even genetics and environmental adaptations may have an influence on the overall health and survival of the mussel spat.

While the initial hypothesis was that UV treatment would reduce mortality rates, the experimental design presented some limitations. The primary concern lies in the differential feeding regime between the two treatment groups. By only feeding the UV-treated group, the experiment introduced a confounding variable that could influence the observed mortality rates. Nutritional stress, or the lack thereof, can significantly impact the survival and health of marine organisms.

Therefore, while the reduced mortality in the UV-treated group could be attributed to the inactivation of potential pathogens, it is equally plausible that the increased food availability contributed to this outcome. To definitively isolate the effects of UV treatment, a more controlled experiment would be necessary, ensuring that both treatment groups receive identical feeding regimes.

The experimental findings align with previous research on the physiological responses of *M. edulis* to environmental stressors (Lynch et al., 2014; Rayssac et al., 2010; Vuorinen et al., 2002) mentioned in Chapter 3. Studies have documented the influence of temperature fluctuations, heavy metal exposure, and salinity variations on mussel health and mortality (Maar et al., 2015; van der Gaag et al., 2016).The observed mortalities in response to environmental challenges only begin to explain the multifaceted interactions between environmental parameters and mussel physiology.

In conclusion, the controlled in situ experiments yielded unexpected results, challenging the initial hypotheses. Contrary to expectations, the artificial seawater control for the Loch Eil dilution series exhibited higher mortality rates than hypothesised. The salinity challenge experiment revealed a non-linear relationship between salinity and mortality, with higher salinity leading to increased spat mortality, contrary to the initial expectation that lower salinity levels were less detrimental to spat survival. The experiment involving UV-treated and untreated water also produced unexpected outcomes, with both Loch Eil and Loch Sunart spat showing varying responses to the different treatments; while showing some potential benefits in reducing mortality, the experiment did not provide conclusive evidence for a pathogen-related cause of mortality.

The results of this study underscore the intricate relationship between environmental parameters, mussel physiology, and the microbial community in influencing spat mortality within Loch Eil. While the experimental findings offer valuable insights into these complex interactions, the precise cause of mass mortality events remains elusive. This highlights the urgent need for further investigation to fully understand the underlying mechanisms and develop effective strategies to mitigate these significant losses and ensure the sustainability of mussel aquaculture in the region.

4.5 Appendix

Appendix 4.1: **Individual spat weights for stocking calculations.** Replicates of spat sample set ($n = 50$ per replicate) from Loch Eil and Loch Sunart were individually weighed on a portable balance (Ohaus Scoutpro) on 14/10/2021. Weighed individuals were separated from the sample sets to avoid duplication.

	Loch Eil			Loch Sunart		
Replicate	A	B	C	A	B	C
n						
1	1.90	1.85	0.35	1.18	0.46	0.91
2	1.43	1.96	1.32	1.25	0.76	0.28
3	1.15	1.37	1.48	0.08	0.24	1.22
4	0.62	0.82	0.84	0.42	0.48	1.58
5	1.02	0.44	0.73	0.74	1.02	1.25
6	1.63	1.36	0.46	1.45	0.52	1.64
7	1.61	0.94	0.52	0.34	0.67	1.01
8	0.29	2.04	0.32	0.45	0.70	0.76
9	0.89	0.22	0.58	1.28	0.12	0.91
10	1.25	1.45	1.08	1.75	0.21	0.63
11	1.39	1.55	0.68	0.68	0.09	0.74
12	1.10	0.88	0.35	1.19	0.74	0.78
13	0.65	1.08	0.36	0.88	0.33	0.79
14	1.02	1.10	0.76	0.99	0.46	0.30
15	0.92	2.20	0.28	1.11	0.53	0.41
16	1.16	1.72	0.17	0.59	0.41	0.38
17	1.99	1.42	0.17	0.58	0.56	0.55
18	0.27	1.82	0.54	0.72	0.86	0.72
19	2.24	0.33	0.25	1.06	0.66	1.26
20	2.43	0.89	0.70	0.28	0.26	0.28
21	1.73	0.45	0.28	1.24	0.39	0.65
22	2.24	1.45	0.53	1.03	0.40	1.06
23	1.30	1.31	0.31	1.18	0.24	0.86
24	1.63	0.71	1.18	0.63	0.46	0.91
25	0.98	1.00	0.30	0.54	0.53	0.54
26	1.36	0.25	0.17	0.50	10.23	0.56

Appendix 4.1 continued

	Loch Eil			Loch Sunart		
Replicate	A	B	C	A	B	C
n						
27	0.40	0.52	0.15	0.46	1.04	0.41
28	1.47	0.17	0.36	1.24	0.58	0.26
29	1.77	0.40	0.11	1.21	0.62	0.62
30	0.25	0.61	0.15	0.89	0.23	0.16
31	1.42	0.20	0.17	0.85	0.36	0.27
32	1.46	0.34	0.19	0.51	0.54	0.25
33	2.51	0.16	1.10	0.66	0.79	0.19
34	2.32	1.40	0.65	0.53	0.17	0.18
35	0.89	1.61	0.80	0.54	0.12	0.23
36	2.07	1.75	0.68	0.46	0.57	0.24
37	1.63	0.85	1.16	0.58	0.15	0.08
38	1.70	0.31	1.71	0.33	0.51	0.49
39	2.07	2.31	0.34	0.54	0.66	0.14
40	1.36	1.90	0.66	0.42	0.41	0.29
41	0.69	1.19	0.95	0.06	0.36	0.17
42	1.87	1.60	0.89	0.46	0.52	0.10
43	1.17	1.51	0.91	1.14	0.35	0.09
44	1.00	1.23	0.61	1.67	0.43	0.09
45	1.05	1.13	0.38	0.88	0.24	0.25
46	0.98	1.13	1.29	0.37	0.13	0.15
47	0.69	0.94	0.23	0.34	0.22	0.07
48	1.60	1.34	0.45	0.74	0.37	0.10
49	0.84	1.18	0.67	0.52	0.50	0.70
50	1.36	1.61	0.94	0.32	0.32	0.79

Appendix 4.2: **Daily temperature measurements of tanks 1 – 30 during the in situ trials 2021.** Temperature was measured and recorded in each tank (1 – 30) daily (1 – 22) with an EcoSense® probe (EC300A). Temperature values are presented in °C.

	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	Trial	*Tank #*	Temperature °C																				
LE Dilution series		*1*	9.4	12.2	13.2	13.3	13.7	12.4	11.8	11.8	12.6	12.6	11.4	11.8	12.6	12.1	11.4	11.4	10.6	11.5	10.1	11.1	11.0	12.2
		2	9.5	12.2	13.2	13.3	13.7	12.2	11.6	11.8	12.5	12.6	11.3	11.7	12.6	12.1	11.3	11.4	10.7	11.3	10.0	10.9	10.9	12.1
		3	9.3	12.0	13.2	13.2	13.7	12.1	11.5	11.7	12.4	12.6	11.2	11.6	12.6	12.1	11.3	11.4	10.7	11.2	9.8	10.7	10.5	12.1
		4	8.3	11.7	13.0	13.2	13.7	11.9	11.4	11.7	12.4	12.5	11.1	11.5	12.7	12.0	11.2	11.3	10.6	11.0	9.6	10.5	10.4	12.0
		5	8.2	11.6	12.8	13.2	13.7	11.9	11.4	11.6	12.3	12.5	11.1	11.4	12.7	12.0	11.2	11.3	10.6	11.1	9.5	10.5	10.4	12.0
		6	8.1	11.4	12.7	13.1	13.7	11.9	11.4	11.6	12.3	12.5	11.0	11.4	12.7	12.0	11.2	11.3	10.5	11.4	9.5	10.6	10.4	12.0
		7	8.0	11.4	12.9	13.2	13.7	12.0	11.4	11.7	12.3	12.5	11.1	11.4	12.6	12.0	11.2	11.3	10.6	11.2	9.6	10.6	10.5	12.0
		8	8.0	11.4	12.9	13.2	13.7	12.0	11.4	11.7	12.4	12.5	11.0	11.4	12.6	12.0	11.2	11.3	10.5	11.0	9.5	10.6	10.4	12.0
		9	7.6	11.3	12.6	13.1	13.7	12.0	11.4	11.7	12.4	12.5	11.1	11.5	12.6	12.1	11.2	11.3	10.6	11.0	9.7	10.7	10.6	12.1
		10	7.2	12.4	13.2	13.3	3.6	12.0	12.1	12.2	12.8	12.7	11.7	12.0	12.5	12.2	11.6	11.6	10.9	11.7	10.5	11.2	11.3	12.5
		11	7.2	12.4	13.2	13.3	3.6	12.0	12.1	12.2	12.8	12.7	11.7	12.0	12.5	12.2	11.6	11.6	10.9	11.7	10.5	11.2	11.3	12.5
		12	6.9	11.7	13.1	13.2	13.7	12.4	11.7	11.9	12.6	12.6	11.5	11.8	12.6	12.1	11.4	11.5	10.8	11.3	9.9	10.9	10.9	12.3
		13	6.9	11.6	13.0	13.2	13.7	12.1	11.6	11.8	12.5	12.6	11.4	11.8	12.6	12.1	11.4	11.4	10.7	11.2	9.8	10.8	10.7	12.3
		14	6.8	11.6	13.0	13.2	13.7	12.2	11.6	11.8	12.5	12.6	11.3	11.7	12.6	12.1	11.4	11.4	10.7	11.3	9.8	10.8	10.9	12.3
		15	6.8	11.4	13.0	13.2	13.7	12.1	11.6	11.7	12.4	12.6	11.3	11.7	12.6	12.1	11.4	11.4	10.7	11.2	9.8	10.8	10.9	12.2
		16	6.8	11.4	13.0	13.2	13.7	12.1	11.6	11.8	12.4	12.6	11.3	11.6	12.6	12.1	11.4	11.4	10.7	11.2	9.9	10.9	10.9	12.2
		17	6.9	11.2	13.0	13.2	13.7	12.2	11.6	11.8	12.5	12.5	11.2	11.6	12.6	12.1	11.4	11.4	10.7	11.1	9.8	10.9	10.8	12.1
		18	7.1	11.1	13.0	13.2	13.7	12.1	11.6	11.8	12.5	12.5	11.2	11.5	12.6	12.2	11.4	11.5	10.7	11.2	9.8	10.9	10.9	12.1

Appendix 4.2 continued

Day		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Trial	*Tank #*	Temperature °C																					
Salinity Challenge	*19*	7.2	12.1	13.1	13.3	13.6	12.4	12.0	12.2	12.8	12.7	11.6	12.0	12.5	12.2	11.5	11.6	10.9	11.5	10.2	11.2	11.1	12.4
	20	7.2	11.7	13.1	13.3	13.7	12.3	11.9	12.2	12.7	12.6	11.5	11.8	12.5	12.2	11.4	11.5	10.8	11.4	10.0	11.0	10.9	12.3
	21	7.2	11.5	13.0	13.2	13.7	12.2	11.8	12.0	12.6	12.6	11.3	11.7	12.6	12.1	11.2	11.4	10.7	11.2	9.8	10.8	10.8	12.2
	22	7.1	11.4	13.0	13.2	13.7	12.2	11.8	11.9	12.6	12.6	11.3	11.7	12.6	12.1	11.3	11.4	10.7	11.3	9.8	10.8	10.7	12.2
	23	7.1	11.2	13.0	13.2	13.7	12.1	11.8	11.9	12.6	12.6	11.2	11.6	12.6	12.1	11.2	11.4	10.6	11.2	9.6	10.7	10.5	12.1
	24	7.1	11.0	13.0	13.2	13.7	12.1	11.6	11.8	12.5	12.6	11.1	11.6	12.6	12.2	11.0	11.4	10.6	11.1	9.6	10.0	10.3	12.0
UV trial	*25*	9.4	13.2	13.5	13.4	13.6	13.2	12.5	12.7	13.0	12.9	12.1	12.5	12.5	12.3	12.0	11.8	11.2	12.1	11.3	12.2	12.2	12.9
	26	9.4	13.2	13.5	13.4	13.6	13.2	12.5	12.7	13.0	12.9	12.1	12.5	12.5	12.3	12.0	11.8	11.2	12.1	11.4	12.2	12.3	12.9
	27	9.4	13.2	13.5	13.4	13.6	13.2	12.5	12.7	13.1	12.9	12.1	12.5	12.5	12.3	12.0	11.8	11.2	12.2	11.3	12.2	12.3	12.9
	28	9.4	13.2	13.5	13.4	13.6	13.2	12.5	12.7	13.1	12.9	12.1	12.5	12.5	12.3	12.0	11.8	11.2	12.2	11.5	12.2	12.3	12.9
	29	9.4	13.2	13.5	13.4	13.6	13.2	12.5	12.5	13.1	12.9	12.1	12.5	12.5	12.3	12.0	11.8	11.2	12.2	11.5	12.2	12.3	12.9
	30	9.4	13.2	13.5	13.4	13.6	13.2	12.5	12.7	13.1	12.9	12.1	12.5	12.5	12.3	12.0	11.8	11.2	12.2	11.5	12.2	12.3	12.6

Appendix 4.3: **Daily salinity measurements of tanks 1 – 30 during the in situ trials 2021.** Salinity was measured and recorded in each tank (1 – 30) daily (1 – 22) with an EcoSense® probe (EC300A). Salinity values are presented in parts per thousand (ppt). Tanks that exhibited salinity values beyond the established limits were adjusted with artificial sea water until measurements were within the specified range.

