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DOCTORAL THESIS

Estimating the dispersal capacity of Scottish blue mussel (*Mytilus edulis*): From hydrodynamic modelling to genetic population structures

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Declaration of Authorship

I, Ana CORROCHANO-FRAILE, declare that this thesis titled, "Estimating the dispersal capacity of Scottish blue mussel (*Mytilus edulis*): From hydrodynamic modelling to genetic population structures" and the work presented in it are my own. I confirm that:

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Abstract

The blue mussel (*Mytilus edulis*) and the Mediterranean mussel (*Mytilus galloprovincialis*) are the primary bivalve species in Europe, with France and Spain being the top producers. In recent years, Scotland has also seen a significant increase in mussel production due to a higher settlement of mussel larvae onto suitable surfaces, which is crucial for cultivation. However, despite the growth of natural spatfall, limited research on mussel farming methods in dynamic areas has led to inefficiencies and fluctuations in production. As a result, there is a pressing need for improved management practices to ensure sustainable growth in the industry.

This study seeks to address knowledge gaps concerning mussel dispersion, crucial for effective mussel farming management, by investigating population genetics and employing hydrodynamic modelling. Presented is a comprehensive genome assembly of the blue mussel, *M. edulis*, identifying multiple whole genome duplication events. This assembly facilitates the development of precise genetic markers, contributing to an improved understanding of the intricate genetic structure of Scottish mussel populations. Additionally, the study utilises a biophysical model to illustrate the high connectivity of *M. edulis* populations, influenced by the rapid water currents and wind direction on Scotland's dynamic West Coast. This interdisciplinary approach integrates population genomics and biophysical modeling, providing valuable insights into various mussel farming areas across Scotland.

The findings suggest that understanding the connectivity of mussel populations and the gene flow is essential for effective management practices. The population study shows a mussel gene flow between key areas, leading to a rapid change in local populations, exemplified by the noticeable alteration of genotypes from one generation to the next. The ocean currents help mussels move around and spread their genes, which creates a complicated network of mussel populations. By understanding this connectivity, mussel farmers can make informed decisions on stocking and harvesting strategies to ensure the sustainability of mussel farming practices in the long term.

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Publications and Conferences

Publications

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- Corrochano-Fraile, A., Davie, A., Carboni, S. and Bekaert M. (2022). Evidence of multiple genome duplication events in *Mytilus* evolution. *BMC Genomics* **23**:340. [doi:10.1186/s12864-022-08575-9]
- Corrochano-Fraile, A., Adams, T. P., Aleynik, D., Bekaert, M., and Carboni, S. (2022). Predictive biophysical models of bivalve larvae dispersal in Scotland. *Frontiers in Marine Science*, **9**:985748. [doi:10.3389/fmars.2022.985748]

Press release

- University of Stirling study could lead to improved mussel production [www.stir.ac.uk/news/2024]
- Moving mussels: new insights into shellfish farming [www.stir.ac.uk/news/2022]

Conferences

- Scientific talk at Association of Scottish Shellfish Growers. "The Importance of Mussel Larvae Dispersion" in Scotland (Oct. 2023)
- Poster presentation at MASTS annual Science Meeting. "Predictive biophysical models of bivalve larvae dispersal in Scotland" (Nov. 2022)
- Scientific talk at Institute of Aquaculture PhD conference (Stirling, Scotland). "Predictive biophysical models of bivalve larvae dispersal in Scotland" (Oct. 2022)

- Poster presentation at the Marine Alliance for Science and Technology for Scotland. "Predictive biophysical models of bivalve larvae dispersal in Scotland" (Oct. 2022)
- Scientific talk at the European Aquaculture Society conference (Rimini, Italy). "Predictive biophysical models of bivalve larvae dispersal in Scotland" (Sep. 2022)
- Poster presentation at the European Aquaculture Society conference (Online). "Evidence of multiple genome duplication events in *Mytilus* evolution" (Oct. 2021)
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Chapter 1

Introduction

Europe's aquaculture industry produces an abundance of nutritious seafood, including the highly sought-after blue mussel (*Mytilus edulis*) and Mediterranean mussel (*Mytilus galloprovincialis*). Spain and France have long been leaders in mussel production, but Scotland has emerged as a prominent player in the industry over the last two decades (Figure 1.1). As the industry is exploring new mussel farming techniques, understanding, and predicting the source of seed mussels is one of the most significant challenges for the expanding industry.

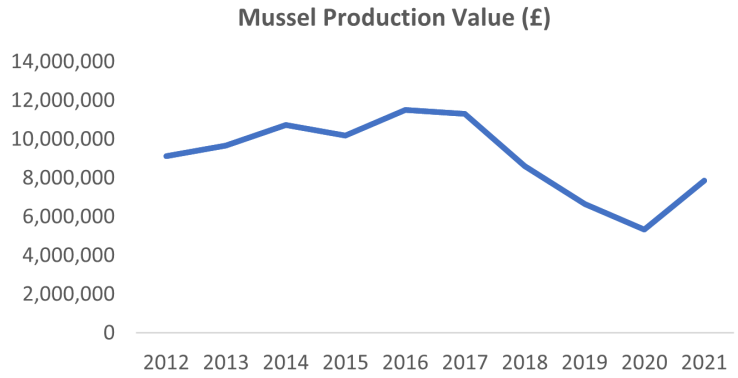

FIGURE 1.1: Total Scottish mussel production from year 2012 to 2021 in pounds (£). Average prices are adjusted for inflation based on 2021 price estimates. Data collected by Munro et al., 2022.

1.1 Blue mussel biology, hybridisation, and roles in the ecosystem

1.1.1 *Mytilus edulis* distribution and reproductive strategy

The blue mussel, *Mytilus edulis* L. 1758, also known as European mussel, belongs to the class Bivalvia (Gosling, 1992). Species within these taxa are described as possessing two shelled valves and together with other species of commercial importance (oysters, cockles, clams, and scallops) are often referred to as marine bivalve molluscs. The genus *Mytilus* is a dominant component of rocky shore communities in cooler waters of the northern and southern hemispheres. On a local scale, mussels (*Mytilus*) dominate the intertidal to subtidal regions of rocky shores. *M. edulis* has the widest distribution pattern in the genus, extending from high intertidal to subtidal regions, from estuarine to fully marine conditions, and from sheltered to extremely wave–exposed shores. This species occurs in European waters from Spitsbergen Island in Svalbard, Norway (Berge et al., 2005) to western France, and on the Atlantic coast of North America from the Canadian Maritimes southward to North Carolina (Gosling, 2003). At exposed sites, the species prefers gently sloping, slow-draining platforms to steep rock faces. When the substrate is firm enough to provide a secure anchorage, mussels settle on a wide variety of substrates, *e.g.,* rock, stones, pebbles, shell, cement. Early spat (young bivalves) either attach to filamentous algae, from which they eventually migrate onto adult mussel beds, or else they settle directly onto adult beds. *M. edulis* spawning times tend to happen late April- May (Chipperfield, 1953), however several environmental factors (*e.g.,* water, temperature, salinity, food availability and local currents) influence the reproductive cycle, in addition to triggering spawning events and determining larval dispersal patterns (Helm et al., 2004) (Figure 1.2).

Mytilus spp employs a reproductive strategy known as broadcast spawning, which involves their eggs being fertilised outside their bodies in the open water. During the reproductive season, mature *Mytilus* individuals exhibit sexual dimorphism, existing either as males or females, and releasing only one type of gametes during spawning. Larval development begins after successful fertilisation, consisting of two fully motile (non- feeding trochophore and feeding veliger) and one partially motile (pediveliger)

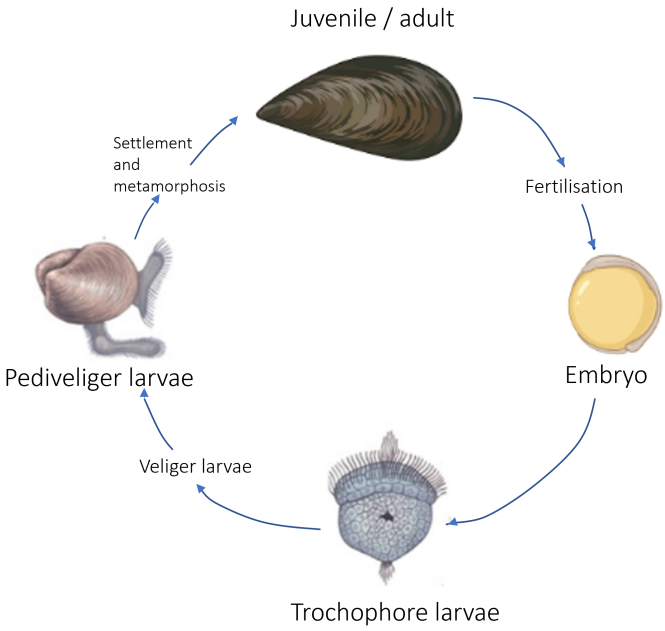

FIGURE 1.2: Diagram of *M. edulis* lifecycle. Showing development from fertilisation to metamorphosis.

stage. This dispersal strategy enhances the changes of survival and colonisation in various geographic areas. Nevertheless, it also exposes the released gametes and developing larvae to an array of environmental variables and challenges, including predation, water quality, and the unpredictable forces of ocean currents, making it a reproductive strategy rich with both advantages and complexities (Eads et al., 2016). The complete larval phases usually span a duration of three-four weeks, marked by the progressive development of crucial organs such as the foot, digestive gland, and gills. After three-four weeks, veliger larvae develops into fully developed pediveligers that are ready to settle, a reversible stage of the mussel life-cycle that precedes metamorphosis. Pediveliger larvae drop down in the water column and onto a substrate, testing the surface with their sensory foot (Helm et al., 2004). Pediveliger larvae can undergo a two-step settlement process, whereby they initially settle on filamentous substrates (*e.g.*, *Polysiphonia* spp. algae or the byssus threads of adult mussels) and then detach and drift in the water column until they find adult beds, or they settle directly into adult beds or other suitable hard surfaces. Primary settlement may avoid competition with adult mussels for food or being inhaled by suspension-feeding adults. In the absence of a suitable susbtrate or conditions, pediveligers can delay settlement; it is not uncommon for planktonic life to extend beyond a two-month period (Bayne, 1965),

but larvae do become less selective of conditions the longer settlement is delayed. Once permanently settled, larvae begin metamorphosis into adult mussels.