	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
	Trial	*Tank #*											Salinity ppt										
LE Dilution series		*1*	25.0	25.1	25.4	24.9	24.9	25.2	25.6	24.7	24.9	25.1	25.2	25.1	25.3	25.3	25.5	24.6	25.3	25.5	25.2	25.5	25.5	25.3
		2	24.8	25.1	25.2	25.4	24.7	25.0	25.3	25.5	25.6	24.9	24.8	25.1	25.0	25.1	25.2	25.3	25.4	24.3	23.6	24.5	24.7	24.7
		3	24.7	24.9	25.0	25.1	25.1	25.4	25.8	24.0	24.2	24.4	24.5	24.3	24.3	24.6	24.8	24.7	24.9	25.1	25.2	25.3	25.5	25.4
		4	24.9	25.3	25.2	25.2	25.4	25.5	25.2	25.4	25.3	25.4	24.6	24.7	24.6	24.6	24.7	24.8	25.0	24.9	25.1	25.1	25.0	23.7
		5	25.0	25.2	25.3	24.8	24.9	25.0	25.6	25.6	24.9	25.1	25.2	25.0	25.2	25.2	25.5	25.2	25.3	25.5	25.2	25.4	24.8	24.8
		6	25.0	25.3	25.3	25.1	25.2	25.4	25.3	24.4	24.9	25.0	25.1	25.2	25.1	25.2	25.3	25.5	24.7	25.0	25.0	25.2	25.3	25.3
		7	25.0	25.2	25.3	25.0	25.0	25.3	25.6	24.2	24.3	24.4	24.5	24.6	24.5	24.6	24.7	24.8	25.0	25.1	25.2	25.2	25.3	25.4
		8	24.9	25.2	25.3	24.9	24.9	25.2	25.5	24.5	24.7	24.8	24.9	25.0	25.0	25.0	25.2	25.2	25.4	24.8	24.9	25.1	25.3	25.2
		9	25.0	25.3	25.5	25.0	25.1	25.6	25.6	24.5	24.6	24.7	24.8	24.9	25.0	25.0	25.2	25.3	25.3	25.6	24.9	25.3	25.5	24.4
		10	25.2	25.5	25.7	25.3	25.3	25.3	25.0	25.2	25.3	25.4	24.7	24.7	24.7	24.9	25.0	25.1	25.2	25.2	25.2	25.6	24.2	24.0
		11	25.2	25.5	25.2	25.7	25.1	25.7	25.4	25.6	24.6	24.7	24.9	24.9	24.7	25.0	25.1	25.2	25.4	24.4	24.7	24.8	25.1	25.0
		12	25.2	25.5	25.6	25.4	25.1	25.4	25.5	24.7	24.7	25.0	25.2	25.3	25.3	25.3	25.4	25.3	25.3	25.5	24.8	25.0	25.2	25.2
		13	25.1	25.5	25.6	25.3	25.3	25.4	24.6	24.8	25.0	25.1	25.2	25.3	25.3	25.4	25.5	25.3	25.4	24.0	24.5	24.4	24.5	24.5
		14	25.1	25.5	25.6	25.1	25.2	25.4	25.1	25.3	25.5	25.1	25.3	25.4	24.6	24.7	24.8	24.9	25.0	25.0	25.3	25.3	25.6	24.7
		15	25.1	25.4	25.6	25.2	25.2	25.5	25.2	25.5	24.9	25.0	25.2	25.2	25.2	25.3	25.5	25.3	25.4	24.4	24.5	24.7	24.8	24.9
		16	25.1	25.5	25.6	25.1	25.2	25.4	25.5	25.0	25.1	25.3	25.4	25.5	24.5	24.6	24.7	24.8	24.9	25.1	25.2	25.5	25.0	25.1
		17	25.2	25.5	25.5	25.5	24.8	25.1	24.7	24.7	25.0	24.8	25.2	25.3	24.8	25.4	25.2	25.4	24.5	24.7	24.8	25.0	25.1	25.2
		18	25.2	25.5	25.6	25.3	25.2	25.4	25.1	25.3	25.4	24.5	25.7	24.9	24.9	24.9	25.0	25.1	25.1	25.2	25.6	24.2	24.4	24.3

Appendix 4.3 continued

Day		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Trial	*Tank #*											Salinity ppt											
Salinity Challenge	*19*	18.8	19.3	18.3	19.1	19.1	19.3	19.1	19.2	19.4	18.8	19.0	19.1	19.0	19.1	19.2	19.3	19.4	19.2	19.3	19.5	19.0	19.1
	20	18.8	19.1	18.8	19.0	19.1	19.4	18.9	19.2	19.4	18.7	18.9	19.0	19.0	19.0	19.2	19.2	19.4	19.0	19.1	19.2	19.5	18.4
	21	18.8	19.1	19.0	19.1	19.2	19.6	19.1	19.1	18.9	19.0	19.2	19.2	19.3	19.4	18.9	18.9	19.0	18.7	19.1	19.0	19.1	19.3
	22	31.8	32.3	31.6	31.6	32.1	32.4	32.1	32.4	32.5	31.9	32.1	32.4	31.0	31.8	32.0	32.1	32.3	31.7	32.0	32.1	32.4	30.7
	23	31.8	32.3	31.9	32.0	32.1	32.5	32.2	32.4	32.5	31.4	31.7	31.8	31.7	31.8	32.0	32.1	32.3	32.4	31.4	31.7	31.9	32.1
	24	31.8	32.3	31.9	32.1	32.1	32.5	31.9	32.2	32.3	31.4	31.3	31.4	31.3	31.4	31.3	31.7	31.7	32.1	32.0	31.7	32.2	32.8
UV Trial	*25*	24.9	24.9	24.9	25.3	26.0	25.8	25.0	26.1	26.1	25.8	22.3	24.6	20.3	16.7	18.5	17.7	13.1	22.0	20.0	23.5	24.5	26.1
	26	24.9	24.8	24.9	25.2	25.9	25.9	25.0	26.1	26.0	25.8	22.3	24.6	20.3	16.7	18.5	17.7	13.1	22.0	20.0	23.4	24.5	26.1
	27	24.9	24.9	25.0	25.3	26.0	25.8	25.0	26.1	26.0	25.8	22.3	24.6	20.3	16.7	18.5	17.7	13.1	22.0	20.0	23.5	24.5	26.1
	28	24.9	24.9	25.0	25.3	26.0	25.8	25.0	26.1	26.0	25.8	22.3	24.6	20.3	16.7	18.5	17.8	13.1	22.0	20.0	23.5	24.7	26.1
	29	24.9	24.9	24.9	25.2	26.0	25.8	25.0	25.0	26.0	25.8	22.3	24.6	20.3	16.7	18.5	17.8	13.1	22.0	20.0	23.5	24.7	26.1
	30	24.9	24.9	24.9	25.3	26.0	25.8	25.0	26.1	26.0	25.8	22.3	24.6	20.3	16.7	18.5	17.8	13.1	22.0	20.0	23.5	24.7	26.1

Appendix 4.4: **Water changes in tanks during 2021 experiment.** Tanks that measured a salinity beyond the established limits were adjusted with artificial sea water, until the salinity measurements returned within the specified range. The upper limit for the Loch Eil dilution series was established at 25.4 ppt. The upper limits for salinity challenge with treatments 18.7 ppt and 31.8 ppt were established at 19.3 ppt and 32.3 ppt respectively.

Tank	Treatment	Date	Salinity [ppt]	Limit [ppt]	Difference [ppt]
1	100% LE	21/10/2021	25.6	25.4	0.2
		29/10/2021	25.5		0.1
		01/11/2021	25.5		0.1
		04/11/2021	25.5		0.1
2		23/10/2021	25.6		0.2
		02/11/2021	23.6		-1.8
3		21/10/2021	25.8		0.4
		04/11/2021	25.5		0.1
4		20/10/2021	25.5		0.1
5	50% LE	22/10/2021	25.6		0.2
		29/10/2021	25.5		0.1
		01/11/2021	25.5		0.1
6		30/10/2021	25.5		0.1
7		21/10/2021	25.6		0.2
8		21/10/2021	25.5		0.1
9	25% LE	17/10/2021	25.5		0.1
		21/10/2021	25.6		0.2
		01/11/2021	25.6		0.2
10		04/11/2021	25.5		0.1
		17/10/2021	25.7		0.3
		03/11/2021	25.6		0.2
11	10% LE	16/10/2021	25.5		0.1
		18/10/2021	25.7		0.3
		20/10/2021	25.7		0.3
		22/10/2021	25.6		0.2
12		17/10/2021	25.6		0.2
		21/10/2021	25.5		0.1
		01/11/2021	25.5		0.1
13		17/10/2021	25.6		0.2
		29/10/2021	25.5		0.1
14	1% LE	17/10/2021	25.6		0.2
		23/10/2021	25.5		0.1
		04/11/2021	25.6		0.2
15		17/10/2021	25.6		0.2
		20/10/2021	25.5		0.1
		22/10/2021	25.5		0.1
		29/10/2021	25.5		0.1

Appendix 4.4 continued

Tank	Treatment	Date	Salinity [ppt]	Limit [ppt]	Difference [ppt]
16	0.1% LE	17/10/2021	25.6	25.4	0.2
		21/10/2021	25.5		0.1
		26/10/2021	25.5		0.1
17		03/11/2021	25.5		0.1
		18/10/2021	25.5		0.1
18		17/10/2021	25.6		0.2
		25/10/2021	25.7		0.3
18		02/11/2021	25.6		0.2
19	18.7 ppt	16/10/2021	19.4	19.3	0.1
		21/10/2021	19.4		0.1
		23/10/2021	19.4		0.1
		31/10/2021	19.4		0.1
		03/11/2021	19.5		0.2
		20/10/2021	19.4		0.1
20		23/10/2021	19.4		0.1
		31/10/2021	19.4		0.1
21		04/11/2021	19.5		0.2
		20/10/2021	19.6		0.3
		28/10/2021	19.4		0.1
22	31.8 ppt	20/10/2021	32.4	32.3	0.2
		23/10/2021	32.5		0.3
		26/10/2021	32.4		0.2
23		04/11/2021	32.4		0.2
		20/10/2021	32.5		0.3
		23/10/2021	32.5		0.3
24		01/11/2021	32.4		0.2
		20/10/2021	32.5		0.3

Appendix 4.5: **Stocking densities and mortality data from the in situ trials during 2021.** During the experimental trials, each treatment was performed in triplicate. All tanks were stocked with mussel spat originating from Loch Eil, whereas the UV/non-UV challenge was additionally supplied with spat from Loch Sunart. The stocking densities within each tank (1 – 30), number of dead spat (total morts) at each time point (T1 – 4 d, T2 – 10 d, T3 – 22 d post stocking) and the surviving spat were counted and recorded throughout the trial period. Percentage values under treatment refer to the quantity of Loch Eil water within the treatment, and salinity units are presented in ppt.

			Spat live count		**Spat mortality count**				
			Start	End					
					Time [d]				
Tank	Treatment		**0**	**22**	**4**	**10**	**18**	**22**	Total
1			132	102	9	8	5	8	30
2	100% Loch Eil		189	124	9	22	3	31	65
3			171	118	10	9	15	19	53
4			194	92	8	38	15	41	102
5	50% Loch Eil		117	83	3	6	3	22	34
6			196	111	14	16	11	44	85
7			164	88	11	11	10	44	76
8	25% Loch Eil		174	110	15	10	3	36	64
9			190	106	10	15	15	44	84
10			209	122	9	18	12	48	87
11	10% Loch Eil		158	98	8	9	7	36	60
12			153	93	2	13	6	39	60
13			153	114	12	8	6	13	39
14	1% Loch Eil		147	114	8	11	4	10	33
15			143	99	7	11	8	18	44
16			103	92	1	3	2	5	11
17	0.1% Loch Eil		111	97	3	5	2	4	14
18			97	78	5	5	4	5	19
19			77	68	1	4	2	2	9
20	18.7 ppt		94	78	4	6	2	4	16
21			77	68	1	6	2	0	9
22			58	42	5	6	1	4	16
23	31.8 ppt		52	41	5	3	2	1	11
24			66	41	7	3	8	7	25
25		LE	34	29	0	2	2	1	5
		LS	*131*	*60*	*0*	*9*	*2*	*60*	*71*
26	UV	LE	31	28	0	2	1	0	3
		LS	*66*	*40*	*0*	*3*	*9*	*14*	*26*
27		LE	28	27	0	0	1	0	1
		LS	*77*	*39*	*0*	*5*	*24*	*9*	*38*
28		LE	43	36	0	4	3	0	7
		LS	*112*	*55*	*0*	*4*	*20*	*33*	*57*
29	Non-UV	LE	61	12	0	2	47	0	49
		LS	*198*	*105*	*0*	*29*	*0*	*64*	*93*
30		LE	51	22	0	5	22	2	29
		LS	*257*	*161*	*0*	*15*	*0*	*81*	*96*

LE – Loch Eil, LS – Loch Sunart

Appendix 4.6: **Statistical analyses on dilution series of Loch Eil data.** Data was transformed into proportions by dividing the count of dead spat from each treatment by the corresponding stocking density, and the inverse sine of the square root was calculated of those proportions. Analysis of variance (ANOVA) was used to determine any statistically significant differences between mortalities and experimental treatments. General linear models (GLM) were used to assess any significant associations between spat mortality (total morts), time (in days post stocking), treatments (in % - quantity of Loch Eil water within the treatment), and interactions thereof (treatment v time, time v total morts). Salinity challenge acted as control for this trial (18.7, 31.8), units are presented in ppt. Data expressed as mean (± SD). Different superscripts imply statistically significant differences ($p < 0.05$). Variation in degrees of freedom (F) indicate the removal of outliers.

				Control		One-Way ANOVA	GLM P-Value	
time [d]	100%	50%	25%	*18.7 ppt*	*31.8 ppt*	P-Value	Treatment v time	Time v total morts
	0.57 (± 0.07) ^{A}	0.70 (± 0.12) ^{A}	0.71 (± 0.05) ^{A}	0.37 (± 0.04)	0.56 (± 0.09)	0.001 ($F_{5, 12}$ = 9.89)		
4	0.24 (± 0.02)	0.21 (± 0.06)	0.26 (± 0.03)			0.15 ($F_{5, 12}$ = 2.04)	0.000 ($F_{1, 9}$ = 177.13)	
18.7	0.24 (± 0.02)	0.21 (± 0.06)	0.26 (± 0.03)	0.15 (± 0.05)		0.07 ($F_{6, 14}$ = 2.62)	0.000 ($F_{1, 11}$ = 131.22)	
31.8	0.24 (± 0.02)	0.21 (± 0.06)	0.26 (± 0.03)		0.32 (± 0.02)	0.02 ($F_{6, 14}$ = 3.94)	0.000 ($F_{1, 9}$ = 177)	
10	0.28 (± 0.06)	0.33 (± 0.12)	0.26 (± 0.02)			0.34 ($F_{5, 12}$ = 1.27)	0.000 ($F_{1, 9}$ = 137.27)	
18.7	0.28 (± 0.06)	0.33 (± 0.12)	0.26 (± 0.02)	0.26 (± 0.03)		0.35 ($F_{6, 14}$ = 1.22)	0.000 ($F_{1, 11}$ = 411.26)	
31.8	0.28 (± 0.06) ^{A,B}	0.33 (± 0.12) ^{A,B}	0.26 (± 0.02) ^{A,B}		0.26 (± 0.06) ^{A}	0.43 ($F_{6, 14}$ = 1.07)	0.000 ($F_{1, 9}$ = 536)	
22	0.41 (± 0.08) ^{B,C}	0.54 (± 0.05) ^{A,B}	0.57 (± 0.06) ^{A}			0.000 ($F_{5, 12}$ = 14.94)	0.000 ($F_{1, 9}$ = 143.55))	0.5 ($F_{5, 9}$ = 0.94)
18.7	0.41 (± 0.08) ^{B,C}	0.54 (± 0.05) ^{A,B}	0.57 (± 0.06) ^{A}	0.22 (± 0.05) ^{D}		0.000 ($F_{6, 14}$ = 21.13)	0.000 ($F_{1, 11}$ = 500.6)	0.28 ($F_{6, 11}$ = 1.46)
31.8	0.41 (± 0.08) ^{A,B,C}	0.54 (± 0.05) ^{A,B}	0.57 (± 0.06) ^{A}		0.35 (± 0.13) ^{C}	0.001 ($F_{6, 14}$ = 8.19)	0.000 ($F_{1, 9}$ = 694)	0.57 ($F_{5, 9}$ = 0.82)

time [d]	10%	1%	0.10%
	0.68 (± 0.02) ^{A}	0.54 (±0.05) ^{A,B}	0.38 (± 0.07) ^{B}
4	0.18 (± 0.06)	0.25 (± 0.03)	0.16 (± 0.07)
18.7	0.18 (± 0.06)	0.25 (± 0.03)	0.16 (± 0.07)
31.8	0.18 (± 0.06)	0.25 (± 0.03)	0.16 (± 0.07)
10	0.28 (± 0.03)	0.26 (± 0.03)	0.20 (± 0.03)
18.7	0.28 (± 0.03)	0.26 (± 0.03)	0.20 (± 0.03)
31.8	0.28 (± 0.03) ^{B}	0.26 (± 0.03) ^{A,B}	0.20 (± 0.03) ^{B}
22	0.56 (± 0.01) ^{A}	0.37 (± 0.06) ^{C}	0.27 (± 0.04) ^{C}
18.7	0.56 (± 0.01) ^{A}	0.37 (± 0.06) ^{C}	0.27 (± 0.04) ^{D}

31.8	0.56 (± 0.01) [A]	0.37 (± 0.06) [A,B,C]	0.27 (± 0.04) [C]

Appendix 4.7: **Statistical analyses on salinity challenge data.** Data was transformed into proportions by dividing the count of dead spat from each treatment by the corresponding stocking density, and the inverse sine of the square root was calculated of those proportions. Analysis of variance (ANOVA) was used to determine any statistically significant differences between mortalities and experimental treatments. General linear models (GLM) were used to assess any significant associations between spat mortality (total morts), time (in days post stocking), salinity treatments (18.7 ppt, 31.8 ppt) and interactions thereof (treatment v time, time v total morts). Data expressed as mean (± SD). Tukey Method was used for post hoc examination. Different superscripts imply statistically significant differences ($p < 0.05$).

			One-Way ANOVA	GLM P-Value	
time [d]	18.7 ppt	31.8 ppt	P-Value	Treatment v time	Time v total morts
	0.37 (± 0.04) [B]	0.56 (± 0.09) [A]	0.03 ($F_{1, 4}$ = 10.24)		0.64 ($F_{1, 1}$ = 0.4)
4	0.14 (± 0.05) [B]	0.31 (± 0.02) [A]	0.007 ($F_{1, 4}$ = 26.38)	0.42 ($F_{1, 1}$ = 1.66)	
10	0.25 (± 0.03)	0.26 (± 0.06)	0.89 ($F_{1, 4}$ = 0.02)	0.29 ($F_{1, 1}$ = 4.19)	
22	0.21 (± 0.05)	0.34 (± 0.12)	0.18 ($F_{1, 4}$ = 2.57)	0.11 ($F_{1, 1}$ = 35.06)	

Appendix 4.8: **Statistical analysis on UV treatment challenge data.** Data was transformed into proportions by dividing the count of dead spat from each treatment by the corresponding stocking density, and the inverse sine of the square root was calculated of those proportions. Analysis of variance (ANOVA) was used to determine any statistically significant differences between mortalities and experimental treatments. General linear models (GLM) were used to assess any significant associations between spat mortality (total morts), time (in days post stocking) , spat origin (Loch Eil – LE, Loch Sunart – LS) within the treatments (UV, non-UV) and interactions thereof (treatment v time, time v total morts). Data expressed as mean (± SD). Tukey Method was used for post hoc examination. Different superscripts imply statistically significant differences ($p < 0.05$). No mortalities were observed during the first time point, 4 days post stocking.

		Loch Eil		Loch Sunart		One-Way ANOVA	GLM P-Value	
time [d]	Treatment	UV	non-UV	UV	non-UV	P-Value	Treatment v time	Time v total morts
	UV LE*LS	0.30 (± 0.1) [B]		0.76 (± 0.08) [A]		0.003 ($F_{1, 4}$ = 39.18)		0.65 ($F_{1, 2}$ = 0.27)
	non-UV LE*LS		0.59 (± 0.15)		0.74 (± 0.07)	0.21 ($F_{1, 4}$ = 2.23)		0.61 ($F_{1, 2}$ = 0.36)
10	UV LE*LS	0.17 (± 0.1)		0.25 (± 0.03)		0.41 ($F_{1, 4}$ = 0.85)	0.017 ($F_{1, 2}$ = 56.83)	
	non-UV LE*LS		0.23 (± 0.09)		0.28 (± 0.10)	0.61 ($F_{1, 4}$ = 0.3)	0.02 ($F_{1, 2}$ = 43.05)	
22	UV LE*LS	0.22 (± 0.07) [B]		0.70 (± 0.06) [A]		0.001 ($F_{1, 4}$ =78.63)	0.016 ($F_{1, 2}$ = 62.12)	
	non-UV LE*LS		0.51 (± 0.22)		0.65 (± 0.09)	0.86 ($F_{1, 4}$ = 0.04)	0.004 ($F_{1, 2}$ = 257.09)	

Chapter 5

General Discussion

This study aimed to investigate the factors contributing to the observed high spat mortality rates in Loch Eil, Scotland. The primary objective was to conduct a multidisciplinary analysis of spat mortality dynamics, investigating the patterns and rates of spat death within this system and comparing the results to an unaffected control site. To achieve this, the study focused on three key objectives:

Characterising environmental factors influencing spat mortality, which aimed to investigate the influence of environmental variables such as temperature, salinity, water quality, and nutrient availability on spat survival.

Integrate environmental and biological factors, which aimed to assess the interactive effects of identified environmental factors and pathogens on spat survival under controlled conditions.

The fundamental question guiding this research was: What are the primary factors contributing to the observed high spat mortality rates in Loch Eil?
This overarching question led to the formulation of several key hypotheses:

1. Spat mortality rates in Loch Eil will be significantly higher than in a control site due to the influence of site-specific environmental factors.
2. Water quality parameters, including heavy metal concentrations and nutrient availability, will differ significantly between Loch Eil and the control site and will correlate with observed spat mortality rates.

Chapter 2 demonstrated significant differences in mortality rates between Loch Eil (68.3%) and the control site in Loch Sunart (0.9%), with Loch Eil experiencing substantially higher mortality rates. This finding strongly supports the hypothesis that site-specific factors within Loch Eil are detrimental to spat survival, thus validating hypothesis 1.

Chapter 3 provided evidence supporting hypothesis 2. Significant differences in water quality parameters were observed between the two sites, including greater salinity variability and potential heavy metal contamination in Loch Eil. These findings, while not explicitly demonstrating a direct correlation with spat mortality, suggest a strong influence of environmental factors on spat survival in Loch Eil. Furthermore, through controlled in situ experiments, Chapter 4 demonstrated that spat mortality in Loch Eil was influenced by a complex interplay of factors, including water quality, salinity fluctuations, and potentially genetic variation. Unexpected results highlighted the difficulty in pinpointing the exact causes of the studied mass mortality events.

Novel Insights: Initial approaches launching the investigation

The original contribution of this study to knowledge lies in the comprehensive approach taken to investigate the factors contributing to spat mortality in Loch Eil. The initial observation of mortality dynamics in Loch Eil revealed the genuine repercussions of the mass mortality event of 2018, providing empirical data crucial for the understanding and necessity for this investigation. The gathered information from the observation led to the use of a multidisciplinary approach involving environmental monitoring to understand the dynamic environmental conditions in Loch Eil, including fluctuations in salinity (Brenko & Calabrese, 1969; Pourmozaffar et al., 2020; Wing & Leichter, 2011), heavy metal concentrations (Boening, 1999; Bryan G. W., 1971; Kumar et al., 2015; McDougall et al., 2022; McLusky et al., 1986; Nelson et al., 1988; Nielsen & Nathan, 1975; Renault, 2015; Strömgren, 1982; Vlahogianni & Valavanidis, 2007), and food availability (Page & Hubbard, 1987; Schalles, 2006; White et al., 2022; Wing & Leichter, 2011), as these factors had previously been identified to have implications on spat health and survival (Parry, 2007). By documenting the detected patterns in environmental conditions between the two lochs, the importance of these factors on spat survival was highlighted and discussed in Chapter 2. The findings from the in situ experiments involving the UV-treatments discussed in Chapter 4, highlighted the importance of understanding the effects of microbial communities and infectious diseases on spat health and survival (Gong et al., 2013; Kunselman et al., 2024; Paillard et al., 2004; Zannella et al., 2017). The combined findings from Chapters 1, 2 and 3 led to controlled in situ

experiments in Loch Eil, focussing on parameters based on information obtained from the chapters, and resulted in significant variations of mortality rates across the different treatments, once again highlighting the complex interplay between environmental factors and spat health (Incze et al., 1980; Kautsky, 1982; Lazo & Pita, 2012; Maar et al., 2015; Murray et al., 2022; Stewart-Sinclair et al., 2020; White et al., 2022). By integrating data from each chapter, the multidisciplinary approach was aided in the understanding of the interactions between environmental parameters and the overall health of the spat (Vieira et al., 2021). Overall, this research has provided useful insights into the dynamic nature of environmental conditions in Loch Eil, which may have influenced the general health of the spat. Additionally, the diagnostics performed within this research have identified potential pathogens, shedding light on the microbial communities associated with spat mortality events. Furthermore, significant variations in spat mortality rates across different treatments of the in situ trial confirmed prior assumptions that the examined factors described in Chapters 2 – 4 all have the potential of influencing spat survival.

Advancements in knowledge: Key insights gained from the investigation

Not only did the results in Chapter 1 provide insights into the actual numbers of mortalities observed during the study, but also led to the detection of patterns that aligned with temporal variations. This outcome suggested a rough estimate of the time frame indicating as to when the mortality events are most likely to occur. By conducting rigorous environmental monitoring and observing spat populations in Loch Eil and Loch Sunart, the quantification and comparison of the mortality rates between the two locations was possible. From this point it was evident that the spat in Loch Eil was succumbing to a phenomenon distinctively localised within that specific body of water. Thus, leading to the examination and comparison of the parameters in both lochs (Chapter 2), underlining the notable differences, in particularly the severe salinity fluctuations observed in Loch Eil (Lynch et al., 2014; Qiu et al., 2002; Wing & Leichter, 2011. Comparing these findings with responses observed in other studies suggests that mussels, despite their wide salinity tolerance, can perceive sudden changes in salinity as a stressor. In some cases, yet more commonly in juveniles, the stressor can be severe enough resulting in mortalities (Hawkins et al., 2013; Kautsky, 1982; Maar et al., 2015;

Vuorinen et al., 2002) which additionally supported the outcome of the in situ experiments involving the salinity treatments.

Opportunities and implications of current findings

The findings from Chapter 2 have challenged the individual assumptions regarding pollution and potential harmful elements, such as heavy metals, as the primary cause for the mortality events. Recent studies by Andreae (1986), Deruytter et al. (2015) and Nordberg et al. (2007) have highlighted the phenomenon of speciation, wherein initially harmless elements can transform into more toxic forms under specific environmental circumstances. This process underscores the importance of understanding how elements interact with their surroundings and how their toxicity can be influenced by various factors. One such factor identified in the literature is salinity, as indicated by McLusky et al., (1986) and Renault (2015). Higher chlorophyll a concentrations in Loch Eil indicate adequate levels of phytoplankton, consequently implying an abundance of food availability in the area (Grantham, 1981; Schalles, 2006; Solórzano & Grantham, 1975).This rejects the assumption that starvation is causing the mortality events.

The approach of incorporating a vast array of analyses, examining a variety of samples, has narrowed down the scope of possible causes for the mortality events, enabling a more targeted approach to further investigations, possibly focusing on specific environmental factors and potential pathogens.

Future directions

There are a few areas where more time would have been beneficial.

Another mortality observation and comprehensive sample collection

Conducting another mortality observation over an extended period could have provided additional data discussed in Chapter 2, to potentially detect the actual onset of the mortality events. Additionally, a comparison of spat and water quality samples from before and after the event could have illuminated the physical response of the animals to any concurrent environmental changes. An additional

investigation of sediment samples from both Loch Eil and Loch Sunart could have offered clues into potential sources of contamination or environmental stressors affecting the spat. Analysing sediment composition and pollutant levels might have helped identify additional factors contributing to the mortality events. Moreover, another observation period would have provided an opportunity to observe and possibly discern emerging trends, contributing to the identification of recurring patterns indicating a source of the mortality events.

Further diagnostic investigation

Diagnostic examinations could have allowed for a deeper exploration of potential diseases affecting mussel health, potentially leading to the identification of specific pathogens responsible for the mortality events in Loch Eil. Further analysis focusing on RNA and viral content could have indicated whether the course of the investigation was proceeding in the right direction. More time dedicated to exploring this possibility would have led to a more in-depth examination, providing more conclusive results, and potentially leading to another course for the investigation.

Genome-wide association studies

Assessing genetic variation and potential hybridisation between spat populations from the different locations could have shed light on the adaptability and resilience of mussel populations to environmental stressors (Mathiesen et al., 2017). Understanding genetic diversity and hybridisation dynamics could have provided important context for interpreting as to why only Loch Eil originating spat seem to be affected (Dias et al., 2011). Additionally, conducting a whole genome association study comparing spat from Loch Eil with spat from Loch Sunart could have elucidated genetic differences between populations and their potential influence on susceptibility to the unknown causative agent responsible for the mortality events.

Concluding remarks

The innovation of this study lies in its comprehensive approach to investigating spat mortality in Loch Eil, including a wide range of environmental monitoring and multidisciplinary diagnostics. Initial observations generated empirical data on mortality dynamics and highlighted the importance of understanding environmental factors such as salinity fluctuations, water quality, and food availability. The diagnostic techniques identified potential pathogens such as *Vibrio spp.,* shedding light on microbial communities associated with spat mortality. *In-situ* experiments confirmed the influence of environmental factors on spat health, underlining the interactions between these factors and spat mortality rates. By cross-referencing findings amongst the chapters, the study refuted assumptions about pollution and starvation as primary causes for mortality events, thereby narrowing down the scope of possible causes for further investigation. The overall research contributed towards a depth of understanding into the dynamic environmental conditions in Loch Eil and their impact on spat health, enabling a framework for future studies into the investigation of the mortality events.