1.1.2 Hybridisation within the genus *Mytilus*

Eight species within the *Mytilus* genus can readily hybridise, and hybrids are observed in the wild wherever their geographic range overlaps globally (Gaitán-Espitia et al., 2016). Hybridisation, a process where distinct species interbreed, has been observed for *Mytilus* species in various regions across the world. Evidence for hybrids between *Mytilus* species is documented along the west coast of the United Kingdom (Gardner, 1996; Hilbish et al., 2002; Vendrami et al., 2020), the northeast Atlantic (Bierne et al., 2002; Bierne et al., 2003a; Fraïsse et al., 2016; Simon et al., 2021), the northwest Atlantic (Koehn et al., 1984; Rawson et al., 2001; Toro et al., 2004), the Baltic Sea (Väinölä & Hvilsom, 1991; Riginos & Cunningham, 2005; Stuckas et al., 2017), the subarctic and arctic regions (Mathiesen et al., 2017), the northeast Pacific (Rawson et al., 1999; Saarman & Pogson, 2015), the south and east Pacific (Larrain et al., 2019; Popovic & Riginos, 2020), and the southwest Atlantic (Zbawicka et al., 2018). This process may result in introgression, a phenomenon where genes flow between species through hybridisation, thereby influencing the genetic makeup of the populations involved (Stuckas et al., 2017). Introgression plays a pivotal role in altering the genetic make up of populations, often blurring species boundaries, and contributing to the creation of novel genetic combinations. Additionally, admixture, sharing similarities with introgression, involves the merging of genetic material from distinct populations within a species (Simon et al., 2020).

In Europe, *M. edulis* together with the Mediterranean mussel, *M. galloprovincialis* Lamarck 1819 and the Baltic *Mytilus trossulus* Gould 1850 readily hybridise wherever their geographical distributions overlap (Figure 1.3), sharing morphological and genetic similarities (Gosling, 1992; Gosling, 2003; Michalek et al., 2016). These species are often grouped together as the "*Mytilus* species complex". In the Northern hemisphere this includes *M. edulis, M. galloprovinciallis* and *M. trossulus* (Koehn et al., 1984; Gardner, 1994; Rawson et al., 1999; Stuckas et al., 2009; Zbawicka et al., 2014; Stuckas

et al., 2017). In the southwest (SW) of the UK an extensive hybrid zone of *M. galloprovincialis* and *M. edulis* has been identified between St Ives (northwest Cornwall) and Thurleston (southeast Devon) (Hilbish et al., 2002). Within this mussel hybrid zone, *M. galloprovincialis* populations have been described as dominating turbulent waters, whilst *M. edulis* are predominant in sheltered, lower-salinity habitats (Schmidt et al., 2008).

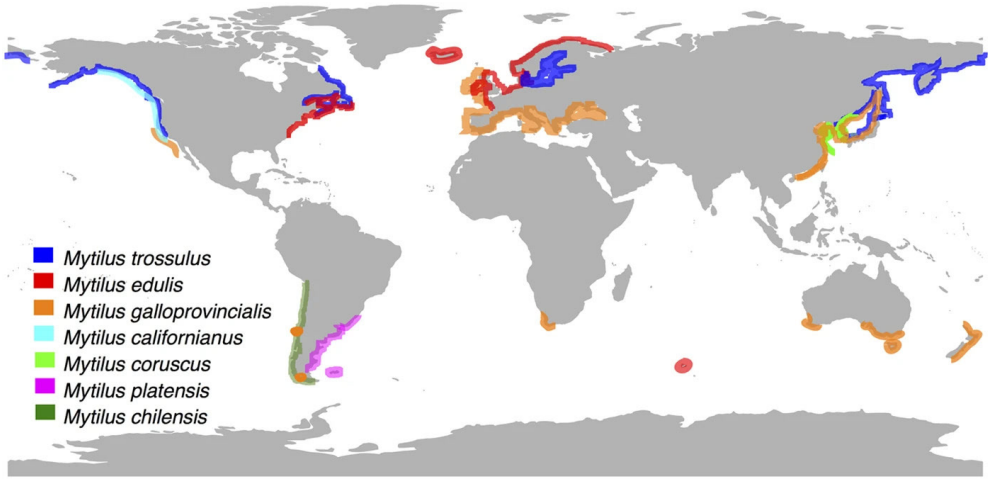

FIGURE 1.3: World map showing the natural distribution of *Mytilus spp.* (Gaitán-Espitia et al., 2016).

Challenges of introgression with *M. trossulus* in the Scottish *Mytilus* complex

The "*Mytilus* species complex" is of interest not only from an evolutionary and ecological perspective but also due to its economic significance in aquaculture and fisheries (Gardner, 1996; Gardeström et al., 2007). In Scotland, hybridisation, and introgression between *M. edulis* and *M. trossulus* is a particular concern for the shellfish sector. While mixed-species stocks of *M. edulis* and *M. galloprovincialis* are successfully farmed along the Scottish west coast, some farming sites have faced challenges with the appearance of mussels that look different from the native stocks.

Compared to *M. edulis* and *M. galloprovincialis, M. trossulus* is often linked to fragile shells that easily break during harvesting and processing, resulting in reduced meat yields and shelf life. Additionally, it is characterized by more elongated shells, which are not desirable traits for mussel production (Mallet, 1995; Penney et al., 2007; Dias et al., 2009). Reports of *M. trossulus* in Loch Etive, Argyll, in 2004 (Beaumont et al., 2008) marked the beginning of its dominance in on-growing facilities, leading to significant

production losses. This situation rendered mussel farming economically unfeasible in the area (Gubbins et al., 2012). Consequently, *M. trossulus* is classified as commercially damaging species in Scotland, and its presence must be reported under 'The Aquaculture and Fisheries (Scotland) Act 2013' to mitigate potential future impacts on the industry (Gubbins et al., 2012). More recently, a study conducted at a Scottish mussel farm aimed to explore the correlation between genetic makeup and shell characteristics along the vertical axis of a cultivation rope (Michalek et al., 2021). The findings revealed that *M. edulis* predominated across all depths, followed by hybrids of *M. edulis* and *M. galloprovincialis*, with fewer occurrences of *M. edulis* and *M. trossulus* hybrids and pure *M. trossulus* individuals. Further analysis indicated that mussels with *M. trossulus* introgression exhibited significantly weaker shells and more elongated shell shapes compared to those without *M. trossulus* genetic influence.

Mechanisms of hybridisation

The broader population structure and dynamics within this species complex primarily arise from the complex interaction between oceanographic forces and the biological characteristics of each species. Both pre- and post-settlement selection drive geographical and ecological segmentation, playing a pivotal role in shaping the distribution of species and the formation of hybrid zones (Bierne et al., 2003b; Knöbel et al., 2021). Environmental factors such as temperature, salinity, and habitat alterations can influence hybridisation rates. Anthropogenic activities, such as the introduction of non-native species, can also increase the likelihood of hybridization by altering natural ecological barriers (Michalek et al., 2021). Related to post-settlement selection, Gilg and Hilbish (2003) researched the distribution of the *Mytilus* parental species and their hybrids. They collected recently settled mussels from 20 sites in southwest England throughout the summer and fall. They noticed that *M. edulis* populations tended to settle earlier compared to *M. galloprovincialis* populations. In hybrid populations, the settlement happened at times that were in-between and overlapped with both parent populations. They found that changes in genetic makeup within a single year were uncommon across most sites, although some differences were observed between different years. On the other hand, they frequently noticed differences in the genetic makeup of young mussels settling in hybrid populations that matched those

of small, young mussels at the same sites. Interestingly, they did not find any consistent changes in the frequency of specific genetic traits over the course of several weeks after settlement, suggesting that the differences in the genetic makeup of adult mussel populations are mainly due to variations in where the larval mussels settle, rather than changes in genetics over time or immediate natural selection after settling. Nascimento-Schulze et al. (2023) studied the makeup of different populations of mussels using a technique called low-coverage whole-genome sequencing (lcWGS). Their analysis revealed some interesting patterns in the ancestry of the sampled populations. Notably, they observed a significant shift in the dominant species composition between two mussel populations in Bodega Bay (California), despite their proximity. They also found evidence of hybrids in Carquinez Harbour (California) that appeared to be a mix of two different species. Similar hybrid patterns have been observed between *M. galloprovincialis* and *M. edulis* in Europe (Simon et al., 2020) , suggesting a possible parallel between the two regions. Additionally, they noticed an advanced mixing pattern in the Baltic population from Finland between *M. edulis* and *M. trossulus*, as well as a mixing of introgressed *M. edulis* and hybrid genotypes in the contact zone between Kiel and Ahrenshoop, also observed in previous literature (Stuckas et al., 2017). The presence of *M.galloprovincialis* in sheltered waters along the northern Pacific coast contrasted with its typical preference for rocky tidal environments along the northeast Atlantic (Bierne et al., 2003a; Hilbish et al., 2002). Their study confirmed previous findings of hybridisation between *M.edulis* and *M.galloprovincialis* in the mussel populations in Exmouth, southern England (Vendrami et al., 2020). In conclusion, they provided further support for existing data on the distribution and hybridisation of blue mussel populations in various regions, including the Baltic Sea, Southwest England, the Mediterranean, the USA, and Chile.

1.1.3 Roles in the ecosystem

Mussels filter enormous amounts of water every day and are generally known to have a positive impact on the environment reducing the seston (suspended particulate matter) concentrations (Newell, 2004), increasing water transparency (Schröder et al., 2014) and improving water quality (Zhou et al., 2006) thus supporting more productive environments which ultimately leads to enhanced sustainable production of

seafood (Smaal, 2002). Supporting this, studies with oysters and mussels have been undertaken in a relatively small bay in France (Marennes-Oléron), showing that bivalves can filter all the water in the bay within 2.7 days and that bivalve communities can filter most of the overlying water column (Dame, 2012).

Bioremediation is another feature of bivalves. They tolerate and accumulate contaminants, and because they are sessile and widely distributed, they can be used in programs for monitoring (O'Connor et al., 2006) and removal of contaminants (Gifford et al., 2007). This tool holds significant importance in aquaculture, where the harvest of bio-accumulated contaminants in bivalves is emerging as a key element in ecosystem management for promoting sustainable water remediation. Because bivalves can rapidly bioaccumulate nutrients, metals, and emerging stressors such as pharmaceuticals and engineered nanomaterials, they can act as model sentinel organisms for monitoring pollution (Zuykov et al., 2013), particularly in coastal areas where they constitute valuable commercial resources in aquaculture (Suarez-Ulloa et al., 2015); *M. edulis* concentrates engineered nanomaterials in its digestive gland, and it has been used to monitor, and as a metal biomonitor (Rocha et al., 2015).

Shells are long-term sinks of nutrients from aquatic environments when buried or harvested and can persist for long time periods. Powell et al. (2011) measured shell degradation over 13 years for 4 bivalve species including ocean quahog (*Arctica islandica*), blue mussel, tiger lucine (*Codakia rbicularis*), and bay scallop (*Argopecten irradians*), with high variation among species and important implications for carbonate cycling and fossil formation. Regarding carbon sequestration, further research linked to carbon sources and bivalves has been made, for example Fodrie et al. (2017) analysed shellfish reefs which contain significant pools of carbon. Carbon sequestration is a crucial service supplied by marine ecosystems (*i.e.*, vegetated coastal habitats, such as salt marshes (Duarte et al., 2004), seagrasses (Fourqurean et al., 2012) and mangroves (Donato et al., 2011) providing protection against global climate change. Fodrie et al. (2017) quantified pools and rates of organic-C and inorganic-C burial within eastern oyster reefs (*Crassostrea virginica*) in North Carolina. The promising results showed that like vegetated blue carbon sinks, these bivalves' reefs can be persistent features of estuarine landscapes over millennial time scales, providing a potential repository for

long-term organic carbon storage. The loss of shellfish reefs can result in the release of formerly dormant organic carbon pools back into the biosphere. Special attention must be paid to the anthropogenic disturbance of shellfish habitat because the disturbance of all these reefs might result in increased atmospheric CO_2.