While a conclusive identification of the causative agent behind the mass mortalities in Loch Eil remains elusive, this study has significantly reduced the scope of potential factors, providing a focused direction for further investigation. The observed mortality patterns suggest a multifactorial aetiology, potentially involving the stress response induced by severe salinity fluctuations, intensified by the presence of pathogenic agents.

References

Acosta, H., & Forrest, B. M. (2009). The spread of marine non-indigenous species via recreational boating: A conceptual model for risk assessment based on fault tree analysis. *Ecological Modelling*, *220*(13–14), 1586–1598. https://doi.org/10.1016/j.ecolmodel.2009.03.026

Adamson, E., Syvret, M., & Woolmer, A. (2018). Shellfish Seed Supply for Aquaculture in the UK. *Views Collected from the Industry in 2017.* London.

Alter, K., Constenla, M., Padrós, F., Sokolova, I. M., & Born-Torrijos, A. (2024). Spawning is accompanied by increased thermal performance in blue mussels. *Journal of Thermal Biology*, *127*. 104018. Advance online publication https://doi.org/10.1016/j.jtherbio.2024.104018

Andreae, M. O. (1986). Chemical species in seawater and marine particulates. *The Importance of Chemical "Speciation" in Environmental Processes,* 301–335. Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-70441-3_17

Artiola, J. F., Walworth, J. L., Musil, S. A., & Crimmins, M. A. (2019). Soil and land pollution. *Environmental and Pollution Science (Third Edition),* 219–235. Elsevier. https://doi.org/10.1016/B978-0-12-814719-1.00014-8

Austin, W. E. N., & Inall, M. E. (2002). Deep-water renewal in a Scottish fjord: temperature, salinity and oxygen isotopes. *Polar Research*, *21*(2), 251–257. https://doi.org/10.3402/polar.v21i2.6485

Avdelas, L., Avdic-Mravlje, E., Borges Marques, A. C., Cano, S., Capelle, J. J., Carvalho, N., Cozzolino, M., Dennis, J., Ellis, T., Fernández Polanco, J. M., Guillen, J., Lasner, T., Le Bihan, V., Llorente, I., Mol, A., Nicheva, S., Nielsen, R., van Oostenbrugge, H., Villasante, S., Visnic, S., Zhelev, K., & Asche, F. (2021). The decline of mussel aquaculture in the European Union: causes, economic impacts and opportunities. *Reviews in Aquaculture*, *13*(1), 91–118. https://doi.org/10.1111/raq.12465

References

Aypa, S. M. (1990). Mussel culture. *Food and Agriculture Organisation of the United Nations.*
Retrieved 16 December 2024, from
https://www.fao.org/4/ab737e/AB737E04.htm

Azra, M. N., Okomoda, V. T., 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

Baez-Ortega, A., & Murchison, E. P. (2022). Searching for transmissible cancers among the mussels of Europe. *Molecular Ecology*, *31*(3), 719–722. https://doi.org/10.1111/mec.16330

Baines, S. B., & Fisher, N. S. (2008). Modelling the effect of temperature on bioaccumulation of metals by a marine bioindicator organism, *Mytilus edulis*. *Environmental Science & Technology*, *42*(9), 3277–3282.
https://doi.org/10.1021/es702336q

Balbi, T., Montagna, M., Fabbri, R., Carbone, C., Franzellitti, S., Fabbri, E., & Canesi, L. (2018). Diclofenac affects early embryo development in the marine bivalve *Mytilus galloprovincialis*. *Science of The Total Environment*, *642*, 601–609.
https://doi.org/10.1016/j.scitotenv.2018.06.125

Bates, C., Moore, C.G., Austin, W. E., & Lyndon, A. (2003). Broad scale mapping of sublittoral habitats in Loch Sunart, Scotland. *Scottish Natural Heritage Commissioned Report*, *006*.

Baxter, J., Boyd, I., Cox, M., Donald, A., Malcolm, S., Miles, H., & Moffat, C. (2011). Scotland's marine atlas: Information for the national marine plan. Edinburgh: *The Scottish Government.*

Bayne, B. L. (1964). Primary and secondary settlement in *Mytilus edulis* L. (Mollusca). *The Journal of Animal Ecology*, *33*, 513–523.

Bayne, B. L. (1965). Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). *Ophelia*, *2*(1), 1–47.
https://doi.org/10.1080/00785326.1965.10409596

Bayne, B. L. (1976). Marine mussels: Their ecology and physiology. *International Biological Programme*, 10. Cambridge University Press: Cambridge.

ISBN 0-521-21058-5. xvii, 506 pp.

Beaumont, A. R., Hawkins, M. P., Doig, F. L., Davies, I. M., & Snow, M. (2008). Three species of *Mytilus* and their hybrids identified in a Scottish Loch: natives, relicts and invaders? *Journal of Experimental Marine Biology and Ecology*, *367*(2), 100–110. https://doi.org/10.1016/j.jembe.2008.08.021

Beaumont, A., Snow, M., Shanks, A. M., Piertney, S. B., Davies, I. M., Bland, M., & Dias, P. J. (2008). *Mytilus* species under rope culture in Scotland: implications for management. *Aquaculture International*, *17*(5), 437–448. https://doi.org/10.1007/s10499-008-9214-6

Beiras, R., & Albentosa, M. (2004). Inhibition of embryo development of the commercial bivalves *Ruditapes decussatus* and *Mytilus galloprovincialis* by trace metals; implications for the implementation of seawater quality criteria. *Aquaculture*, *230*(1–4), 205–213. https://doi.org/10.1016/S0044-8486(03)00432-0

Beiras, R., & His, E. (1995). Effects of dissolved mercury on embryogenesis, survival and growth of *Mytilus galloprovincialis* mussel larvae. *Marine Ecology Progress Series*, *126*, 185–189. https://doi.org/10.3354/meps126185

Benabdelmouna, A., Garcia, C., Ledu, C., Lamy, P., Maurouard, E., & Dégremont, L. (2018). Mortality investigation of *Mytilus edulis* and *Mytilus galloprovincialis* in France: An experimental survey under laboratory conditions. *Aquaculture*, *495*, 831–841. https://doi.org/10.1016/j.aquaculture.2018.06.075

Benabdelmouna, A., & Ledu, C. (2016). The mass mortality of blue mussels (*Mytilus* spp.) from the Atlantic coast of France is associated with heavy genomic abnormalities as evidenced by flow cytometry. *Journal of Invertebrate Pathology*, *138*, 30–38. https://doi.org/10.1016/j.jip.2016.06.001

Benadelmouna, A., Saunier, A., Ledu, C., Travers, M.-A., & Morga, B. (2018). Genomic abnormalities affecting mussels (*Mytilus edulis-galloprovincialis*) in France are related to ongoing neoplastic processes, evidenced by dual flow cytometry and cell monolayer analyses. *Journal of Invertebrate Pathology*, *157*, 45–52.

https://doi.org/10.1016/j.jip.2018.08.003

Betzer, S. B., & Yevich, P. P. (1975). Copper toxicity in *Busycon canaliculatum* L. *The Biological Bulletin*, *148*(1), 16–25. https://doi.org/10.2307/1540646

Bhagde Rupendra V. (2013). Study of condition indices of the green mussel *Perna viridis* from Mandi shore in Ratnagiri district of Maharashtra state, India. *Trend of Fisheries Research*, *2*(1), 2319–2474.

Boening, D. W. (1999). An Evaluation of bivalves as biomonitors of heavy metals pollution in marine waters. *Environmental Monitoring and Assessment*, *55*(3), 459–470. https://doi.org/10.1023/A:1005995217901

Bogart, S. J., Azizishirazi, A., & Pyle, G. G. (2019). Challenges and future prospects for developing Ca and Mg water quality guidelines: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1764), 20180364. https://doi.org/10.1098/rstb.2018.0364

Boroda, A. V., Kipryushina, Y. O., & Odintsova, N. A. (2020). The effects of cold stress on *Mytilus* species in the natural environment. *Cell Stress & Chaperones*, *25*(6), 821–832. https://doi.org/10.1007/s12192-020-01109-w

Botta, R., Asche, F., Borsum, J. S., & Camp, E. V. (2020). A review of global oyster aquaculture production and consumption. *Marine Policy*, *117*, 103952. https://doi.org/10.1016/j.marpol.2020.103952

Boudry, P., Blanco, A., Beaumont, A., Cornette, F., Pincot, L., Galley, T., Batista, F. M., Dominguez, L., Fuentes, J., McCombie, H., & Kamermans, P. (2013). Blue mussel hatchery technology in Europe. *Advances in Aquaculture Hatchery Technology, 242,* 339–373. https://doi.org/10.1533/9780857097460.2.339

Bourget, E. (1983). Seasonal variations of cold tolerance in intertidal molluscs and relation to environmental conditions in the St. Lawrence Estuary. *Canadian Journal of Zoology*, *61*(6), 1193–1201. https://doi.org/10.1139/z83-162

References

Boyd, C. E. (2000). Particulate matter, turbidity, and colour. *Water Quality,* 95–103. Boston, MA: Springer US. https://doi.org/10.1007/978-1-4615-4485-2_6

Braby, C. E., & Somero, G. N. (2006). Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *Journal of Experimental Biology*, *209*(13), 2554–2566. https://doi.org/10.1242/jeb.02259

Brenko, M. Hrs., & Calabrese, A. (1969). The combined effects of salinity and temperature on larvae of the mussel *Mytilus edulis*. *Marine Biology*, *4*(3), 224–226.

Breuer, M. E. G., & Twisk, D. D. (2024, April). Aquaculture production in the European Union. *Fact sheets on the European Union.* European Parliament Retrieved 12 December 2024, from https://www.europarl.europa.eu/factsheets/en/sheet/120/aquaculture-production-in-the-european-union#:~:text=Whereas%20there%20was%20a%20considerable,produced%20mainly%20seabass%20and%20seabream.

Bryan G. W. (1971). The effects of heavy metals (other than mercury) on marine and estuarine organisms. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *177*(1048), 389–410. https://doi.org/10.1098/rspb.1971.0037

Buck, B. H. (2007). Experimental trials on the feasibility of offshore seed production of the mussel *Mytilus edulis* in the German Bight: installation, technical requirements and environmental conditions. *Helgoland Marine Research*, *61*(2), 87–101. https://doi.org/10.1007/s10152-006-0056-1

Buer, A.-L., Taylor, D., Bergström, P., Ritzenhofen, L., & Klemmstein, A. (2020). Nitrogen and phosphorous content in blue mussels (*Mytilus* spp.) Across the Baltic Sea. *Frontiers in Marine Science*, *7*, 705. https://doi.org/10.3389/fmars.2020.00705

Capelle, J. J., Garcia, A. B., Kamermans, P., Engelsma, M. Y., & Jansen, H. M. (2021). Observations on recent mass mortality events of marine mussels in the Oosterschelde, the Netherlands. *Aquaculture International*, *29*(4), 1737–1751.

https://doi.org/10.1007/s10499-021-00713-6

Carbonell, G., & Tarazona, J. V. (1994). Toxicokinetics of copper in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology*, *29*(3–4), 213–221. https://doi.org/10.1016/0166-445X(94)90069-8

Carlton, J. T. (1996). Biological invasions and cryptogenic species. *Ecology*, *77*(6), 1653–1655. https://doi.org/10.2307/2265767

Centre for Environment, Fisheries & Aquaculture Science. (no date). UV disinfection in depuration: Theory and practice [PDF]. Retrieved 21 February 2024, from https://www.cefas.co.uk/media/im3dgqpe/uv-disinfection-in-depuration-theory-and-practice-dj-passed.pdf

Centre for Environment, Fisheries & Aquaculture Science (2014). Scottish sanitary survey report sanitary survey report Loch Sunart V1.0 Retrieved 13 February 2024, from https://cefaswebsitedev.cefastest.co.uk/media/1vjcdpc3/loch-sunart-sanitary-survey-report-v10.pdf

Centre for Environment, Fisheries & Aquaculture Science. (no date). Harmful aquatic toxins. Retrieved 17 December 2024, from https://www.cefas.co.uk/icoe/seafood-safety/science/harmful-aquatic-biotoxins/#:~:text=Biotoxins%20are%20hazards%20produced%20naturally,poisoning%20(DSP)%20or%20azaspiracid%20shellfish

Chalkiadaki, O., Dassenakis, M., & Lydakis-Simantiris, N. (2014). Bioconcentration of Cd and Ni in various tissues of two marine bivalves living in different habitats and exposed to heavily polluted seawater. *Chemistry and Ecology*, *30*(8), 726–742. https://doi.org/10.1080/02757540.2014.917172

Charles, M., Bernard, I., Villalba, A., Oden, E., Burioli, E. A. V., Allain, G., Trancart, S., Bouchart, V., & Houssin, M. (2020). High mortality of mussels in northern Brittany – Evaluation of the involvement of pathogens, pathological conditions and pollutants. *Journal of Invertebrate Pathology*, *170*, 107308. https://doi.org/10.1016/j.jip.2019.107308

References

Chu, F.-L. E., & Hale, R. C. (1994). Relationship between pollution and susceptibility to infectious disease in the eastern oyster, *Crassostrea virginica. Marine Environmental Research*, *38*(4), 243–256. https://doi.org/10.1016/0141-1136(94)90026-4

Clarke Murray, C., Pakhomov, E. A., & Therriault, T. W. (2011). Recreational boating: a large unregulated vector transporting marine invasive species. *Diversity and Distributions*, *17*(6), 1161–1172. https://doi.org/10.1111/j.1472-4642.2011.00798.x

Cledon, M., Tremblay, L. A., Griffiths, C., Fadhlaoui, M., Champeau, O., Albentosa, M., Besada, V., Fernandez, V. H., McKindsey, C.W., Bendell, L. I., Zhang, B., Garcia-Esquivel, Z., Curiel, S., Brar, S. K., Kumar, P., Laroche, O & Couture, P. (2021). Trace metal residues in marine mussels: A global survey. *Environmental Toxicology and Chemistry*, *40*(12), 3434–3440. https://doi.org/10.1002/etc.5228

Connor, D. W., Allen, J. H., Golding, N., Howell, K. L., Lieberknecht, L. M., Northen, K. O., & Reker, J. B. (2004). The marine habitat classification for Britain and Ireland. Version 04.05. JNCC, Peterborough
ISBN 1-861-07561-8 (internet version)
Retrieved 13 February 2024, from
www.jncc.gov.uk/MarineHabitatClassification

Corrochano-Fraile, A., Carboni, S., Green, D. M., Taggart, J. B., Adams, T. P., Aleynik, D., & Bekaert, M. (2024). Estimating blue mussel (*Mytilus edulis*) connectivity and settlement capacity in mid-latitude fjord regions. *Communications Biology*, *7*(1), 166. https://doi.org/10.1038/s42003-023-05498-3

Corsi, S. R., Graczyk, D. J., Geis, S. W., Booth, N. L., & Richards, K. D. (2010). A fresh look at road salt: Aquatic toxicity and water-quality impacts on local, regional, and national scales. *Environmental Science & Technology*, *44*(19), 7376–7382. https://doi.org/10.1021/es101333u

Dailianis, S. (2011). Environmental impact of anthropogenic activities: the use of mussels as a reliable tool for monitoring marine pollution. *Mussels: anatomy, habitat and environmental impact,* 43-72. Nova Sciences Publishers Inc. New York.

References

Dales, R. P. (1979). Biology of the intertidal zone. *Nature*, *279*(5715), 744–744. https://doi.org/10.1038/279744a0

De Rijcke, M., Vandegehuchte, M. B., Vanden Bussche, J., Nevejan, N., Vanhaecke, L., De Schamphelaere, K. A. C., & Janssen, C. R. (2015). Common European harmful algal blooms affect the viability and innate immune responses of Mytilus edulis larvae. *Fish and Shellfish Immunology*, *47*(1), 175–181. https://doi.org/10.1016/j.fsi.2015.09.003

DeForest, D. K., & Schlekat, C. E. (2013). Species sensitivity distribution evaluation for chronic nickel toxicity to marine organisms. *Integrated Environmental Assessment and Management*, *9*(4), 580–589. https://doi.org/10.1002/ieam.1419

Dégremont, L., Maurouard, E., Rabiller, M., & Glize, P. (2019). Response to selection for increasing resistance to the spring mortality outbreaks in *Mytilus edulis* occurring in France since 2014. *Aquaculture*, *511*, 734269. https://doi.org/10.1016/j.aquaculture.2019.734269

Deruytter, D., Vandegehuchte, M. B., Garrevoet, J., De Laender, F., Vergucht, E., Delbeke, K., Blust, R., De Schamphelaere, K. A. C., Vincze, L., & Janssen, C. R. (2015). Salinity and dissolved organic carbon both affect copper toxicity in mussel larvae: Copper speciation or competition cannot explain everything. *Environmental Toxicology and Chemistry*, *34*(6), 1330–1336. https://doi.org/10.1002/etc.2924

Dias, P. J., Malgrange, B., Snow, M., & Davies, I. M. (2011). Performance of Mussels, *Mytilus edulis*, *Mytilus trossulus*, and their hybrids in cultivation at three Scottish Lochs. *Journal of the World Aquaculture Society*, *42*(1), 111–121. https://doi.org/10.1111/j.1749-7345.2010.00450.x

Dias, P. J., Piertney, S. B., Snow, M., & Davies, I. M. (2011). Survey and management of mussel *Mytilus* species in Scotland. *Hydrobiologia*, *670*(1), 127–140. https://doi.org/10.1007/s10750-011-0664-x

Dickerson, K. K., Hubert, W. A., & Bergman, H. L. (1996). Toxicity assessment of water from lakes and wetlands receiving irrigation drain water. *Environmental Toxicology and Chemistry*, *15*(7), 1097–1101. https://doi.org/10.1002/etc.5620150712

Dixon-Anderson, I. S., & Science, M. (2021). Effect of Magnesium Concentration in Seawater on Marine Invertebrate Calcification [University of Otago]. *https://ourarchive.otago.ac.nz/esploro/outputs/graduate/Effect-of-Magnesium-Concentration-in-Seawater/9926480168701891#file-0*

Dobretsov, S., & Miron, G. (2001). Larval and post-larval vertical distribution of the mussel *Mytilus edulis* in the White Sea. *Marine Ecology Progress Series*, *218*, 179–187. https://doi.org/10.3354/meps218179

Edwards, A., Edelsten, D. J., Saunders, M. A., & Stanley, S. O. (1980). Renewal and entrainment in Loch Eil; A periodically ventilated Scottish fjord. *Freeland, H.J., Farmer, D.M., Levings, C.D. (eds) Fjord Oceanography. NATO Conference Series, vol 4. Springer, Boston, MA.* https://doi.org/10.1007/978-1-4613-3105-6_47

Eisler, R., (1988). Arsenic hazards to fish, wildlife, and invertebrates: A synoptic review. *Contaminant Hazard Reviews Report 12; Biological Report 85(1.12).* U.S. Department of the Interior, Fish and Wildlife Service Retrieved 8 February 2024, from https://pubs.usgs.gov/publication/5200037

Eisler, R., (1998). Copper hazards to fish, wildlife and invertebrates: A synoptic review. *Contaminant Hazard Reviews Report 33, Biological Science Report USGS/BRD/BSR-1997-0002.* U.S. Department of the Interior, Fish and Wildlife Service Retrieved 9 February 2024, from https://pubs.usgs.gov/publication/5200199

European Commission: Directorate-General for Maritime Affairs and Fisheries. (2024). *The EU fish market: 2024 edition.* Publications Office of the European Union. https://data.europa.eu/doi/10.2771/9420236

Faury, N., Tourbiez, D., Renault, T., Bourgougnon, N., Benabdelmouna, A., Segarra, A., & Baillon, L. (2014). Ostreid herpesvirus type 1 replication and

host response in adult Pacific oysters, *Crassostrea gigas*. *Veterinary Research*, *45*(1), 30–32. https://doi.org/10.1186/s13567-014-0103-x

Figueiras, F. G., Labarta, U., & Reiriz, M. J. F. (2002). Coastal upwelling, primary production and mussel growth in the Rías Baixas of Galicia. *Sustainable Increase of Marine Harvesting: Fundamental Mechanisms and New Concepts,* 121–131. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-017-3190-4_11

Findlay, K. (2018). New record for mussel production. *The Press and Journal.* Retrieved 16 February 2018, from https://www.pressandjournal.co.uk/fp/business/scotland-business/1489086/new-record-for-mussel-production/

Fisher, C., & Skibinski, D. O. F. (1990). Sex-Biased Mitochondrial DNA Heteroplasmy in the Marine Mussel *Mytilus*. *Proceedings: Biological Sciences*, *242*(1305), 149–156. https://doi.org/10.1007/BF02221509

Fitridge, I., Dempster, T., Guenther, J., & de Nys, R. (2012). The impact and control of biofouling in marine aquaculture: A review. *Biofouling*, *28*(7), 649–669. https://doi.org/10.1080/08927014.2012.700478

Flander-Putrle, V., & Malej, A. (2008). The evolution and phytoplankton composition of mucilaginous aggregates in the northern Adriatic Sea. *Harmful Algae*, *7*(6), 752–761. https://doi.org/10.1016/j.hal.2008.02.009

Food and Agriculture Organization of the United Nations FAO. (2018). The state of world fisheries and aquaculture. 2018: meeting the sustainable development goals. *State of World Fisheries and Aquaculture.* Rome, 2018 ISBN 9-789-25130-562-1

Food and Agriculture Organization of the United Nations FAO. (2022). The State of World Fisheries and Aquaculture 2022: towards blue transformation. *State of World Fisheries and Aquaculture.* Rome, 2022 ISBN 9-789-25136-364-5 https://doi.org/10.4060/cc0461en

References

Food and Agriculture Organization of the United Nations FAO GLOBEFISH. (2019). European mussel production good this year. *Information and Analysis on Markets and Trade of Fisheries and Aquaculture Products.* Retrieved 3 March 2019, from European mussel production good this year

Food Standards Agency (FSA). (no date). Biotoxin and phytoplankton monitoring. *Food Standards Agency.* Retrieved 17 December 2024, from https://www.food.gov.uk/business-guidance/biotoxin-and-phytoplankton-monitoring

Forrest, B., & Atalah, J. (2017). Significant impact from blue mussel *Mytilus galloprovincialis* biofouling on aquaculture production of green-lipped mussels in New Zealand. *Aquaculture Environment Interactions*, *9*, 115–126. https://doi.org/10.3354/aei00220

Freitas, R., Coppola, F., Henriques, B., Wrona, F., Figueira, E., Pereira, E., & Soares, A. M. V. M. (2017). Does pre-exposure to warming conditions increase *Mytilus galloprovincialis* tolerance to Hg contamination? *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *203*, 1–11. https://doi.org/10.1016/j.cbpc.2017.09.010

Fuentes, J., Reyero, I., Zapata, C., & Alvarez, G. (1992). Influence of stock and culture site on growth rate and mortality of mussels (*Mytilus galloprovincialis* Lmk.) in Galicia, Spain. *Aquaculture*, *105*(2), 131–142. https://doi.org/10.1016/0044-8486(92)90125-5

Galbraith, L., Mackenzie, M., Sinclair, D., Garner, F., Scottish Government, Scottish Water, Adrian, A., Lewis, S., & Byrne, A. (2012). Loch Eil Sanitary Survey Report Final V1.0. *Scottish Sanitary Survey Programme*, CEFAS & Food Standards Agency Scotland.

Gardner, J. P. A., & Skibinski, D. O. F. (1991). Mitochondrial DNA and allozyme covariation in a hybrid mussel population. *Journal of Experimental Marine Biology and Ecology*, *149*(1), 45–54. https://doi.org/10.1016/0022-0981(91)90115-D

Gilg, M. R., & Hilbish, T. J. (2003). The geography of marine larval dispersal: coupling genetics with fine-scale physical oceanography. *Ecology*, *84*(11), 2989–2998. https://doi.org/10.1890/02-0498

Gillibrand, P. A., Turrell, W. R., & Elliott, A. J. (1995). Deep-water renewal in the upper basin of Loch Sunart, a Scottish fjord. *Journal of Physical Oceanography*, *25*(6), 1488–1503.

Gleason, L. U., Strand, E. L., Hizon, B. J., & Dowd, W. W. (2018). Plasticity of thermal tolerance and its relationship with growth rate in juvenile mussels (*Mytilus californianus*). *Proceedings of the Royal Society B: Biological Sciences*, *285*(1877). 20172617. https://doi.org/10.1098/rspb.2017.2617

Gokul, T., Kumar, K. R., Prema, P., Arun, A., Balaji, P., & Faggio, C. (2023). Particulate pollution and its toxicity to fish: An overview. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *270*, 109646. https://doi.org/10.1016/j.cbpc.2023.109646

Golterman, H. L., R.S. Clymo, & Ohnstad, M. A. M. (1978). *Methods for physical and chemical analysis of fresh waters - 2nd ed.* (Vol. 8). Oxford, Blackwell.

Gong, N., Ellis, R. P., Russ, S., Baden, S. P., Hernroth, B. E., & Asplund, M. E. (2013). Ocean acidification and host-pathogen interactions: blue mussels, *Mytilus edulis*, encountering *Vibrio tubiashii*. *Environmental Microbiology*, *16*(4), 1029–1039. https://doi.org/10.1111/1462-2920.12307

Gosling, E. (2003a). Bivalve culture. *Bivalve Molluscs*: *Biology, Ecology and Culture,* 284–332. Blackwell Publishing Ltd.

Gosling, E. (2003b). Ecology of bivalves. *Bivalve Molluscs*: *Biology, Ecology and Culture,* 44–86. Blackwell Publishing Ltd.

Gosling, E. (2003c). Reproduction, settlement and recruitment. *Bivalve Molluscs*: *Biology, Ecology and Culture,* 131–168. Blackwell Publishing Ltd.

Gosling, E. (2003d). Bivalve growth. *Bivalve Molluscs*: *Biology, Ecology and Culture*, 169–200) Blackwell Publishing Ltd.

Gosling, E. (2015a). Bivalve culture. *Marine Bivalve Molluscs*, *2nd Edition*, 325–382. John Wiley & Sons, Ltd.

Gosling, E. (2015b). Ecology of bivalves. *Marine Bivalve Molluscs, 2nd Edition*, 44–98. John Wiley & Sons, Ltd.

Gosling, E. (2015c). Reproduction, settlement and recruitment. *Marine Bivalve Molluscs*, *2nd Edition*, 157–202. John Wiley & Sons, Ltd.

Gosling, E. (2015d). Growth. *Marine Bivalve Molluscs*, *2nd Edition*, 203–242. John Wiley & Sons, Ltd.

Gosling, E. M., & McGrath, D. (1990). Genetic variability in exposed-shore mussels, *Mytilus* spp., along an environmental gradient. *Marine Biology*, *104*(3), 413–418. https://doi.org/10.1007/BF01314344

Goulletquer, P. (2009). *Mytilus edulis* (Linnaeus, 1758). *Cultured aquatic species fact sheets*. Food and Agriculture Organization of the United Nations FAO. Retrieved 16 June 2021, from https://www.fao.org/fishery/docs/CDrom/aquaculture/I1129m/file/en/en_bluemussel.htm

Goulletquer, P., & Heral, M. (1997). Marine molluscan production trends in France: From fisheries to aquaculture. *NOAA Technical Reports NMFS* (129th ed., pp. 137–164). U.S. Department of Commerce.

Granato, G., Church, P., & Stone, V. (1995). Mobilisation of major and trace constituents of highway runoff in groundwater potentially caused by deicing chemical migration. *Transportation Research Record, 1483,* 92–104.