1.1.4 Factors affecting distribution and abundance

Limited distribution of *Mytilus*, both in subtidal and intertidal habitats, is primarily influenced by biological factors such as predation, competition, and environmental conditions like water temperature, salinity, food availability and local currents, competition, and predation. Essentially the *Mytilus* species can survive in subtidal habitats, but the presence of predators and competition restricts their distribution. In sheltered areas they experience a refuge from predation, thus upper distribution limits are usually governed by physical factors, primarily temperature, while predators are mainly responsible for setting lower limits (Seed & Suchanek, 1992).

Studies have shown that the recruitment of blue mussel larvae may be hindered in regions with highly turbulent flow. During the initial stages of attachment, young *M. edulis* larvae use their foot instead of their byssus. As they mature into post-larvae and juveniles, they produce long drifting byssus threads to improve their capability to settle in favourable locations (Abelson & Denny, 1997; Railkin, 2004).

Environmental variables play a critical role in shaping the distribution and abundance of bivalves in marine ecosystems, their tolerance limits can vary depending on the mussel species and their geographical location. These variables can influence various aspects of bivalve life history, including growth, reproduction, and survival (Yund & McCartney, 2016). Temperature is one environmental variable that can have a significant impact on bivalve populations. For example, warmer temperatures can enhance the growth and development of some bivalve species, while others may be negatively impacted, the optimal temperature for growth and survival around 10-20°C (Anestis et al., 2007). Additionally, changes in temperature can affect the timing of reproductive events in bivalves, potentially leading to shifts in their seasonal patterns of abundance. Bivalves, such as mussels, oysters, and clams, have the ability to tolerate a wide range of salinities, making them euryhaline (Muller et al., 2014) . However, salinity is

a critical variable that can strongly influence bivalve populations. Variations in salinity levels can disrupt the osmotic regulation of bivalves, affecting their ability to filter feed and maintain proper physiological functions (Wu & Sokolova, 2021). Additionally, salinity levels can impact bivalve growth and reproduction, with varying degrees of tolerance among different species (Gosling, 2003). The presence of oxygen in the water is crucial for bivalve populations, and its levels can significantly impact their survival. Mussels are classified as facultative anaerobes and possess adaptations that enable them to withstand aerial exposure, allowing them to endure periods of extremely low oxygen levels. Nonetheless, the duration of exposure to such conditions plays a pivotal role in their ability to thrive and survive. When oxygen levels dip below a critical threshold, typically around 2 mg/L, the metabolic rates and respiration of bivalves can be significantly affected, resulting in diminished growth and decreased survival rates. In regions where oxygen levels fall below this critical point, bivalves may experience mass mortality events due to the inadequate supply of oxygen necessary for proper physiological functioning. Changes in food availability can also impact bivalve populations, with reduced food availability potentially leading to lower densities of bivalves in affected areas (Gosling, 1992; Cranford et al., 2011). Wave exposure through both wave force and changes in immersion patterns has an important influence on patterns of zonation and abundance on bivalves. Mussels are wave-exposed on shores and are subjected to high water velocities from breaking waves (often as high as 25 $\mathrm{m\,s^{-1}}$; Denny et al., 2003). Wave action also has a controlling influence on mussel bed communities by causing dislodgement through lift and drag. When dislodgement occurs, new space is created for colonisation (Gosling, 2015). In *M. edulis* this risk increases with flow speed and mussel size and decreases with mussel tenacity, or attachment strength (Carrington, 2002).

The introduction of invasive species into marine environments disrupts native predator-prey relationships, causing declines in prey populations and impacting the entire food web. Invasive species' lack of natural predators allows them to rapidly compete for resources with native species, altering food web dynamics, habitats, and directly preying upon native species. Bivalves are affected by invasive species through

resource competition, direct predation, and changes in ecosystem dynamics. This disruption significantly harms native bivalves, influencing their distribution, abundance, and overall well-being within marine ecosystems, while also impacting nutrient cycling. Invasive species, like M. *galloprovincialis*, can not only disrupt ecosystems but also cause economic damage by fouling structures such as boats and water intake pipes and reducing the productivity of aquaculture operations (Lowe & Fossato, 2000; Molnar et al., 2008). These invasive species, face intense pressures to adapt to new environments, including developing traits to withstand varying temperatures. *M. galloprovincialis*, comes from the Mediterranean Sea and the eastern Atlantic, spreading globally over the past century to regions like the west coast of North America, East Asia, and South Africa, with its larvae often transported through ballast water (Geller et al., 1994). Upon its introduction to Southern California in the early 1900s, *M. galloprovincialis* swiftly adapted to the new surroundings, displacing the native *M. trossulus* (Lockwood et al., 2010; Tomanek & Zuzow, 2010).

Predators are an important source of natural mortality in bivalve molluscs and have the potential to influence population size structure in addition to overall abundance and local distribution patterns (Gosling, 2015). Gastropods are significant predators of mussels worldwide, the dog whelk, *Nucella lapillus*, is widely distributed on exposed shores in Northern Europe and on the east coast of North America, where it feeds extensively on barnacles and small mussels (Gosling, 2015). As numbers on mussel beds on the low and mid-shore start to increase in the spring, the profitability (energy assimilated from a food item relative to handling time) for dog whelks feeding on mussels increases with prey size (Hughes & De, 1984). It has been shown that dog whelks choose mussels with the maximum average profitability, mainly targeting damaged mussels via olfaction. Tactile stimuli also play a key role when selecting prey (Hughes & De, 1984). Starfish are also important predators, influencing the distribution and abundance of mussels on the lower shore and in the sublittoral zone. In Northern Europe, *Asterias rubens* is a serious predator of *M. edulis* by aggregating seasonally on mussel beds in large numbers and often completely destroying local mussel populations (Dare, 1982). Mussel beds with solid interconnected structure restricts predation to only those mussels located at the bed surface, giving refuge from

predation for smaller mussels deeper down (Dolmer, 1998). Reimer and Tedengren, 1996 carried out a study where *M. edulis* was cultured in close vicinity to *A. rubens*, showing morphological changes (adaptive value in that predator-exposed mussels) in the population such as smaller shell length, height, and width but had larger posterior abductor muscles, thicker shell and more meat/shell volume.

1.2 Mussel aquaculture and limitations of expansion

Marine food production encompasses a diverse array of organisms, with macro-algae, fish, and shellfish (comprising crustaceans and molluscs) emerging as central components of this vital sector (Wijsman et al., 2019a). The future of food production anticipates a significant expansion of aquaculture (McKindsey et al., 2011a), with molluscs, in particular, occupying a substantial share. In 2019, molluscs constituted more than 22% of the total global aquaculture harvest, equivalent to 17.6 million tonnes by live weight (FAO Fisheries and Aquaculture Department, 2021). Driven by their reputation as nutritious and sustainable food sources, the demand for molluscs has experienced steady growth in recent years (FAO Fisheries and Aquaculture Department, 2022). Notably, a substantial portion of marine bivalve production (89%) is derived from aquaculture, contributing significantly to the sector's economic viability, with an estimated annual value of approximately US$ 20.6 billion (FAO Fisheries and Aquaculture Department, 2021). Shellfish aquaculture operations are typically situated in well-defined coastal zones, occupying intertidal or subtidal regions, where they are cultivated directly on the substrate or suspended from rafts or stakes, often with protective netting or on racks. Beyond their economic significance, shellfish culture plays a vital role in fostering biodiversity by introducing structure and habitats that benefit various marine species (Shumway et al., 2003).

1.2.1 Europe

In Europe, responsible for 5.5% of the world production of marine bivalves, the production has decreased since 2000. This decrease is mainly due to a decrease in mussel production by aquaculture activities, from about 780 thousand tonnes per year in 2000 to about 580 thousand tonnes per year in 2020 (Regan et al., 2021). The production is

limited by a reduction in physical space due to competing claims with nature conservation and occasional recruitment failures. Juveniles for many species of culture bivalves are obtained from wild spat, but due to decreasing seed resources and environmental issues with the seed fishery, more and more of the seed resources for marine bivalve aquaculture are produced within land-based hatcheries. The direct capture production of marine bivalves remained relatively constant since the 1970's (1.78 million tonnes per year), but the aquaculture production of marine bivalves increased from 1.18 million tonnes per year in the period 1970–1974 to 16.00 million tonnes per year in the period 2015-2020 (FAO Fisheries and Aquaculture Department, 2022).

Related to *Mytilus* mussels and its role in marine ecosystems, their high abundance in coastal waters and the demand for human consumption has made them target species for aquaculture. In 2020, the European Union's 27 member states collectively produced 430,748 tonnes of mussels. Spain holds the top spot as the largest producer, accounting for 47% of the EU's total production in 2020. France comes in second with 14% and followed by Italy with 12% (Smaal, 2002; Kijewski et al., 2006; Mussel & Eu, 2022). In Spain, mussels (*M. galloprovincialis*) are cultured on ropes suspended in the water column and attached to rafts. Mussel seed collection is based on natural spatfall on rocky shores and on special ropes (Cáceres-Martínez & Figueras, 1998). In France, *M. edulis* are grown on poles (bouchots), longlines and on the bottom. There is also harvesting from wild stocks. Spat for bouchots and ropes are collected on special collector ropes (Goulletquer & Heral, 1997). And mussel (*M. galloprovincialis*) farming in Italy combines traditional and modern methods, the farming process starts with the collection of wild mussel seeds which attach to cables and buoys. Mussel seeds are placed in plastic mesh socks that hang from suspended long-lines kept 2-3 meters deep by buoys during the grow-out phase. Long-line plants, which use vertically oriented ropes attached to parallel cables, enable efficient and large-scale farming (Cefalo et al., 2020).

1.2.2 United Kingdom and Scotland

The shellfish industry is an important part of the UK economy, contributing 37% of total landings by value in 2018 (Hyder et al., 2018). Between 1994 and 2017, the UK produced 163,000 tonnes of shellfish annually, with 133,000 t coming from wild caught shellfish and 30,000 tonnes from shellfish aquaculture. England and Scotland are the highest producers of shellfish with 66,000 tonnes and 61,000 tonnes per year, respectively. In terms of value, the wild capture of shellfish is worth £203 million per year and shellfish aquaculture £28 million per year, whilst molluscs and crustaceans together contribute £183 million per year. The UK mollusc wild capture is dominated by scallops (92%) while *Nephrops* (55%), brown crabs (25%) and lobsters (19%) are the dominant crustacean species landed. Similarly, UK aquaculture is dominated by mussels (95%) and Pacific oysters (4%). Whilst the UK exports large amounts of shellfish across Europe (including crabs, oysters, mussels, scallops and lobsters), a significant amount is also consumed locally. Each year UK households buy 46,000 t of shellfish comprised of 4,000 t of molluscs and 42,000 t of crustaceans (FAO Fisheries and Aquaculture Department, 2022). Focusing on *Mytilus* mussels, in England and Wales the major blue mussel culture consists of rope culture. Notably, the UK's largest mussel farm, situated in Devon, employs offshore rope culture techniques. In many estuaries wild beds occur, but harvesting is prohibited because of poor sanitary quality, or purification is required In Northern-Ireland, wild fisheries occur in Belfast and Carlingford Lough (MacKenzie, 1997). Limitations are recruitment failure, predation by crabs and starfish and Eider ducks (Ross & Furness, 2000). Mussel farming in Scotland is generally off-bottom and depends on the settlement of spat on ropes suspended from longline systems (McKindsey et al., 2011b), the recruitment process involves both natural spawning from wild populations and seeding from existing mussel farms (Cockrell et al., 2015; Stirling & brahim Okumus, 1995). Low levels of spat settlement with temporal and environmental variation, and importing of variable quality spat, have been partly responsible for production fluctuations since 2010 (Mayes & Fraser, 2011; Gubbins et al., 2012; Seuront et al., 2019). Following the Scottish Shellfish Farm Production Survey in 2016 (Munro & Wallace, 2017) the Scottish shellfish farming industry was estimated to be worth £9.5 million at first sale value (a decrease of 23%). In

the year 2015, the total revenue from Scottish shellfish farming was estimated at £10.1 million and the bulk of which was generated from *M. edulis* production 7,270 tonnes of mussel worth £8.8 million was produced, in 2016 the total value increased to approximately £11.7 million (Munro & Wallace, 2017). Despite high production, this was a 5% decrease from production in 2014. Indeed, yearly production values in Scotland have fluctuated over the last five years due to inconsistent yields and variable market values. Studies predicted a production growth up to 7,200 tonnes in 2021, and a 13% decline to 6,277 tonnes in 2023 (Munro & Wallace, 2017), however, in the latest Scottish Shellfish Farm Production Survey (Munro et al., 2022) the previously predicted numbers are left behind, showing an actual production growth of 8,590 tonnes in 2021 (Table 1.1).

TABLE 1.1: Bivalve production trends in Scotland from years 2012 to 2021. Pacific oyster (*Crassostrea gigas*), Native oyster (*Ostrea edulis*), Queen (*Aequipecten opercularis*), Scallop (*Pecten maximus*), and Blue mussel (*Mytilus edulis*). The values represented in the table are in thousands of tonnes for all the species, except for the values corresponding to the blue mussel that are in tonnes. Data collected by Munro et al., 2022.

	2012	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**
Pacific oyster	2,706	1,891	3,392	2,693	3,534	5,034	4,031	4,393	2,863	4,853
Native oyster	317	260	242	200	201	200	142	103	35	8
Queen	9	33	18	33	155	273	18	18	0.5	0.5
Scallop	58	40	48	30	35	47	31	26	19	27
Blue mussel	6,277	6,757	7,683	7,270	7,732	8,232	6,874	6,699	5,661	8,590

1.2.3 Mussel farming practices

There are several types of mussel farming practices, each with their own advantages and limitations. The most common ones are rope-grown, bottom-grown, raft-grown, and offshore.

In rope-grown mussels are grown on ropes that are suspended in the water, usually by attaching them to buoys or a longline system. The mussels feed on plankton in the water and grow until they reach market size. Rope-grown mussel farming has several advantages, including low capital costs and easy harvesting. It is also considered environmentally friendly, as it has a low impact on the surrounding ecosystem. However, the main limitation is that the mussels are vulnerable to predation by birds, crabs, and starfish, which can reduce the overall yield (Richard et al., 2006).

In bottom-grown mussels are grown on the seafloor, either directly or in mesh bags. The mussels feed on nutrients in the sediment and grow until they reach market size. Bottom-grown mussel farming is favoured for its high yields and low labour costs, making it a cost-effective option. Additionally, this method has a low impact on the environment, as it utilizes the natural nutrients available in the sediment. However, the mussels are susceptible to disease and predation by bottom-dwelling animals, which can affect the overall yield (Stokesbury et al., 2011).

In raft-grown mussels are grown on rafts that are suspended in the water and made of plastic or metal. The rafts are anchored to the seafloor, and the mussels feed on plankton in the water, growing until they reach market size. Raft-grown mussel farming is known for its high yields and easy harvesting, as the rafts can be easily moved and the mussels are conveniently accessible. Like rope-grown mussel farming, it is also environmentally friendly. However, as with the rope-grown method, the mussels are vulnerable to predation by birds, crabs, and starfish, which can reduce the overall yield (Karayücel & Karayücel, 2000).

And in offshore mussel farming, where mussels are grown in deeper water, several miles from the shore. They are grown on ropes or in mesh bags and are suspended at various depths. Offshore mussel farming has several advantages, including higher yields and fewer environmental impacts than other methods. It is also less susceptible

to disease and predation, as it is far removed from shore-based predators. However, the capital costs for offshore mussel farming are higher than other methods, and the harvesting process can be more challenging due to the distance from shore (Brenner et al., 2009).

In summary, rope-grown mussel farming, the predominant practice in Scotland, holds critical implications for wild spat recruitment. Predation on rope-grown mussels, primarily by birds, starfish, and crabs, presents a potential factor contributing to recruitment instability. These predators target vulnerable spat during their pivotal settlement phase, when they must anchor themselves to substrates like ropes. While spat naturally attach themselves to the ropes in rope culture, environmental variables like strong currents or turbulence can disrupt this settlement process. Additionally, ropes used in mussel culture are susceptible to bio-fouling, as various marine organisms, including filter-feeders like barnacles and tunicates, compete for space and resources. Striking the right balance in rope spacing is crucial, as overcrowding may hinder effective spat settlement and growth, while excessive spacing can lead to inefficiencies in spat culture.

1.3 Marine connectivity

The overlapping distribution of species of the genus *Mytilus*, known for readily interbreeding in specific areas, intensifies the complexities associated with the genetic contribution of diverse parent populations to hybrid offspring. Despite prolonged genetic mixing, the factors upholding species boundaries in such scenarios remain incompletely understood. This dependence on the ability to reproduce highlights the intricate challenge of distinguishing between mussel species, particularly when hybridisation blurs distinctions and yields viable, reproductive hybrid offspring (Cowen & Sponaugle, 2009). Therefore, achieving a comprehensive understanding of various factors influencing mussel populations—such as dispersal abilities, ocean currents, and environmental conditions (Mallet, 1995)—becomes crucial in comprehending how hybridisation impacts the distinctiveness of mussel species. Assessing the genetic compositions and reproductive behaviours of mussel populations, particularly in regions where hybridisation is prevalent, offers an avenue to establish clear

taxonomical boundaries and ensure accurate classification within this group.

1.3.1 Larval connectivity

A population consists of a group of individuals that belong to the same species and are present in the same geographic area. In the marine environment, populations are dynamic and can be influenced by a wide range of factors, including dispersal ability, ocean currents, and environmental conditions (Mallet, 1995). Marine populations can be categorized into open and closed populations based on their exchange of individuals. Open populations have a higher degree of exchange and receive and export individuals to local populations, while closed populations have little exchange of individuals. Considering subpopulation as a set of individuals that live in the same habitat patch and interact with each other, and metapopulation as an assemblage of discrete local populations with some measure of shared migration among them, Cowen and Sponaugle (2009) focused on the drivers of larval dispersal, pointing at the necessity to understand the biophysical processes related to successful larval dispersion in benthic species. The four stages of successful larval dispersal (Swearer et al., 2019) in benthic marine organisms (Figure 1.4) are:

- Initiation or spawning period, where the most important parameters are related to reproductive output, including adult abundance, fecundity, egg quality, and fertilisation success.
- Transport and movement of larvae, where the disperser's trajectory is determined both by potential advection and turbulence of currents and the mortality and behaviour of individuals. The transition through this stage depends on larval survival and development rates, particularly sensory and motility capabilities that determine behaviour, and the extrinsic roles of currents and the spatial structure of the pelagic environment.
- Settlement, marking the end of the dispersal period, occurring when dispersers actively settle into some suitable habitat, which can be biophysically complex and governed by intrinsic and extrinsic factors that influence the likelihood of survival during this habitat transition.

- Recruitment happening when some settlers survive and mature to reproduce, contributing to subpopulation demographics and gene flow. Recruitment into the adult stage is determined by individual growth and survival to maturation, influenced by habitat quality, disturbance, local ecology, and individual conditions and phenotype.

Biophysical processes incorporate biological and physical elements, biological in the sense of processes that influence offspring production, growth, development, and survival; physical in the sense of advection and diffusion properties of water circulation; and biophysical in the sense of interactions between certain larval traits (*e.g.*, vertical swimming behaviour) and physical properties of the environment that operate at various scales (*e.g.*, coastal topography, tidal forces, surface waves, turbulence) (Palumbi, 2003; Cowen & Sponaugle, 2009; Paris et al., 2013). As mentioned before, for benthic species, the primary dispersal phase is typically associated with the earliest life history stage (spore, egg, or larva). The concept of larvae settling into a local population from a well-mixed larval pool led to the belief that marine populations are demographically open, potentially over hundreds to thousands of kilometres. Thus, marine connectivity encompasses the dispersal phase from reproduction to the completion of the settlement process (including habitat choice and metamorphosis), if reproductive connectivity happens, then the dispersal of individuals among subpopulations has survived to reproduce (Cowen & Sponaugle, 2009).

The pelagic larval stage is relevant to understand population dispersal patterns as well as the timing and magnitude of recruitment, nevertheless barriers to gene flow in the marine pelagic environment are often not clearly identified (Cowen & Sponaugle, 2009) hence the biogeography of species must be studied in relation to the geological history of the oceans (Hellberg, 2009). Ecological barriers can be geographic, *e.g.* seas, land masses, mountains, or environmental, *e.g.*, temperature and salinity gradients, light, currents, or oxygen levels (Selkoe & Toonen, 2011). Yet, many mobile marine organisms are also known to utilise seasonally dynamic oceanographic features to move between known breeding and foraging habitats (Guilford et al., 2009).