Grantham, B. (1981). The Loch Eil project: Chlorophyll a and nutrients in the water column of Loch Eil. *Journal of Experimental Marine Biology and Ecology*, *55*(2–3), 283–297. https://doi.org/10.1016/0022-0981(81)90118-0

Griffith, M. B. (2017). Toxicological perspective on the osmoregulation and ionregulation physiology of major ions by freshwater animals: Teleost fish, crustacea, aquatic insects, and *Mollusca*. *Environmental Toxicology and Chemistry*, *36*(3), 576–600. https://doi.org/10.1002/etc.3676

Hall, M. R., Moffett, J. W., & Gracey, A. Y. (2020). RNAseq reveals sensitive, concentration-dependent transcriptional markers of copper in *Mytilus californianus* larvae and adults. *Frontiers in Marine Science*, *7*. 572496. https://doi.org/10.3389/fmars.2020.572496

References

Hamilton, D. J. (2000). Direct and indirect effects of predation by common eiders and abiotic disturbance in an intertidal community. *Ecological Monographs*, *70*(1), 21. https://doi.org/10.2307/2657166

Hawkins, A. J. S., Pascoe, P. L., Parry, H., Brinsley, M., Black, K. D., McGonigle, C., Moore, H., Newell, C. R., O'Boyle, N., Ocarroll, T., O'Loan, B., Service, M., Smaal, A. C., Zhang, X. L., & Zhu, M. Y. (2013). Shellsim: A generic model of growth and environmental effects validated across contrasting habitats in bivalve shellfish. *Journal of Shellfish Research*, *32*(2), 237–253. https://doi.org/10.2983/035.032.0201

Hilbish, T. J., Bayne, B. L., & Day, A. (1994). Genetics of physiological differentiation within the marine mussel genus *Mytilus*. *Evolution*, *48*(2), 267–286. https://doi.org/10.1111/j.1558-5646.1994.tb01311.x

Hilbish, T. J., Mullinax, A., Dolven, S. I., Meyer, A., Koehn, R. K., & Rawson, P. D. (2000). Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): routes and timing of trans equatorial migration. *Marine Biology*, *136*(1), 69–77. https://doi.org/10.1007/s002270050010

Hilgerloh, G. (1997). Predation by birds on blue mussel *Mytilus edulis* beds of the tidal flats of Spiekeroog (southern North Sea). *Marine Ecology Progress Series*, *146*, 61–72. https://doi.org/10.3354/meps146061

Hintz, W. D., & Relyea, R. A. (2017). Impacts of road deicing salts on the early-life growth and development of a stream salmonid: Salt type matters. *Environmental Pollution*, *223*, 409–415. https://doi.org/10.1016/j.envpol.2017.01.040

HMSO. (1986). The determination of chlorophyll a in aquatic environments. *Methods for the Examination of Water and Associated Materials*, *4*, 51–109. Her Majesty's Staionary Office

Hofmann, G. E., & Somero, G. N. (1995). Evidence for protein damage at environmental temperatures: Seasonal changes in levels of ubiquitin conjugates and Hsp70 in the intertidal mussel *Mytilus Trossulus*. *Journal of Experimental Biology*, *198*(7), 1509–1518.

https://doi.org/10.1242/jeb.198.7.1509

Hrs-Brenko, M., Claus, C., & Bubic, S. (1977). Synergistic effects of lead, salinity and temperature on embryonic development of the mussel *Mytilus galloprovincialis. Marine Biology*, *44*(2), 109–115. https://doi.org/10.1007/BF00386951

Huang, J., & Zhang, R. (2022). The Mineralisation of molluscan shells: Some unsolved problems and special considerations. *Frontiers in Marine Science*, *9*, 2022-2026. https://doi.org/10.3389/fmars.2022.874534

Hung T.-C., Meng P.-J., & Chuang A. (1992). Relationships among the species of copper in water, sediments and biomass along the Taiwan Erhjin Chi coastal area. *Journal of the Institute of chemistry, Academia Sinica.* (39), 77–90. https://doi.org/10.6522/BICAS.1992.39.10

Incze, L. S., Lutz, R. A., & Watling, L. (1980). Relationships between effects of environmental temperature and seston on growth and mortality of *Mytilus edulis* in a temperate northern estuary. *Marine Biology*, *57*(3), 147–156. https://doi.org/10.1007/BF00390733

Jha, A. N., Dogra, Y., Turner, A., & Millward, G. E. (2005). Impact of low doses of tritium on the marine mussel, *Mytilus edulis*: Genotoxic effects and tissue-specific bioconcentration. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *586*(1), 47–57. https://doi.org/10.1016/j.mrgentox.2005.05.008

Johnston, R., & Topping, G. (1972). The effects of a new pulp mill on two interconnecting sea lochs. *DAFS Mar. Lab. Internal Report, 48 MM 72*, 43.

Karayücel, S., Karayücel, I., Erdem, M., Saygun, S., & Uyan, O. (2003). Growth and production in long-Line cultivated Mediterranean mussel (*Mytilus Galloprovincialis*) In Sinop, Black Sea. *Israeli Journal of Aquaculture - Bamidgeh*, *55*(3), 169–178. https://doi.org/10.46989/001c.20348

Kautsky, N. (1982). Growth and size structure in a Baltic *Mytilus edulis* population. *Marine Biology*, *68*(2), 117–133. https://doi.org/10.1007/BF00397599

Kautsky, N., Johannesson, K., & Tedengren, M. (1990). Genotypic and phenotypic differences between Baltic and North Sea populations of *Mytilus edulis* evaluated through reciprocal transplantations. Growth and morphology. *Marine Ecology Progress Series*, *59*(3), 203–210.

Kefaloyianni, E., Gourgou, E., Ferle, V., Kotsakis, E., Gaitanaki, C., & Beis, I. (2005). Acute thermal stress and various heavy metals induce tissue-specific pro-or anti-apoptotic events via the p38-MAPK signal transduction pathway in *Mytilus galloprovincialis* (Lam.). *Journal of Experimental Biology*, *208*(23), 4427–4436.
https://doi.org/10.1242/jeb.01924

Kerambrun, E., Rioult, D., Delahaut, L., Evariste, L., Pain-Devin, S., Auffret, M., Geffard, A., & David, E. (2016). Variations in gene expression levels in four European zebra mussel, *Dreissena polymorpha*, populations in relation to metal bioaccumulation: A field study. *Ecotoxicology and Environmental Safety*, *134*, 53–63.
https://doi.org/10.1016/j.ecoenv.2016.08.018

King, P. A., McGrath, D., & E. M. (1989). Reproduction and settlement of *Mytilus edulis* on an exposed rocky shore in Galway Bay, west coast of Ireland. *Journal of the Marine Biological Association of the United Kingdom*, *69*(2), 355–365.
https://doi.org/10.1017/S0025315400029465

Koehn, R. K. (1991). The genetics and taxonomy of species in the genus *Mytilus*. *Aquaculture*, *94*(2–3), 125–145.
https://doi.org/10.1016/0044-8486(91)90114-M

Koehn, R. K., Hall, J. G., Innes, D. J., & Zera, A. J. (1984). Genetic differentiation of *Mytilus edulis* in eastern North America. *Marine Biology*, *79*(2), 117–126.
https://doi.org/10.1007/BF00951820

Koehn, R. K., Milkman, R., & Mitton, J. B. (1976). Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel *Mytilus edulis*. *Evolution*, *30*(1), 2.
https://doi.org/10.2307/2407669

Koehn, R. K., & Mitton, J. B. (1972). Population genetics of marine pelecypods. I. Ecological heterogeneity and evolutionary strategy at an enzyme locus. *The American Naturalist*, *106*(947), 47–56.

https://doi.org/10.1086/282750

Kolyuchkina, G. A., & Ismailov, A. D. (2011). Morpho-functional characteristics of bivalve molluscs under the experimental environmental pollution by heavy metals. *Oceanology*, *51*(5), 804–813. https://doi.org/10.1134/S0001437011050092

Kumar, V., Sinha, A. K., Rodrigues, P. P., Mubiana, V. K., Blust, R., & De Boeck, G. (2015). Linking environmental heavy metal concentrations and salinity gradients with metal accumulation and their effects: A case study in 3 mussel species of Vitória estuary and Espírito Santo bay, Southeast Brazil. *Science of the Total Environment*, *523*, 1–15.

Kunselman, E., Wiggin, K., Diner, R. E., Gilbert, J. A., & Allard, S. M. (2024). Microbial threats and sustainable solutions for molluscan aquaculture. *Sustainable Microbiology*, *1*(1), qvae002 https://doi.org/10.1093/sumbio/qvae002

Landes, A., Dolmer, P., Poulsen, L. K., Petersen, J. K., & Vismann, B. (2015). Growth and respiration in blue mussels (*Mytilus* spp.) from different salinity regimes. *Journal of Shellfish Research*, *34*(2), 373–382. https://doi.org/10.2983/035.034.0220

Langan, R., & Horton, F. (2003). Design, operation and economics of submerged longline mussel culture in the open ocean. *Bulletin of the Aquaculture Association of Canada 103*(3): 11–20

Langston, W. J., O'Hara, S., Pope, N. D., Davey, M., Shortridge, E., Imamura, M., Harino, H., Kim, A., & Vane, C. H. (2012). Bioaccumulation surveillance in Milford Haven Waterway. *Environmental Monitoring and Assessment*, *184*(1), 289–311. https://doi.org/10.1007/s10661-011-1968-z

Lazo, C. S., & Pita, I. M. (2012). Effect of temperature on survival, growth and development of *Mytilus galloprovincialis* larvae. *Aquaculture Research*, *43*(8), 1127–1133. https://doi.org/10.1111/j.1365-2109.2011.02916.x

Lee, C.-H. (2003). Effects of the red tide and toxic dinoflagellates on the survival and growth of larvae of the mussel, *Mytilus galloprovincialis. The Korean Journal of Malacology*, *19*, 25–32.

References

Lengyel, N. (2009). The invasive colonial ascidian *Didemnum vexillum* on Georges Bank — Ecological effects and genetic identification. *Aquatic Invasions*, *4*(1), 143–152. https://doi.org/10.3391/ai.2009.4.1.15

Leuthold, S. J., Haddix, M. L., Lavallee, J., & Cotrufo, M. F. (2023). Physical fractionation techniques. *Encyclopedia of Soils in the Environment*, 68–80. Elsevier. https://doi.org/10.1016/B978-0-12-822974-3.00067-7

Levinton, J. S., & Suchanek, T. H. (1978). Geographic variation, niche breadth and genetic differentiation at different geographic scales in the mussels *Mytilus californianus* and *M. edulis*. *Marine Biology*, *49*(4), 363–375. https://doi.org/10.1007/BF00455031

Lin, C.-H., Yeh, P.-L., & Lee, T.-H. (2016). Ionic and amino acid regulation in hard clam (*Meretrix lusoria*) in response to salinity challenges. *Frontiers in Physiology*, *7*, 368. https://doi.org/10.3389/fphys.2016.00368

Lussier, S. M., Boothman, W. S., Poucher, S., Champlin, D., & Helmstetten, A. (1999). Comparison of dissolved and total metals concentrations from acute tests with saltwater organisms. *Environmental Toxicology and Chemistry*, *18*(5), 889–898. https://doi.org/10.1002/etc.5620180511

Lynch, S. A., Morgan, E., Carlsson, J., Mackenzie, C., Wooton, E. C., Rowley, A. F., Malham, S., & Culloty, S. C. (2014). The health status of mussels, *Mytilus* spp., in Ireland and Wales with the molecular identification of a previously undescribed haplosporidian. *Journal of Invertebrate Pathology*, *118*, 59–65. https://doi.org/10.1016/j.jip.2014.02.012

Maar, M., Saurel, C., Landes, A., Dolmer, P., & Petersen, J. K. (2015). Growth potential of blue mussels (*M. edulis*) exposed to different salinities evaluated by a dynamic energy budget model. *Journal of Marine Systems*, *148*, 48–55. https://doi.org/10.1016/j.jmarsys.2015.02.003

Malone, T., Azzaro di Rosamarina, M., Bode, A., Brown, E., Duce, R., Kamykowski, D., Kang, S., Kedong, Y., Thorndyke, M., Wang, J., Park, C., Calumpong, H., & Eghtesadi, P. (2016). Primary production, cycling of

nutrients, surface layer and plankton. *First Global Marine Assessment* (p. 67). Oceans and Laws of the Sea, United Nations

Mandić, M., Nikolić, S., Kokić, I., & Jokanović, S. (2024). Mass mortality of farmed mussels - a phenomenon without explanation? *Studia Marina*, *2024*(1), 5–21. https://doi.org/10.5281/zenodo.12749090

Martin, M., Osborn, K. E., Billig, P., & Glickstein, N. (1981). Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and *Cancer magister* larvae. *Marine Pollution Bulletin*, *12*(9), 305–308. https://doi.org/10.1016/0025-326X(81)90081-3

Mascorda Cabre, L., Hosegood, P., Attrill, M. J., Bridger, D., & Sheehan, E. V. (2021). Offshore longline mussel farms: a review of oceanographic and ecological interactions to inform future research needs, policy and management. *Reviews in Aquaculture*, *13*(4), 1864–1887. https://doi.org/10.1111/raq.12549

Mathiesen, S. S., Thyrring, J., Hemmer-Hansen, J., Berge, J., Sukhotin, A., Leopold, P., Bekaert, M., Sejr, M. K., & Nielsen, E. E. (2017). Genetic diversity and connectivity within *Mytilus* spp. in the subarctic and Arctic. *Evolutionary Applications*, *10*(1), 39–55. https://doi.org/10.1111/eva.12415

McDonald, J. H., & Koehn, R. K. (1988). The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. *Marine Biology*, *99*(1), 111–118. https://doi.org/10.1007/BF00644984

McDougall, D. R., Chan, A., McGillivray, D. J., de Jonge, M. D., Miskelly, G. M., & Jeffs, A. G. (2019). Examining the role of ethylenediaminetetraacetic acid (EDTA) in larval shellfish production in seawater contaminated with heavy metals. *Aquatic Toxicology*, *217,* 105330. https://doi.org/10.1016/j.aquatox.2019.105330

McDougall, D. R., Kihara, S., Reinhardt, J., Miskelly, G. M., McGillivray, D. J., & Jeffs, A. G. (2020). Biodegradable chelating agent improves the survival of early larvae for shellfish aquaculture. *Aquatic Toxicology*, *228,* 105645. https://doi.org/10.1016/j.aquatox.2020.105645

McDougall, D. R., Toone, T. A., & Jeffs, A. G. (2022). Natural heavy metal concentrations in seawater as a possible cause of low survival of larval mussels. *Journal of Trace Elements in Medicine and Biology*, *74,* 127071. https://doi.org/10.1016/j.jtemb.2022.127071

McDougall, D. R., Vignier, J., Ragg, N. L. C., Finnie, B., Jeffs, A., & Adams, S. (2020). The value of EDTA treatment of hatchery water to rear Greenshell™ mussel (*Perna canaliculus*) larvae. *Aquaculture International*, *28*(4), 1579–1592. https://doi.org/10.1007/s10499-020-00543-y

McGeer, J. C., Niyogi, S., & Scott Smith, D. (2011). Cadmium. *Fish Physiology, 31*, Part B, 125–184. https://doi.org/10.1016/S1546-5098(11)31025-4

Mcleod, D. A., & Mcleod, C. (2019). Review of the contribution of cultivated bivalve shellfish to ecosystem services. *A review of the scientific literature commissioned by Crown Estate Scotland*. Crown Estate Scotland

McLusky, D. S., Bryant, V., & Campbell, R. (1986). The effects of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. *Oceanography and Marine Biology Annual Review*, *24*, 481–520.