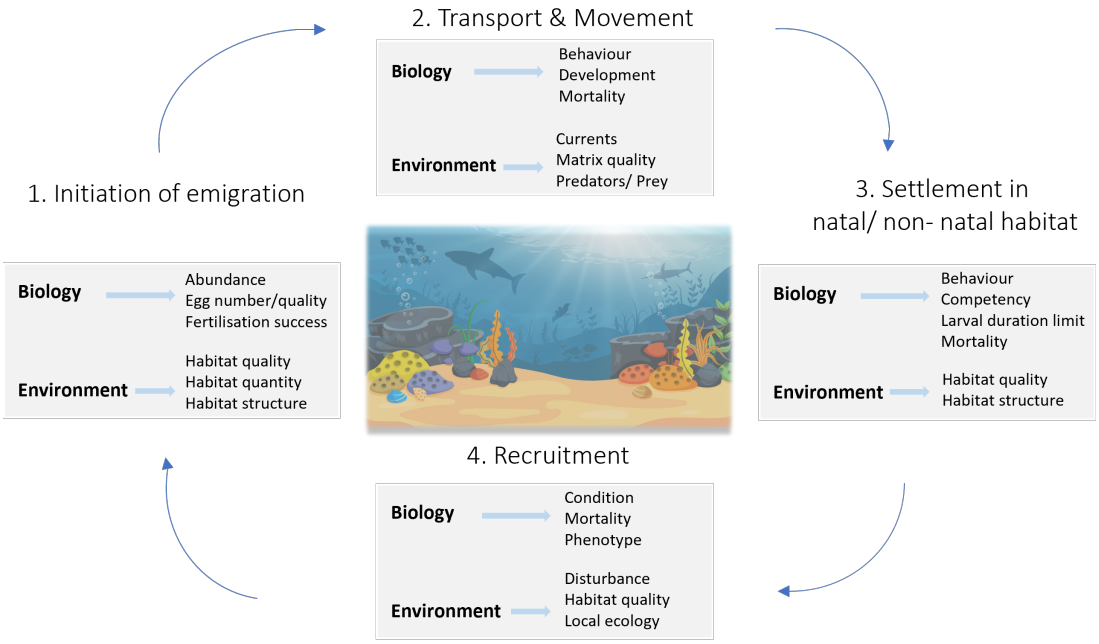

FIGURE 1.4: Four stages of successful larval dispersal, resulting in population connectivity in benthic marine organisms. Adapted from Swearer et al., 2019.

In pelagic systems dynamic oceanographic features like currents, eddies, fronts, filaments, or changes in vertical mixing are the major sources of this environmental variability leading to the development of short-lived corridors or ecological bridges. The timing and location of ecological bridges and barriers may change over space and time, connecting or disconnecting animals (Briscoe et al., 2017). For example, regions of upwelling and coastal heterogeneity have for long been recognised as influencing the transport and settlement of larvae (Banks et al., 2007).

1.3.2 Larvae and changes in environmental conditions

Many larvae of most benthic marine organisms are associated with particular substrate types such as rocks or sediments and determined environmental differences, such as tides and currents, which can create heterogeneity within substrate types. These differences in substrate properties can affect the distribution of benthic marine organisms that have specific preferences for certain types of substrate (Thrush et al., 2006). For example, Munguia et al. (2011) investigated how the distribution and density of organisms changed across different environments on the ocean floor. The researchers created a model with two different types of habitats and found that

when there was more variation in resources and environmental conditions, mussels and other species were found to be in the highest density. This means that the presence of different types of resources and environmental conditions within a habitat can support a greater diversity and abundance of organisms. On the other hand, when the habitats were more homogeneous, lacking variation in spatial and temporal resources, disturbance, and abiotic conditions, the density of mussels decreased, suggesting that the lack of environmental variability can limit the survival and growth of mussels and other species. Habitat heterogeneity can allow for the partitioning of resources and space among different species, which reduces competition and facilitates coexistence. This can be particularly important at larger scales, such as across entire regions, where the availability of different habitats can support a greater diversity of species (Mouquet & Loreau, 2003). Furthermore, results on Munguia et al. (2011) showed how changes in heterogeneity affect local populations, and how habitat connectivity can affect these changes. When a heterogeneous habitat shifts to a homogeneous system, the broadcast spawning species tended to disappear from the system, reducing diversity. But in a heterogeneous habitat, all the species, including *M. edulis* were able to coexist locally. Constant change in environmental conditions affects species fitness, population dynamics and eventually geographic distributions and when changes occur at rates faster than previously experienced, species invasions as well as population extinctions may arise depending on the context of habitat changes (Brown & Lomolino, 2000). The study of population connectivity plays an important role to understand how populations are maintained in time.

1.3.3 Mussel farming management practices and connectivity

Mussel farming practices can have implications for connectivity in marine ecosystems. Connectivity is important for marine conservation and decision-making management, as it helps to establish successful sink populations by managing heterogeneity levels of habitat. The different types of mussel farming practices, such as rope-grown, bottomgrown, raft-grown, and offshore, each have their own advantages and limitations that can affect connectivity in marine ecosystems (Weersing & Toonen, 2009; Schunter et al., 2011). For example, rope-grown and raft-grown methods are environmentally

friendly but are vulnerable to predation by birds, crabs, and starfish, which can reduce the overall yield. Bottom-grown mussel farming has high yields and low labour costs, but is susceptible to disease and predation by bottom-dwelling animals. Offshore mussel farming has higher yields and fewer environmental impacts than other methods, but the capital costs are higher, and the harvesting process can be more challenging due to the distance from shore. The settlement of larvae into a benthic population can determine the characteristics of future populations and influence existing individuals, and population persistence is linked to rates of larval delivery and recruitment, and hence connectivity among subpopulations (Thorrold et al., 2002).

Mussel farmers in the UK employ various strategies and practices to manage their stock efficiently, particularly in the absence of mussel hatcheries (Maguire et al., 2007) and given the specific challenges posed by wild spat recruitment and the timing of mussel harvesting (Avdelas et al., 2021; Regan et al., 2021).

- Collecting Wild Seed (Spat) : Many mussel farms in the UK rely on collecting wild mussel spat, often using specialised spat collectors. These collectors are structures or materials placed in the water that provide suitable settlement substrates for mussel spat. The spat naturally settle on these collectors, and farmers periodically collect them once they reach an appropriate size for on-growing. .
- Moving Stock: Mussel farmers may move their stock, including spat, to optimize growth conditions. This could involve relocating spat collectors to areas with better water quality or nutrient availability. Moving stock can also help mitigate the risk of predation in some cases.
- Purchasing Seed: In situations where wild spat recruitment is insufficient or unpredictable, some mussel farmers purchase mussel seed (juvenile mussels) from other sources. These seed suppliers may use hatchery-produced spat or wild-caught seed from other locations.
- Harvest Timing: Timing the harvest of mussels is critical. Harvesting is often done before the mussels reach sexual maturity and undergo spawning, as mature mussels may lose condition and market value during this process. Harvesting at the right size and before spawning is essential to obtain the best quality

and yield.

- Monitoring and Research: Mussel farmers closely monitor environmental conditions, spat settlement patterns, and growth rates to make informed decisions about managing their stocks. They may collaborate with researchers and marine scientists to better understand local conditions and improve spat collection and management methods.
- Adaptation: Given the variability of wild spat recruitment, mussel farmers need to be adaptable and responsive to changing conditions. They may adjust their farming practices based on factors like water temperature, currents, and the availability of spat.

The absence of mussel hatcheries in the UK means that farmers must rely on natural sources of mussel spat. They may invest in spat collection and spat management techniques, such as optimising collector design and deployment locations. Some farmers also participate in research and conservation efforts to better understand and enhance wild spat recruitment (Regan et al., 2021).

1.4 Tools to investigate connectivity

Marine dispersal is a challenging process to measure, and it is difficult to quantify the exchange between populations of marine organisms. This is because the distance between populations is determined not only by geography but also by the trajectory and fate of offspring, which are hard to track. Even when using genetically inferred patterns of connectivity, the match between currents and dispersal patterns is often poor (Hellberg, 2009), and the number of larvae decreases rapidly with distance and time from the spawning location due to the mixing and stirring of currents and high mortality rates (Cowen & Sponaugle, 2009). Thus, it is important to develop tools to better understand these processes for conservation and management decisions (Weersing & Toonen, 2009).

In the context of mussel farming, it is important to have a clear understanding of the local and regional hydrodynamics. This knowledge is crucial in comprehending the forces that either promote or hinder the dispersal of larvae, which ultimately affects

the level of gene flow between different sites (van der Reis et al., 2022). When there is limited gene flow between populations, this can lead to a reduction in genetic diversity, which can result in poor quality spat. This is because low gene flow increases the chances of inbreeding, which can ultimately lead to decreased fitness and heightened vulnerability to environmental stressors and diseases. Moreover, low gene flow can also reduce the population's ability to adapt to new environmental conditions. This further contributes to reducing the quality of the spat, as it becomes increasingly challenging for the population to cope with changing environmental conditions. For example, Bierne et al. (2003a) explored the genetic structure of the mosaic hybrid zone formed by *M. edulis* and *M. galloprovincialis* along the Atlantic coast of Europe. They utilised three length-polymorphic PCR loci as neutral and diagnostic markers in the analysis of 32 samples. Their findings revealed the frequency of alleles typical of *M. galloprovincialis* initially decreased in some areas, increased in others, and remained high in most of Brittany, and then decreased again in South Normandy. Notably, within the mosaic hybrid zone, two enclosed patches exhibited distinct genetic characteristics. These patches, predominantly resembling *M. edulis* and *M. galloprovincialis*, demonstrated differentiated allele frequencies compared to reference external populations of each species. Furthermore, each patch displayed partial introgression of alleles from the other species. When considering introgression, the presence of strong genetic barriers within all transition zones became evident. Their research provided evidence of a recent migratory 'short-cut' connecting *M. edulis*-like populations to an external *M. edulis* population in Normandy, likely reflecting the artificial transfer of spat for aquaculture purposes. In summary, gene flow plays a significant role in maintaining genetic diversity and ensuring the long-term survival of mussel populations. Hence, it is essential to understand and manage the factors that affect gene flow, such as hydrodynamics, to ensure the sustainability and success of mussel farming practices (Stuckas et al., 2017). Previous studies have explored various pre- and post-zygotic isolation mechanisms, including habitat specialization, spawning asynchrony, assortative fertilization, and hybrid depression. However, the comprehensive assessment of their role in shaping observed patterns remains incomplete (Bierne et al., 2003a). Within mixed populations, alleles specific to *M. galloprovincialis* tend to

increase in frequency as individuals grow and mature, potentially influenced by environmental factors like wave erosion on exposed shores. While certain instances suggest an initial advantage for *M. edulis*-like genotypes during settlement, a shift towards *M. galloprovincialis* genotypes occurs in adulthood, indicating an advantage for the latter rather than a disadvantage for hybrids. Early in the life cycle, mechanisms such as spawning asynchrony and assortative fertilization contribute to restricting hybrid mussel production (Marshall et al., 2010).

A study conducted by Demmer et al. (2022) developed a lagrangian particle tracking model to simulate larval dispersal and connectivity between distinct mussel populations in the northern part of the Irish Sea, revealing that wind-driven surface currents and tidal currents influence larval connectivity, leading to complex spatial patterns of connectivity between mussel beds.

In many cases, when there is a management of valuable fisheries along with periods of mass mortalities, the necessity for evidence-based management and improved plans to promote the recovery of the affected species are needed (Malham et al., 2009). For instance, the common cockle *Cerastoderma edule* is a long-lived, widespread bivalve occurring in intertidal soft-sediment locations along the coast of North-Western Europe, forming some of the most commercially valuable fisheries in the UK and Ireland. However, the population dynamics were poorly understood, with minimal stock management and suffering annual mass mortalities in southern Ireland. To understand the physical and biological factors that influenced the common cockle larval dispersal in the southern Irish Sea, Coscia et al. (2013) used 12 species-specific markers and analysed the genetic variation. They also used a biophysical model based on simulations of larval transport in the southern Irish Sea coupled with a 3D hydrodynamic model to reproduce the observed barotropic and baroclinic circulation. The markers were employed to assess population structure, comparing results with estimates of population connectivity through larval dispersal, calculated using a biophysical modelling approach. The model predicted connectivity between common cockle populations on the Welsh and Irish coasts, and was supported by genetic data indicating that allele frequencies were similar. The oceanographic and particle tracking model showed that

residual currents (caused mainly by atmospheric events) were important for shelf-scale larval transport.

Similarly, Pastor et al. (2021) studied the connectivity of the blue mussel in Denmark. They coupled a 3D physical model system with an agent-based model to examine connectivity of the marine system in terms of mussel larval dispersal and settling potential. Combined with the genetic analysis, they identified different clusters grouped together, with some exchange of simulated larvae observed among the clusters. They were able to identify genetic low differentiation and to support the 3D physical model output, highlighting the complexity and importance of using both methods to understand species connectivity.

Both studies provided evidence that a combination of multiple methods can upgrade our understanding of species connectivity and their dispersal. The researchers used genetic analysis to assess population structure and compared the results with estimates of population connectivity through simulations of larval dispersal, calculated using biophysical models. The results of both studies showed that residual currents and other physical factors play an important role in larval dispersal and population connectivity, highlighting the importance of using a combination of genetic and hydrodynamic modelling approaches.

1.5 Knowledge gaps in mussel larvae dispersal

Marine larval dispersal is a complex process that involves many factors. Despite significant advances in this field, there are still several knowledge gaps that need to be addressed. Studying larval behaviour in situ is a challenging task due to several factors, including the small size and transparent nature of the larvae, which makes them difficult to track in the ocean. Furthermore, the absence of reference genomes for key species can hinder genetic studies that aim to understand larval dispersal and connectivity. Additionally, spatial and temporal gaps in sampling can limit our ability to accurately assess these patterns, as they may not capture the complete extent of larval transport over time and space (Li et al., 2020). Another important knowledge gap is the limited understanding of the influence of climate change on larval dispersal

and subsequent population dynamics. As the climate changes, the impacts on larval dispersal and connectivity are not well understood, and more research is needed to address this gap (Mathiesen et al., 2017). Although physical and environmental factors are recognised to be major contributors to larval dispersal, the significance of biotic interactions, such as predation and competition, is still not well understood. Therefore, the absence of knowledge on the role of biotic interactions remains a significant gap in our understanding of larval dispersal. Studying rare and threatened species presents another challenge, as their low abundance and patchy distribution make them difficult to study (Pineda et al., 2010). As a result, more research is needed to understand their larval dispersal patterns and population connectivity.

1.5.1 Larvae transport

The lack of information in larvae dispersal is linked to the unpredictable recruitment of *M. edulis* in unstable pulses (Suchanek, 1981), as the larvae stay in the plankton for periods between 20 days and 2 months, depending on water temperature and currents (Lane et al., 1985). This unpredictable recruitment pattern is due to the larvae's dependence on environmental conditions and the length of time they spend in the plankton, which can delay metamorphosis for up to 6 months in unfavourable conditions (Mainwaring et al., 2014). As a result, the only mechanism for *M. edulis* to recover from mass mortality, predation, and challenging environmental conditions, is through larval recruitment to the bed or the area where previously a bed existed.

Understanding the transport of mussel larvae is crucial for predicting and managing mussel populations and improving the success of mussel aquaculture (Yund et al., 2015). However, limited knowledge on mussel larvae transport is due to a lack of understanding of the mechanisms that influence the transport of mussel larvae, such as water currents, temperature, and other environmental factors, and how these mechanisms affect the timing and pattern of larval recruitment in mussel populations. This lack of knowledge has significant implications, as mussel aquaculture relies on the availability of suitable seed material, which can be influenced by the transport of larvae from different populations (Alexander et al., 2021). Furthermore, the transport of larvae from other areas can affect the genetic diversity and overall health of wild

mussel populations. Thus, a deeper understanding of the factors that influence mussel larvae transport is essential for effective management and conservation of these important resources.

By studying the transport of mussel larvae, researchers can gain insights into the factors that influence recruitment and population dynamics, which can help inform management and conservation strategies. Some key research questions that need to be addressed include: what are the mechanisms that influence the transport of mussel larvae? How do environmental factors, such as temperature and water currents, affect larval transport and recruitment? How do genetic and physiological traits of mussel populations influence larval transport and recruitment? Answering these questions will require interdisciplinary research efforts, combining expertise in marine ecology, oceanography, genetics, and aquaculture, among other fields.

1.5.2 Limited genetic data

In general, fish species have received more attention in genetic research than bivalves, primarily because of their economic and ecological importance. Additionally, fish are popular model organisms for genetic research, making them a common subject of investigation in both commercial and academic settings (Ward et al., 2009). In contrast, bivalves have received relatively less attention in genetic research. Although bivalves are also economically important, they have not been studied as extensively as fish. While there are some bivalve species with complete or nearly complete genomes available, such as the Pacific oyster (Wang et al., 2014) or the Mediterranean mussel (Murgarella et al., 2016) this is not as common as in the case of fish.

The lack of complete genomes in fisheries species can have significant implications for the industry's productivity and sustainability. One of the primary issues is that it can make it challenging to understand the genetic makeup of these species fully. Partial genomes, or a lack of complete genomes, can limit our ability to analyse and interpret the genetic diversity of fish populations accurately. This genetic diversity is crucial for conservation and management purposes, as it informs us of the population's health, genetic structure, and potential for adaptation (Li et al., 2020).

Without reference genomes, it can be difficult to identify important genes that are associated with traits of interest, such as growth rate, disease resistance, or adaptation to different environments. These genes are critical to the breeding and selection of desirable traits in fisheries species, and the lack of information can ultimately impact the industry's productivity and sustainability. Without access to this information, fish populations may struggle to adapt to changing environmental conditions, making them more susceptible to disease and other stressors (Modak et al., 2021).

Another implication of the absence of reference genomes is the hindered development of tools for genetic identification and traceability. With incomplete genetic data, it is challenging to develop accurate markers that can be used to identify specific populations or individuals, making it difficult to track the origin of seafood products (Davey et al., 2011). This makes it harder for fisheries management organisations to monitor fish populations and ensure that fishing practices are sustainable and responsible.

The absence of reference genomes in fisheries species can limit our ability to understand, manage, and conserve these populations. It can make it difficult to analyse and interpret genetic diversity, limit the identification of important genes associated with desirable traits, and hinder the development of tools for genetic identification and traceability (Vendrami et al., 2020) such as selective breeding (Michalek et al., 2016) which holds great promise for enhancing various aspects of mussel production.

Breeding programs for bivalves globally employ mass and family selection methods (Hollenbeck & Johnston, 2018). Mass selection, based on individual performance relative to the population's mean for a specific trait, risks inbreeding depression and suits a narrow focus on few traits. Family selection, using pedigree data, selects top performing families to maintain genetic diversity. The application of successful selection strategies in bivalve breeding has significantly improved growth rate, disease resistance, and environmental resilience (Hershberger et al., 1984; Naciri-Graven et al., 1998; Degremont et al., 2015; de Melo et al., 2016). However, bivalve aquaculture predominantly relies on wild strains that may not be well-suited for farming environments (Hollenbeck & Johnston, 2018). Despite these challenges, focusing on specific traits in breeding programs can yield numerous advantages. For instance, it can expedite growth rates, reducing the time required for mussels to reach market size, thereby

increasing productivity and conserving resources. Moreover, this approach can enhance mussel populations' resistance to prevalent diseases, thereby improving overall survival rates. Genomics advancements hold significant promise, offering a potential pathway to optimize productivity in bivalve aquaculture globally

1.5.3 Advancements towards comprehending larvae dispersal

The development of coupled biological-physical models, such as Lagrangian particle tracing methods, has made it possible to investigate larval movement and connectivity (Adams et al., 2014, 2016). Regional-scale hydrodynamic models can be implemented using a regular grid for computation to resolve the study area. However, past studies have shown limitations in the scope of the study area, as the regular grid includes a specific area and has limits as such (Salama & Rabe, 2013).

For example, Salama and Rabe (2013), compared different models to study sea lice dispersal in Loch Linnhe on the West Coast of Scotland. They found that the limitations of the hydrodynamic Proudman Oceanographic Laboratory Coastal Ocean Model System (POLCOMS) were linked to resolution constraints and the complicated bathymetry and topography around the study area. Although good bathymetry data was available, the coarse model grid led to some simplifications, resulting in a few features in the topography being simplified or omitted.

To overcome these limitations, biophysical models can be used to simulate the release of passive particles in an ocean general circulation model to track the fate of drifters as carried by the currents (Lavelle & Mohn, 2010). These passive drifters can represent larvae and identify likely dispersal pathways, highlighting mechanisms of dispersal and oceanographic barriers to dispersal (Edwards et al., 2007; Adams et al., 2014).

Another successful approach to studying connectivity is genetic methods (Hedgecock et al., 2007). Genetic similarities and differences can be tested using genetic markers. However, in the case of fish, shellfish, and corals, genomic introgression from hybridisation between species can impede species assignment (Harrison et al., 2017). Therefore, developing custom genetic databases of reference species to supplement genetic repositories and using taxon-specific primers can greatly improve species assignment.

In summary, the combination of biophysical models and genetic methods can provide a comprehensive understanding of larval dispersal and connectivity in the marine environment, overcoming the limitations of traditional hydrodynamic models

1.6 Objectives

The overarching objective of this thesis is to advance current knowledge in mussel larval dispersal to advise mussel farming planning, conservation, and management practices. This has been achieved by using biophysical models in combination with genome-wide sequencing to investigate larval dispersal and population connectivity. Tracing and understanding the source of seed mussels is both a national and international problem and the most significant challenge for the expanding industry.

To accomplish the objective, the following three goals will be pursued:

- Obtain the whole genome assembly of the blue mussel to correctly identify molecular markers for evolutionary, population genetics, and conservation studies.
- Use an unstructured 3D hydrodynamic model to examine patterns of larval movement on the West coast of Scotland. Allowing the quantification of variability and connectivity between regions and the identification of the most probable sources of larvae.
- Conduct a blue mussel population structure study and validate the hydrodynamic model for key bivalve production locations in dynamic places such as the West coast of Scotland.

Chapter 2

Mytilus edulis whole genome sequencing

This chapter has been adapted from the research paper published by the Scientific Journal 'BMC Genomics' as:

Corrochano-Fraile, A., Davie, A., Carboni, S. and Bekaert M. (2022). Evidence of multiple genome duplication events in *Mytilus* evolution. *BMC Genomics* **23**:340. [doi:10.1186/s12864-022-08575-9]

I was involved in sourcing and preparing the biological materials, performing the functional analysis for all orthologs, and writing the manuscript.

2.1 Abstract

Molluscs remain one significantly under-represented taxa amongst available genomic resources, despite being the second-largest animal phylum and the recent advances in genomes sequencing technologies and genome assembly techniques. With the present work, we want to contribute to the growing efforts by filling this gap, presenting a new high-quality reference genome for *Mytilus edulis* and investigating the evolutionary history within the Mytilidae family, in relation to other species in the class Bivalvia.