Metzger, M. J., & Goff, S. P. (2016). A sixth modality of infectious disease: contagious cancer from devils to clams and beyond. *PLOS Pathogens*, *12*(10): e1005904. https://doi.org/10.1371/journal.ppat.1005904

Metzger, M. J., Reinisch, C., Sherry, J., & Goff, S. P. (2015). Horizontal transmission of clonal cancer cells causes leukaemia in soft-shell clams. *Cell*, *161*(2), 255–263. https://doi.org/10.1016/j.cell.2015.02.042

Micallef, S., & Tyler, P. A. (1990). Effect of mercury and selenium on the gill function of *Mytilus edulis*. *Marine Pollution Bulletin*, *21*(6), 288–292. https://doi.org/10.1016/0025-326X(90)90592-V

Michalek, K., Vendrami, D. L. J., Bekaert, M., Green, D. H., Last, K. S., Telesca, L., Wilding, T. A., & Hoffman, J. I. (2021). *Mytilus trossulus* introgression and consequences for shell traits in longline cultivated mussels. *Evolutionary Applications*, *14*(7), 1830–1843.

https://doi.org/10.1111/eva.13245

Michalek, K., Ventura, A., & Sanders, T. (2016). *Mytilus* hybridisation and impact on aquaculture: A minireview. *Marine Genomics*, *27*, 3–7. https://doi.org/10.1016/j.margen.2016.04.008

Mičić, M., Bihari, N., Labura, Ž., Müller, W. E. G., & Batel, R. (2001). Induction of apoptosis in the blue mussel *Mytilus galloprovincialis* by tri-n-butyltin chloride. *Aquatic Toxicology*, *55*(1–2), 61–73. https://doi.org/10.1016/S0166-445X(01)00156-4

Milik, J., & Pasela, R. (2018). Analysis of concentration trends and origins of heavy metal loads in stormwater runoff in selected cities: A review. *E3S Web of Conferences*, *44*, 00111. https://doi.org/10.1051/e3sconf/20184400111

Milne, P. H. (1972). Hydrography of Scottish West Coast Sea Lochs. *Marine Research, No. 3,* 50.

Miramand, P., & Ünsal, M. (1978). Acute toxicity of vanadium to some marine benthic and phytoplanktonic species. *Chemosphere, 10,* 827–832.

Mizuta, D. D., & Wikfors, G. H. (2019). Depth selection and in situ validation for offshore mussel aquaculture in northeast United States federal waters. *Journal of Marine Science and Engineering*, *7*(9), 293. https://doi.org/10.3390/jmse7090293

Monfort, M.-C. (2014) The European market for mussels. *Globefish Research Programme,* Rome, FAO, *115,* 65.

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

Morgan, J. D., Mitchell, D. G., & Chapman, P. M. (1986). Individual and combined toxicity of manganese and molybdenum to mussel, *Mytilus edulis*, larvae. *Bulletin of Environmental Contamination and Toxicology*, *37*(1), 303–307. https://doi.org/10.1007/BF01607765

Mubiana, V. K., & Blust, R. (2007). Effects of temperature on scope for growth and accumulation of Cd, Co, Cu and Pb by the marine bivalve *Mytilus edulis. Marine Environmental Research*, *63*(3), 219–235.

https://doi.org/10.1016/j.marenvres.2006.08.005

Munro, L. (2019). Scottish shellfish farm production survey 2018. *Marine Scotland Science.* The Scottish Government
ISBN: 978-1-78781-836-1

Munro, L., & Wallace, I. (2018). Scottish shellfish farm production survey 2017. *Marine Scotland Science.* The Scottish Government.

Murphy, J. M., & Munro, L. A. (2024). Scottish shellfish farm production survey 2023. *Marine Scotland Science.* The Scottish Government.

Murphy, J., & Munro, L. (2023). Scottish shellfish farm production survey 2022. *Marine Scotland Science.* The Scottish Government.

Murray, A., Falconer, L., Clarke, D., & Kennerley, A. (2022). Climate change impacts on marine aquaculture relevant to the UK and Ireland. *MCCIP Science Review 2022*, 2.

Murray, A. G., Munro, L. A., & Matejusova, I. (2020). The network of farmed Pacific oyster movements in Scotland and routes for introduction and spread of invasive species and pathogens. *Aquaculture*, *520*, 734747. https://doi.org/10.1016/j.aquaculture.2019.734747

Myint, U. M., & Tyler, P. A. (1982). Effects of temperature, nutritive and metal stressors on the reproductive biology of Mytilus edulis. *Marine Biology*, *67*(2), 209–223. https://doi.org/10.1007/BF00401287

Myrand, B., & Gaudreault, J. (1995). Summer mortality of blue mussels (*Mytilus edulis* Linneaus, 1758) in the Magdalen Islands (southern Gulf of St Lawrence, Canada). *Journal of Shellfish Research*, *14*(2), 395–404.

Myrand, B., Guderley, H., & Himmelman, J. (2000). Reproduction and summer mortality of blue mussels *Mytilus edulis* in the Magdalen Islands, southern Gulf of St. Lawrence. *Marine Ecology Progress Series*, *197*, 193–207. https://doi.org/10.3354/meps197193

Nadella, S. R., Fitzpatrick, J. L., Franklin, N., Bucking, C., Smith, S., & Wood, C. M. (2009). Toxicity of dissolved Cu, Zn, Ni and Cd to developing embryos of the blue mussel (*Mytilus trossolus*) and the protective effect of dissolved organic carbon. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, *149*(3), 340–348. https://doi.org/10.1016/j.cbpc.2008.09.001

References

Naylor, R. L., Williams, S. L., & Strong, D. R. (2001). Aquaculture--A Gateway for Exotic Species. *Science*, *294*(5547), 1655–1656. https://doi.org/10.1126/science.1064875

Nelson, D. A., Miller, J. E., & Calabrese, A. (1988). Effect of heavy metals on bay scallops, surf clams, and blue mussels in acute and long-term exposures. *Archives of Environmental Contamination and Toxicology*, *17*(5), 595–600. https://doi.org/10.1007/BF01055828

Newell, R. I. E., Hilbish, T. J., Koehn, R. K., & Newell, C. J. (1982). Temporal variation in the reproductive cycle of Mytilus edulis L. (Bivalvia, Mytilidae) from localities on the East Coast of the United States. *The Biological Bulletin*, *162*(3), 299–310. https://doi.org/10.2307/1540985

Nicholson, S., & Lam, P. K. S. (2005). Pollution monitoring in Southeast Asia using biomarkers in the mytilid mussel Perna *viridis* (*Mytilidae: Bivalvia). Environment International*, *31*(1), 121–132. https://doi.org/10.1016/j.envint.2004.05.007

Nielsen, S. A., & Nathan, A. (1975). Heavy metal levels in New Zealand molluscs. *New Zealand Journal of Marine and Freshwater Research*, *9*(4), 467–481. https://doi.org/10.1080/00288330.1975.9515582

Nordberg, G. F., Fowler, B. A., Nordberg, M., & Friberg, L. (2007). *Handbook on the Toxicology of Metals, 3,* Academic Press.

Oduor, N. A., Cristina, S. C., & Costa, P. (2023). Sources of anthropogenic nutrients and their implications on nutrient chemistry and ecological conditions of Ria Formosa lagoon, Portugal. *Regional Studies in Marine Science*, *61*, 102843. https://doi.org/10.1016/j.rsma.2023.102843

O'Shaughnessy, K., Lyons, D., Ashelby, C., Counihan, R., Pears, S., Taylor, E., Davies, R., & Stebbing, P. (2023). Rapid assessment of marine non-native species in Irish marinas. *Management of Biological Invasions*, *14*(2), 245–267. https://doi.org/10.3391/mbi.2023.14.2.05

Page, H. M., & Hubbard, D. M. (1987). Temporal and spatial patterns of growth in mussels *Mytilus edulis* on an offshore platform: relationships to water temperature and food availability. *Journal of Experimental Marine Biology and Ecology*, *111*(2), 159–179.

https://doi.org/10.1016/0022-0981(87)90053-0

Paillard, C., Le Roux, F., & Borrego, J. J. (2004). Bacterial disease in marine bivalves, a review of recent studies: Trends and evolution. *Aquatic Living Resources*, *17*(4), 477–498. https://doi.org/10.1051/alr:2004054

Pan, J., Plant, J. A., Voulvoulis, N., Oates, C. J., & Ihlenfeld, C. (2010). Cadmium levels in Europe: implications for human health. *Environmental Geochemistry and Health*, *32*(1), 1–12. https://doi.org/10.1007/s10653-009-9273-2

Parry, H. E. (2007). The immune function of developmental stages of *Mytilus edulis* and effects of stressors [Doctoral dissertation], *University of Wales Sansea.* ProQuest LLC

Pavičić, J., Škreblin, M., Kregar, I., Tušek-Žnidarič, M., & Stegnart, P. (1994). Embryo-larval tolerance of *Mytilus galloprovincialis*, exposed to the elevated sea water metal concentrations: I. Toxic effects of Cd, Zn and Hg in relation to the metallothionein level. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, *107*(2), 249–257. https://doi.org/10.1016/1367-8280(94)90048-5

Pearson, T. H. (1971). The benthic ecology of Loch Linnhe and Loch Eil, a sea-loch system on the west coast of Scotland. III. The effect on the benthic fauna of the introduction of pulp mill effluent. *Journal of Experimental Marine Biology and Ecology*, *6*(3), 211–233. https://doi.org/10.1016/0022-0981(71)90020-7

Penney, R. W., Hart, M. J., & Templeman, N. D. (2007). Shell strength and appearance in cultured blue mussels *Mytilus edulis, M. trossulus*, and *M. edulis* × *M. trossulus* hybrids. *North American Journal of Aquaculture*, *69*(3), 281–295. https://doi.org/10.1577/A06-044.1

Penrose, W. R., & Woolson, E. A. (1974). Arsenic in the marine and aquatic environments: Analysis, occurrence, and significance. C R C Critical Reviews*, Environmental Control*, *4*(1–4), 465–482. https://doi.org/10.1080/10643387409381621

Perceval, O., Couillard, Y., Pinel-Alloul, B., Giguère, A., & Campbell, P. G. C. (2004). Metal-induced stress in bivalves living along a gradient of Cd

contamination: relating sub-cellular metal distribution to population-level responses. *Aquatic Toxicology*, *69*(4), 327–345. https://doi.org/10.1016/j.aquatox.2004.06.009

Pérez-Camacho, A., Labarta, U., Vinseiro, V., & Fernández-Reiriz, M. J. (2013). Mussel production management: Raft culture without thinning-out. *Aquaculture*, *406–407*, 172–179. https://doi.org/10.1016/j.aquaculture.2013.05.019

Petton, B., Pernet, F., Robert, R., & Boudry, P. (2013). Temperature influence on pathogen transmission and subsequent mortalities in juvenile pacific oysters *Crassostrea gigas*. *Aquaculture Environment Interactions*, *3*(3), 257–273. https://doi.org/10.3354/aei00070

Pourmozaffar, S., Tamadoni Jahromi, S., Rameshi, H., Sadeghi, A., Bagheri, T., Behzadi, S., Gozari, M., Zahedi, M. R., & Abrari Lazarjani, S. (2020). The role of salinity in physiological responses of bivalves. *Reviews in Aquaculture*, *12*(3), 1548–1566. https://doi.org/10.1111/raq.12397

Prato, E., & Biandolino, F. (2007). Combined toxicity of mercury, copper and cadmium on embryogenesis and early larvaL stages of the *Mytilus galloprovincialis*. *Environmental Technology*, *28*(8), 915–920. https://doi.org/10.1080/09593332808618851

Purbonegoro, T., & Hindarti, D. (2019). Larvae abnormality of green mussel (*Perna viridis*) due to cadmium (Cd) and copper (Cu) exposure. *AIP Conference Proceeding.* 3 July 2019; 2120 (1): 040001. https://doi.org/10.1063/1.5115639

Qiu, J., Tremblay, R., & Bourget, E. (2002). Ontogenetic changes in hyposaline tolerance in the mussels *Mytilus edulis* and *M. trossulus*: implications for distribution. *Marine Ecology Progress Series*, *228*, 143–152. https://doi.org/10.3354/meps228143

Rainbow, P. S. (1985). The biology of heavy metals in the sea. *International Journal of Environmental Studies*, *25*(3), 195–211.

Rajagopal, S., Van Der Velde, G., Venugopalan, V. P., & Jenner, H. A. (2004). Dose-response of mussels to chlorine. *Water Encyclopedia*, 401–406. Wiley. https://doi.org/10.1002/047147844X.wq171

Rayssac, N., Pernet, F., Lacasse, O., & Tremblay, R. (2010). Temperature effect on survival, growth, and triacylglycerol content during the early ontogeny of *Mytilus edulis* and *M. trossulus*. *Marine Ecology Progress Series*, *417*, 183–191. https://doi.org/10.3354/meps08774

Renault, T. (2015). Immunotoxicological effects of environmental contaminants on marine bivalves. *Fish and Shellfish Immunology*, *46*(1), 88–93. https://doi.org/10.1016/j.fsi.2015.04.011

Renault, T., Bouquet, A. L., Maurice, J.-T., Lupo, C., & Blachier, P. (2014). Ostreid Herpesvirus 1 Infection among Pacific oyster (*Crassostrea gigas*) spat: Relevance of water temperature to virus replication and circulation prior to the onset of mortality. *Applied and Environmental Microbiology*, *80*(17), 5419–5426. https://doi.org/10.1128/AEM.00484-14

Riemann, B., & Ernst, D. (1982). Extraction of chlorophylls a and b from phytoplankton using standard extraction techniques. *Freshwater Biology*, *12*(3), 217–223. https://doi.org/10.1111/j.1365-2427.1982.tb00617.x

Riisgård, H. U., Egede, P. P., & Barreiro Saavedra, I. (2011). Feeding behaviour of the mussel, *Mytilus edulis* : New observations, with a minireview of current knowledge. *Journal of Marine Biology*, *2011*, 1–13. https://doi.org/10.1155/2011/312459

Robert, R., Sanchez, J. L., Perez-Paralle, L., Ponis, E., Kamermans, P., & O'mahoney, M. (2013). A glimpse on the mollusc industry in Europe. *Aquaculture Europe*, *38*(1), 5–11.

Rodney, E., Herrera, P., Luxama, J., Boykin, M., Crawford, A., Carroll, M. A., & Catapane, E. J. (2007). Bioaccumulation and tissue distribution of arsenic, cadmium, copper and zinc in *Crassostrea virginica* grown at two different depths in Jamaica Bay, New York. *In Vivo*, *29*(1), 16–27.

Schalles, J. (2006). Optical remote sensing techniques to estimate phytoplankton Chlorophyll a concentrations in coastal waters with varying suspended matter and CDOM concentrations. *Remote Sensing of Aquatic Coastal Ecosystem Processes: Science and Management Applications*, *2*(2), 27–79. https://doi.org/10.1007/1-4020-3968-9_3

Schroeder, H. A., Nason, A. P., Tipton, I. H., & Balassa, J. J. (1966). Essential trace metals in man: Copper. *Journal of Chronic Diseases*, *19*(9), 1007–1034.
https://doi.org/10.1016/0021-9681(66)90033-6

Scottish Government. (2023). Spread of invasive species into Scotland: Study. *The Scottish Gornement,* Environment and Forestry Directorate & Marine Directorate.
ISBN 9-781-83521-094-9
Retrieved 18 December 2024, from https://www.gov.scot/publications/provision-horizon-scanning-analysis-pathways-spread-invasive-species-scotland/pages/6/

Seed, R. (1968). Factors influencing shell shape in the mussel *Mytilus Edulis*. *Journal of the Marine Biological Association of the United Kingdom*, *48*(3), 561–584.
https://doi.org/10.1017/S0025315400019159

Seed, R. (1969). The ecology of *Mytilus edulis* L. (Lamellibranchiata) on exposed rocky shores. *Oecologia*, *3*(3–4), 277–316.
https://doi.org/10.1007/BF00390380

Seed, R. (1992). Systematics evolution and distribution of mussels belonging to the genus *Mytilus* - An overview. *American Malacological Bulletin*, *9*(2), 123–137.