Here we present, for the first time, the discovery of multiple whole genome duplication events in the Mytilidae family and, more generally, in the class Bivalvia. In

addition, the calculation of evolution rates for three species of the Mytilinae subfamily sheds new light onto the taxa evolution and highlights key orthologs of interest for the study of *Mytilus* species divergences.

The reference genome presented here will enable the correct identification of molecular markers for evolutionary, population genetics, and conservation studies. Mytilidae have the capability to become a model shellfish for climate change adaptation using genome-enabled systems biology and multi-disciplinary studies of interactions between abiotic stressors, pathogen attacks, and aquaculture practises.

2.2 Introduction

The family Mytilidae constitutes a diverse group of bivalves, broadly distributed in marine environments. *Mytilus edulis* and *Mytilus galloprovincialis* are the common species cultivated in Europe and both hybridise with *Mytilus trossulus* where their geographical distribution overlaps (Gosling, 1992; Riginos & Cunningham, 2004) forming the European *Mytilus* Species Complex (Wilson et al., 2018). Nonetheless, a number of environmental and genetic barriers work together to maintain genetic discontinuities between the different species of the complex (El Ayari et al., 2019). *M. edulis* and *M. galloprovincialis* can be considered cosmopolitan species while *M. trossulus* remains more geographically confined to the northernmost regions of the Pacific and Atlantic oceans and to the Baltic Sea (Gosling, 1994). At a finer geographical scale, mussel species display an extraordinary capability of environmental adaptation, extending from high inter-tidal to sub-tidal regions, from estuary to fully marine conditions, and from sheltered to extremely wave-exposed shores. Mussels are furthermore exposed to a wide range of potentially pathogenic microorganisms and pollutants, and yet they display a remarkable resilience to stress and infections (Gerdol et al., 2020). Of particular interest are observations of a relatively high heterozygosity, rapid evolutionary responses to environmental threats, including predation (Freeman, 2006), and recent suggestions that widespread relaxed selection in "low locomotion" molluscs, such as bivalves, and high copy number variants (Modak et al., 2021) could underpin observed high resilience and rapid adaptation to new environments (Sun et al., 2017).

The Phylum Mollusca remains significantly under-represented amongst those with available genomic resources (Sigwart et al., 2021). To date, only two high-quality genomes and associated gene models are available within the Mytilidae family: *M. galloprovincialis*, first sequenced by Murgarella et al., 2016 and then improved by Gerdol et al., 2020, and *M. coruscus* recently sequenced by Li et al., 2020 and Yang et al., 2021. Comparative genomics provides an opportunity to investigate the "signatures" that natural selection has left on the genomes of related species. By analysing the frequency distribution of synonymous substitution per synonymous sites, it is possible to identify major evolutionary events, including Whole-Genome Duplications (WGDs). While WGDs are rare within animal lineages, they deeply shaped vertebrate evolution and represent important evolutionary landmarks from which some major lineages have diversified (Berthelot et al., 2014). Furthermore, comparisons among related species adapted to contrasting niches, can provide an opportunity to investigate how their genomes diverge in response to different habitat conditions (Oliver et al., 2010). In cases where specific amino acids are known to affect protein function, analyses of intra-specific polymorphism and divergence can be used to directly study function variation in natural populations (Dean & Thornton, 2007; Storz et al., 2015). Both whole genome duplication analysis and positive selection genome-wide analysis can therefore expose the strength and direction of selection on genes' functional variation and corroborate the adaptive significance of the loci under study (Linnen et al., 2009; Natarajan et al., 2015).

With this work, we want to contribute to the growing efforts in filling the gap in Molluscs genomic resources (Davison & Neiman, 2021) by presenting a new high-quality reference genome for *M. edulis* and investigating the evolutionary history within the Mytilidae family and in relation to other species in the class Bivalvia. Here we present a new reference genome for *M. edulis*; we introduce the first evidence of WGD events in the Mytilidae family and in Bivalvia more generally; finally, we identify gene clusters under significant positive selection within each species of the Mytilidae family for which suitable reference genomes are available (*M. edulis*, *M. galloprovincialis* and *M. coruscus*).

The availability of this reference genome will not only increase interest in Mytilidae as a model for ecological and evolutionary research, but will be also a valuable tool for breeding programmes (Regan et al., 2021). The discovery of multiple duplication events will enable the correct identification of molecular markers for evolutionary, population genetics, and conservation studies. The Mytilidae have the capability to become a model shellfish for climate change adaptation using genome-enabled systems biology and multi-disciplinary studies of interactions between abiotic stressors, pathogen attacks, and aquaculture practises.

2.3 Material and Methods

2.3.1 Material collection

The *M. edulis* used in this work was obtained from a female adult blue mussel gill tissue from St Andrews Bay (Scotland, UK), a location that previously reported only the presence of a pure *M. edulis* population (Wilson et al., 2018). Gill tissue was dissected, stored in 95% ethanol and shipped to Novogene Ltd (Cambridge, UK) for DNA extraction and sequencing. A sub-sample was tested to confirm the species identification using Wilson et al., 2018 test panel and protocol.

2.3.2 Library construction and sequencing

High-quality DNA was used for subsequent library preparation and sequencing using both the PromethION and Illumina platforms at Novogen UK (Novogene UK Company Ltd, UK). To obtain long non-fragmented sequence reads, 15 µg of genomic DNA was sheared and size-selected (30-80 kb) with a BluePippin and a 0.50% agarose Gel cassette (Sage Science, USA). The selected fragments were processed using the Ligation Sequencing 1D Kit (Oxford Nanopore, UK) as directed by the manufacturer's instructions and sequenced using the PromethION DNA sequencer (Oxford Nanopore, UK) for 48 hours. For the estimation and correction of genome assembly, an Illumina DNA paired-end library with an insert size of 350 bp was built in compliance with the manufacturer's protocol and sequenced on an Illumina HiSeq X Ten platform (Illumina Inc., USA) with paired-end 150 nt read layout.

2.3.3 RNA isolation, cDNA library construction and sequencing

The total RNA was extracted using the TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. The preparation and sequencing reactions of cDNA library were done by Novogene Ltd. Briefly, the poly (A) messenger RNA was isolated from the total RNA with oligo (dT) attached magnetic beads (Illumina Inc., USA). Fragmentation was carried out using divalent cations under elevated temperature in Illumina proprietary fragmentation buffer. Double-stranded cDNAs were synthesised and sequencing adaptors were ligated according to the Illumina manufacturer's protocol (Illumina Inc., USA). After purification with AMPureXP beads, the ligated products were amplified to generate high quality cDNA libraries. The cDNA libraries were sequenced on an Illumina Hiseq 4000 platform (Illumina Inc., USA) with paired-end reads of 150 nucleotides.

2.3.4 Genome assembly

Reads from the two types of sequencing libraries were used independently during assembly stages. Long-reads were filtered for length ($>$ 5,000 nt) and complexity (entropy over 15), while all short reads were filtered for quality (QC $>$ 25), length (150 nt), absence of primers / adaptors and complexity (entropy over 15) using fastp v0.20.1 (Chen et al., 2018). Using Jellyfish v2.3.0 (Marçais & Kingsford, 2011), the frequency of 17-mers and 23-mers in the Illumina filtered data was calculated with a 1 bp sliding window (Vurture et al., 2017) to evaluate genome size. Long-reads were then assembled using wtdbg2 v2.5 (Ruan & Li, 2020) which uses fuzzy Bruijn graph as well as raven v1.5.0 (Vaser & Šikić, 2021). As it assembles raw reads without error correction and then creates a consensus from the intermediate assembly outputs, several error corrections, gap closing, and polishing steps have been implemented. The initial outputs have been combined using Quickmerge v0.3 (Solares et al., 2018). The combined output was re-aligned to the long-read and polished using Minimap2 v2.17 (Li, 2018) and Racon v1.4.3 (Vaser et al., 2017), first with filtered reads, to bridge potential gaps, then with the filtered reads to correct for error. Finally, Pilon v1.23 (Walker et al., 2014) was used to polish and correct for sequencing error using the short-reads. The redundant contigs due to diploidy were reduced by aligning the long reads back to the

assembly with Minimap2 v2.17 (Li, 2018) and by passing the alignment through the Purge Haplotigs pipeline v1.1.1 (Roach et al., 2018). This reduced the artefact scaffolds and created the final haploid representation of the genome. Scaffolds were ordered with Medusa v1.6 (Bosi et al., 2015) using *M. galloprovincialis* (Gerdol et al., 2020; Murgarella et al., 2016) and *M. coruscus* (Li et al., 2020) genome scaffolds. Mitochondrial genome was annotated using MITOS r999 (Bernt et al., 2013) and manually curated.

2.3.5 Repeat sequences

The transposable elements were annotated using a *de novo* prediction using RepeatModeler v2.0.1 (Flynn et al., 2020) and LTR-Finder v1.07 (Stanke et al., 2008). The repetitive sequences yielded from these two programs were combined into a non-redundant repeat sequence library. With this library, the *M. edulis* genome was scanned using RepeatMasker v4.10 (Smit et al., 2019) for the representative sequences.

2.3.6 Gene models

RNA-seq reads of poor quality (*i.e.* with an average quality score less than 20) or displaying ambiguous bases or too short and PCR duplicates were discarded using fastp v0.20.1 (Chen et al., 2018). Ribosomal RNA was further removed using SortMeRNA v3.0.2 (Kopylova et al., 2012) against the Silva version 119 rRNA databases (Quast et al., 2012). The cleaned RNA-seq reads were pooled and mapped to the genome using the using HiSat2 v2.2.0 (Kim et al., 2019). A combined approach that integrates ab initio gene prediction and RNA-seq-based prediction was used to annotate the protein-coding genes in *M. edulis* genome. We used Braker v2.1.5 (Hoff et al., 2019) to make *de novo* gene predictions. The accuracy and sensitivity of the predicted model was improved by applying iterative self-training with transcripts. The predicted coding sequences were been annotated using the InterProscan v5.46-81.0 (Jones et al., 2014; Mitchell et al., 2019), Swiss-Prot release 2020_02 (Bateman et al., 2017) and Pfam release 33.1 (El-Gebali et al., 2019) databases. For classification, the transcripts were handled as queries using Blast+/BlastP v2.10.0 (Altschul et al., 1990), E-value threshold of 10^{-5}, against Kyoto Encyclopedia of Genes and Genomes (KEGG) r94.1 (Kanehisa et al., 2019). Gene Ontology (Ashburner et al., 2000) was recovered from the

annotations of InterPro, KEGG and SwissProt. Subsequently, the classification was performed using R v4.0.0 (R Core Team, 2021) and the Venn diagram was produced by jvenn (Bardou et al., 2014). The completeness of gene regions was further tested using BUSCO v4.0.2 (Simão et al., 2015) with a Metazoa (release 10) benchmark of 954 conserved Metazoa genes.

2.3.7 Phylogenetic Tree

Concatenated alignments constructed from all mitochondrial shared CDS sequences were used to construct a phylogenetic tree. Sequences were aligned using MACSE v10.02 (Ranwez et al., 2011). A Maximum Likelihood (ML) tree was inferred under the GTR model with gamma-distributed rate variation (Γ) and a proportion of invariable sites (I) using a relaxed (uncorrelated log-normal) molecular clock in RAxML v8.2.12 (Stamatakis, 2014).