Seed, R. (1993). Invertebrate predators and their role in structuring coastal and estuarine populations of filter feeding bivalves. *Dame, R.F. (eds) Bivalve Filter Feeders. Nato ASI Series, 33,* Springer, Berlin, Heidelberg.
https://doi.org/10.1007/978-3-642-78353-1_5

Seed, R., & Suchanek, T. (1992). Population and community ecology of *Mytilus*. *Gosling, E., Ed., The Mussel Mytilus: Ecology, Physiology, Genetics and Culture*, Elsevier, London, 87-169

Scottish Environment Protection Agency SEPA (2011). Loch Eil. Report 117, *Scottish Environment Protection Agency (SEPA)*

Sokolova, I. M., Evans, S., & Hughes, F. M. (2004). Cadmium-induced apoptosis in oyster haemocytes involves disturbance of cellular energy balance but no mitochondrial permeability transition. *Journal of Experimental Biology*, *207*(19), 3369–3380.

https://doi.org/10.1242/jeb.01152

Solórzano, L., & Grantham, B. (1975). Surface nutrients, chlorophyll a and phaeopigment in some Scottish sea lochs. *Journal of Experimental Marine Biology and Ecology*, *20*(1), 63–76. https://doi.org/10.1016/0022-0981(75)90102-1

Somero, G. N. (2011). Comparative physiology: a “crystal ball” for predicting consequences of global change. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *301*(1), R1–R14. https://doi.org/10.1152/ajpregu.00719.2010

Sprague, M., Fawcett, S., Betancor, M. B., Struthers, W., & Tocher, D. R. (2020). Variation in the nutritional composition of farmed Atlantic salmon (*Salmo salar* L.) fillets with emphasis on EPA and DHA contents. *Journal of Food Composition and Analysis*, *94*, 103618. https://doi.org/10.1016/j.jfca.2020.103618

Stachowicz, J. J., Whitlatch, R. B., & Osman, R. W. (1999). Species diversity and invasion resistance in a marine ecosystem. *Science*, *286*(5444), 1577–1579. https://doi.org/10.1126/science.286.5444.1577

Stevens, C., Plew, D., Hartstein, N., & Fredriksson, D. (2008). The physics of open-water shellfish aquaculture. *Aquacultural Engineering*, *38*(3), 145–160. https://doi.org/10.1016/j.aquaeng.2008.01.006

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

Stirling, H. P., & Okumuş, İ. (1994). Growth, mortality and shell morphology of cultivated mussel (*Mytilus edulis*) stocks cross-planted between two Scottish sea lochs. *Marine Biology*, *119*(1), 115–123. https://doi.org/10.1007/BF00350113

Stobo, L. A., Lacaze, J.-P. C. L., Scott, A. C., Petrie, J., & Turrell, E. A. (2008). Surveillance of algal toxins in shellfish from Scottish waters. *Toxicon*, *51*(4), 635–648. https://doi.org/10.1016/j.toxicon.2007.11.020

Streit, B. (1998). Bioaccumulation of contaminants in fish. *Fish Ecotoxicology* 353–387. Basel: Birkhäuser Basel.

https://doi.org/10.1007/978-3-0348-8853-0_12

Strömgren, T. (1982). Effect of heavy metals (Zn, Hg, Cu, Cd, Pb, Ni) on the length growth of *Mytilus edulis*. *Marine Biology*, *72*(1), 69–72. https://doi.org/10.1007/BF00393949

Talbot, V., Magee, R. J., & Hussain, M. (1976). Lead in Port Phillip Bay mussels. *Marine Pollution Bulletin*, *7*(12), 234–237. https://doi.org/10.1016/0025-326X(76)90269-1

Tan, K., Yan, X., Julian, R., Lim, L., Peng, X., Fazhan, H., & Kwan, K. Y. (2023). Effects of climate change induced hyposalinity stress on marine bivalves. *Estuarine, Coastal and Shelf Science*, *294*, 108539. https://doi.org/10.1016/j.ecss.2023.108539

Thompson, R. (1984). Production, reproductive effort, reproductive value and reproductive cost in a population of the blue mussel *Mytilus edulis* from a subarctic environment. *Marine Ecology Progress Series*, *16*, 249–257. https://doi.org/10.3354/meps016249

Tillin, H., & Mainwaring, K. (2016.). *Mytilus edulis* beds on sublittoral sediments*, H Marine Life Information Network: Biology and Sensitivity Key Information Reviews*, [online]. Plymouth: Marine Biological Association of the United Kingdom. Retrieved 16 February 2024, from https://www.marlin.ac.uk/habitats/detail/36 https://dx.doi.org/10.17031/marlinhab.36.1

Tillin, H., & Mainwaring, K. (2024.). *Mytilus edulis* beds on littoral sediments. *Tyler-Walters, H Marine Life Information Network: Biology and Sensitivity Key Information Reviews*, [online]. Plymouth: Marine Biological Association of the United Kingdom. Retrieved 24 November 2024, from https://www.marlin.ac.uk/habitat/detail/269 https://doi.org/10.17031/marlinhab.269.1

Travers, M.-A., Boettcher Miller, K., Roque, A., & Friedman, C. S. (2015). Bacterial diseases in marine bivalves. *Journal of Invertebrate Pathology*, *131*, 11–31. https://doi.org/https://doi.org/10.1016/j.jip.2015.07.010

Tremblay, R., Myrand, B., Sevigny, J.-M., Blier, P., & Guderley, H. (1998). Bioenergetic and genetic parameters in relation to susceptibility of blue

mussels, *Mytilus edulis* (L.) to summer mortality. *Journal of Experimental Marine Biology and Ecology*, *221*(1), 27–58. https://doi.org/10.1016/S0022-0981(97)00114-7

Tyler-Walters, H. (2008). *Mytilus edulis* Common mussel. *Tyler-Walters H. Marine Life Information Network: Biology and Sensitivity Key Information Reviews*, [online]. Plymouth: Marine Biological Association of the United Kingdom. Retrieved 28 January 2024, from https://www.marlin.ac.uk/habitats/detail/269/mytilus_edulis_beds_on_littoral_sediments

Tyler-Walters, H., Williams, E., Mardle, M. J., & Lloyd, K. A. (2022). Sensitivity assessment of contaminant pressures - approach development, application, and evidence reviews. *Plymouth, Marine Biological Association of the United Kingdom,* 192. Retrieved 14 February 2024, from https://www.marlin.ac.uk/publications

Väinölä, R., & Hvilsom, M. M. (1991). Genetic divergence and a hybrid zone between Baltic and North Sea *Mytilus* populations (*Mytilidae: Mollusca*). *Biological Journal of the Linnean Society*, *43*(2), 127–148. https://doi.org/10.1111/j.1095-8312.1991.tb00589.x

Vallee, B. L., Ulmer, D. D., & Wacker, W. E. C. (1960). Arsenic toxicology and biochemistry. *Journal of Occupational and Environmental Medicine*, *2*(7), 358.

van der Gaag, M., van der Velde, G., Wijnhoven, S., & Leuven, R. S. E. W. (2016). Salinity as a barrier for ship hull-related dispersal and invasiveness of dreissenid and mytilid bivalves. *Marine Biology*, *163*(7), 147. https://doi.org/10.1007/s00227-016-2926-7

Vieira, H., Rodrigues, A., Pires, S., Oliveira, J., Rocha, R., Soares, A., & Bordalo, M. (2021). Ocean warming may enhance biochemical alterations induced by an invasive seaweed exudate in the mussel *Mytilus galloprovincialis. Toxics*, *9*(6), 121. https://doi.org/10.3390/toxics9060121

Viviani, R., Boni, L., Cattani, O., Milandri, A., Poletti, R., Pompei, M., & Sansoni, G. (1995). ASP, DSP, NSP and PSP monitoring in 'mucilaginous aggregates' and in mussels in a coastal area of the Nothern Adriatic Sea facing Emilia-

Romagna in 1988, 1989 and 1991. *Science of The Total Environment*, *165*(1–3), 203–211. https://doi.org/10.1016/0048-9697(95)04553-D

Vlahogianni, T. H., & Valavanidis, A. (2007). Heavy-metal effects on lipid peroxidation and antioxidant defence enzymes in mussels *Mytilus galloprovincialis*. *Chemistry and Ecology*, *23*(5), 361–371. https://doi.org/10.1080/02757540701653285

Vuorinen, I., Antsulevich, A. E., & Maximovich, N. V. (2002). Spatial distribution and growth of the common mussel *Mytilus edulis* L. in the archipelago of SW-Finland, northern Baltic Sea. *Boreal Environment Research*, *7*(1), 41–52.

Wang, Q., Liu, B., Yang, H., Wang, X., & Lin, Z. (2009). Toxicity of lead, cadmium and mercury on embryogenesis, survival, growth and metamorphosis of *Meretrix meretrix* larvae. *Ecotoxicology*, *18*(7), 829–837. https://doi.org/10.1007/s10646-009-0326-1

Wang, Q., Liu, B., Yang, H., Wang, X., & Lin, Z. (2009). Toxicity of lead, cadmium and mercury on embryogenesis, survival, growth and metamorphosis of *Meretrix meretrix* larvae. *Ecotoxicology*, *18*(7), 829–837. https://doi.org/10.1007/s10646-009-0326-1

Weiss, I. M., Tuross, N., Addadi, L., & Weiner, S. (2002). Mollusc larval shell formation: amorphous calcium carbonate is a precursor phase for aragonite. *Journal of Experimental Zoology*, *293*(5), 478–491. https://doi.org/10.1002/jez.90004

Wenne, R., Zbawicka, M., Prądzińska, A., Kotta, J., Herkül, K., Gardner, J. P. A., Apostolidis, A. P., Poćwierz-Kotus, A., Rouane-Hacene, O., Korrida, A., Dondero, F., Baptista, M., Reizopoulou, S., Hamer, B., Sundsaasen, K. K., Árnyasi, M., & Kent, M. P. (2022). Molecular genetic differentiation of native populations of Mediterranean blue mussels, *Mytilus galloprovincialis* Lamarck, 1819, and the relationship with environmental variables. *The European Zoological Journal*, *89*(1), 755–784. https://doi.org/10.1080/24750263.2022.2086306

Westerbom, M., Kilpi, M., & Mustonen, O. (2002). Blue mussels, *Mytilus edulis,* at the edge of the range: population structure, growth and biomass along a salinity gradient in the north-eastern Baltic Sea. *Marine Biology*, *140*(5), 991–999.

https://doi.org/10.1007/s00227-001-0765-6

White, D. E. J., Haag, W. R., McGregor, M. A., & Price, S. J. (2022). Effects of food abundance on juvenile freshwater mussel survival and growth in aquaculture, and comparison with growth in streams. *Aquaculture*, *560*, 738473. https://doi.org/10.1016/j.aquaculture.2022.738473

Wijsman, J. W. M., Troost, K., Fang, J., & Roncarati, A. (2019). Global production of marine bivalves. Trends and challenges. *Goods and Services of Marine Bivalves*, 7–26.

Wing, S., & Leichter, J. (2011). Variation in environmental conditions in a subtidal prey refuge: effects of salinity stress, food availability and predation on mussels in a fjord system. *Marine Ecology Progress Series*, *422*, 201–210. https://doi.org/10.3354/meps08911

Winogradow, A., Mackiewicz, A., & Pempkowiak, J. (2019). Seasonal changes in particulate organic matter (POM) concentrations and properties measured from deep areas of the Baltic Sea. *Oceanologia*, *61*(4), 505–521. https://doi.org/10.1016/j.oceano.2019.05.004

Wong, W. H., & Levinton, J. S. (2004). Culture of the blue mussel *Mytilus edulis* (Linnaeus, 1758) fed both phytoplankton and zooplankton: a microcosm experiment. *Aquaculture Research*, *35*(10), 965–969. https://doi.org/10.1111/j.1365-2109.2004.01107.x

Yang, H., Sturmer, L. N., & Baker, S. (2016). Molluscan Shellfish Aquaculture and Production (FA191). *Fisheries and Aquatic Sciences of the School of Forest Resources and Conservation,* IFAS Extension, University of Florida.

Yaroslavtseva, L. M., & Sergeeva, E. P. (2007). Effect of temperature on early development of the Pacific mussel *Mytilus trossulus* (*Bivalvia: Mytilidae*) in sea water polluted by copper ions. *Russian Journal of Marine Biology*, *33*(6), 375–380. https://doi.org/10.1134/S106307400706003X

Yarra, T., Blaxter, M., & Clark, M. S. (2021). A bivalve biomineralization toolbox. *Molecular Biology and Evolution*, *38*(9), 4043–4055. https://doi.org/10.1093/molbev/msab153

Young, T. (2009). Pharmacological induction of larval settlement in the New Zealand mussel *Perna canaliculus*. *Masters Thesis, Auckland University of Technology, New Zealand (2009),* 346

Zannella, C., Mosca, F., Mariani, F., Franci, G., Folliero, V., Galdiero, M., Tiscar, P., & Galdiero, M. (2017). Microbial diseases of bivalve molluscs: Infections, immunology and antimicrobial defense. *Marine Drugs*, *15*(6). https://doi.org/10.3390/md15060182

Zhao, L., Milano, S., Tanaka, K., Liang, J., Deng, Y., Yang, F., Walliser, E., & Schöne, B. R. (2020). Trace elemental alterations of bivalve shells following transgenerational exposure to ocean acidification: Implications for geographical traceability and environmental reconstruction. *Science of The Total Environment*, *705*, https://doi.org/10.1016/j.scitotenv.2019.135501

Zitoun, R., Hassler, C., Clearwater, S. J., Thompson, K. J., Albert, A., & Sander, S. G. (2019). Investigating the fate of copper in a laboratory-based toxicity test with embryos of *Mytilus galloprovincialis*: Copper mass balance of a closed bioassay. *Environmental Toxicology and Chemistry*, *38*(3), 561–574. https://doi.org/10.1002/etc.4345

Zuykov, M., Pelletier, E., & Harper, D. (2013) Bivalve molluscs in metal pollution studies: From bioaccumulation to biomonitoring. *Chemosphere*, *93*(2), 201–208. https://doi.org/10.1016/j.chemosphere.2013.05.001