2.3.8 Calculating Ka, Ks, and Ka/Ks values

Complete annotated nuclear genomes of Bivalvia (class) were collected. Blast+ / BlastP v2.10.0 (Altschul et al., 1990) was used to search for duplicated sequences in protein-coding genes between each genome. Duplicate pairs were identified as sequences that demonstrated over 70% sequence similarity, mutual protein coverage $>$ 80%, protein length $>$ 30 amino-acid from an all-against-all search. Duplicated sequences were aligned accounting for codon and coding frame, using MACSE v10.02 (Ranwez et al., 2011). Finally, the Ka (number of non-synonymous substitutions per non-synonymous site) and Ks (number of synonymous substitutions per synonymous site) for each pair was calculated using an MPI version of KaKs_Calculator (Zhang et al., 2006) under the MLPB model (Tzeng, 2004). The Ks values $>$ 5.0 were excluded from further analysis due to the saturated substitutions at synonymous sites. Univariate mixture models were fitted to the distributions of Ks by expectation maximisation that uses the finite mixture expectation maximisation algorithm (Benaglia et al., 2009; Tiley et al., 2018).

2.3.9 Estimation of evolution rates

A set of core-orthologs was constructed from the three complete annotated nuclear genomes of Mytilinae (subfamily), *M. edulis*, *M. galloprovincialis* (Gerdol et al., 2020) and *M. coruscus* (Li et al., 2020) and were used to identity cluster of orthologous genes with a 1:1:1 ratio. Ka and Ks estimation were reported pairwise between species after MACSE v10.02 (Ranwez et al., 2011) and KaKs_Calculator (Zhang et al., 2006) under the MLPB model (Tzeng, 2004) as above. From a literature search of comparative mussel biology studies, we identified genes relevant to core physiological functions, specifically immunity, stress response and shell formation. Subsequently, a local BLAST search was conducted on NCBI to identify the genes of interest in the available genomes of the three species with a cut-off point of 80% sequences similarity.

2.4 Results

2.4.1 Sequencing results

After sequencing with the PromethION platform, a total of 15.95 million long-reads were generated and used for the genome assembly. The mean length of the sequences was 7,002 nt. The Illumina HiSeq X Ten platform produced 652.47 million paired-ended short reads (150 nt). Based on the presumption that the genome size will be similar to that of closely related taxa: *M. galloprovincialis* (Murgarella et al., 2016) and *M. coruscus* (Li et al., 2020) with an estimated genome size of 1.60 Gb and 1.90 Gb respectively; therefore, the estimated sequencing coverage was 64x and 113x, for long and short reads respectively (Table A.1).

2.4.2 *De novo* assembly of the *M. edulis* genome

Using Jellyfish, the frequency of 17-mers and 23-mers in the Illumina filtered data were determined (Figure 2.1) and followed the theoretical Poisson distribution typical of a diploid species (Benadelmouna et al., 2018). The proportion of heterozygosity in the *M. edulis* genome was evaluated as being 3.69% and 4.84% respectively, and the genome size was estimated as 1.01 Gb and 1.10 Gb, with a repeat content of 68.13% and 39.91% respectively (Table 2.1).

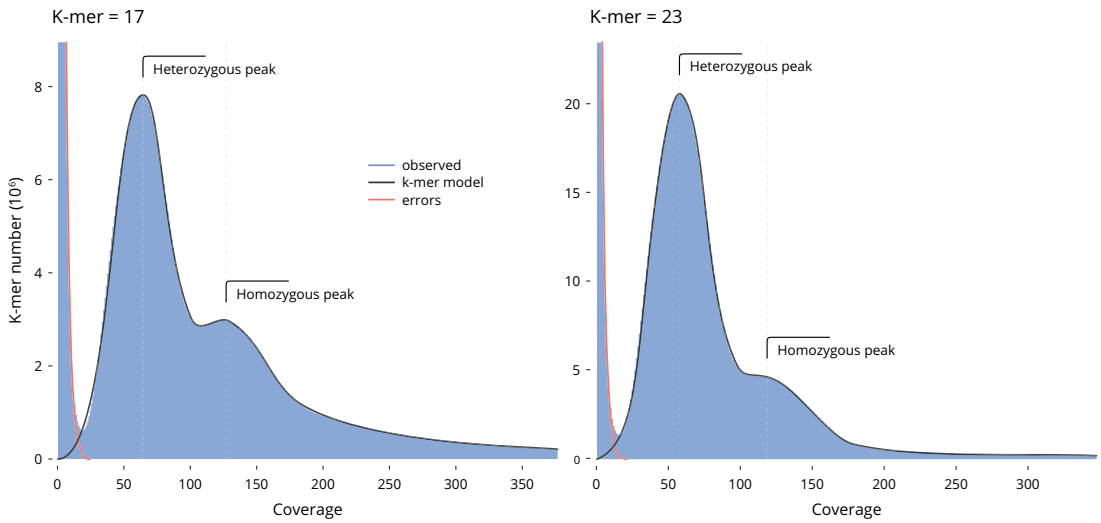

FIGURE 2.1: The k-mer distribution used for the estimation of genome size. The heterozygous and homozygous peaks of k-mer depth are clearly markers, suggesting a high-complexity genome. (**A**) The 17-mer distribution. Predicted genome size, 1,010,184,781 nt; (**B**) The 23-mer distribution. Predicted genome size, 1,096,306,163 nt.

Long-reads were assembled, polished with Racon and short-read sequence were corrected with Pilon, creating an assembled genome of 3,339 contigs with a total length and contig N_{50} of 1.83 Gb and 1.10 Mb, respectively (Table 2.1). The realignment of the short-reads also provided descriptions of the mean observed heterozygosity of 0.48%, which is consistent with the most recent evidence collected from *de novo* RAD analysis (Vendrami et al., 2020) and microsatellite loci study (Coolen et al., 2020). A second *M. edulis* genome was recently released, NCBI Accession GCA_019925275.1. This chromosome level *de novo* assembly was only based on long-reads (PacBio Sequel platform), where fewer error corrections are possible; but produced a comparable genome size of 1.65 Gb and contig N_{50} of 0.49 Mb.

2.4.3 Repeat sequences and Gene models

The transposable elements and repetitive sequences have been annotated using RepeatMasker and LTR-Finder. In total, we have found 1.03 Gb (56.33%) of repetitive sequences (Table A.2). We used a combined method that integrates *ab initio* gene prediction and RNA-seq-based prediction to annotate the protein-coding genes in *M. edulis* genome. A total of 69,246 distinct gene models and 73,842 transcripts were annotated.

TABLE 2.1: Statistics of the genome assembly of *M. edulis*.

Category	Number/length
K-mer = 17	
Estimated genome size	1,010,184,781 nt
Estimated repeats	688,190,885 nt
Estimated hetorizygosity	3.69%
K-mer = 23	
Estimated genome size	1,096,306,163 nt
Estimated repeats	437,569,400 nt
Estimated hetorizygosity	4.84%
Number of contigs	3,339
Total length	1,827,085,763 nt
Total repeats	1,029,206,554 nt
Observed hetorizygosity	0.48%
Largest contig	10,529,124 nt
N_{50}	1,097,279 nt
GC	32.17%
Read Mapped	91.35%
Avg. coverage depth	152x
Coverage over 10x	99.99%
N's per 100 kbp	13.73
BUSCO recovered	98.9%
Predicted rRNA genes	132
Predicted protein coding genes	69,246

The completeness of gene regions was further assessed using BUSCO using a Metazoa (release 10) benchmark of 954 conserved Metazoa genes, of which 93.8% had complete gene coverage (including 29.4% duplicated ones), 5.1% were fragmented and only 1.1% were classified as missing (Figure 2.2A).

These data largely support a high-quality *M. edulis* genome assembly, which can be used for further investigation. The predicted proteins from the reconstructed genes were subjected to BlastP similarity searches against SwissProt, Pfam, InterPro, KEGG and GO databases. Of the total 69,246 gene models annotated by at least one database, 9,005 (13.0%) were annotated in all five databases used (Figure 2.2B and Table A.3). A total of 31,620 predicted genes were annotated to three major GO classes: "biological processes", "cellular components" and "molecular functions" (Figure 2.2C).

2.4.4 Mitochondrial genome

The mitochondrial genome was retrieved manually from the genome assembly. The sequence of 16.74 kb was validated for continuity and circularity, and fully annotated.

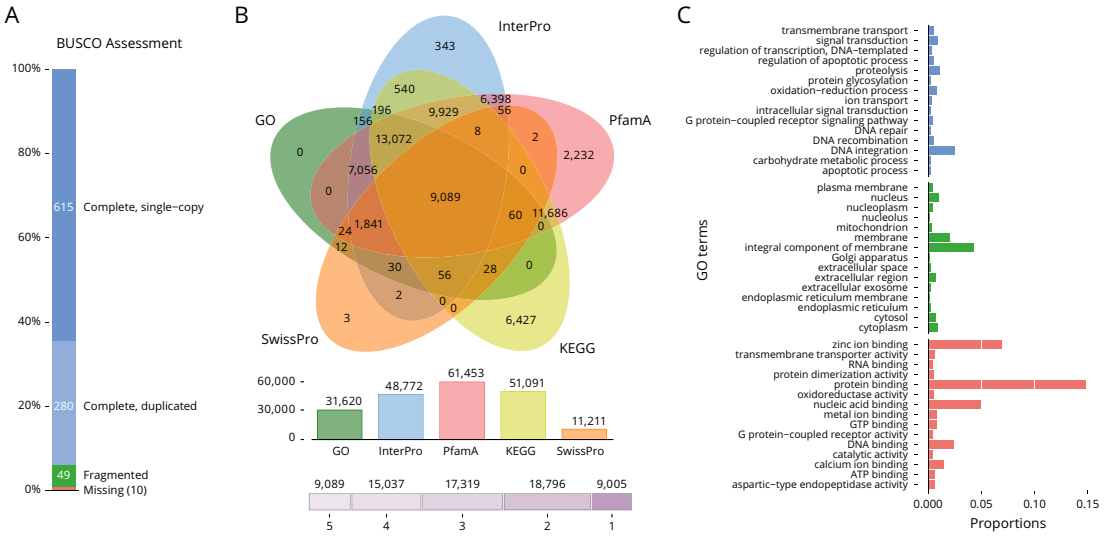

FIGURE 2.2: Gene composition and annotation estimations. (**A**) BUSCO evaluation (Metazoa database; number of framework genes 954), 98.9% of the gene were recovered; (**B**) A five-way Venn diagram. The figure shows the unique and common genes displaying predicted protein sequence similarity with one or more databases (details in Supplementary Table A.3); (**C**) Level 2 GO annotations using the gene ontology of assembled transcripts.

The full mitochondrial genome (Figure 2.3A) was compared to the reference *M. edulis* genome (Boore et al., 2004). Only one haplotype was recovered which is identical at 99% (85 SNPs) with the reference genomes (EBI Accession NC_006161.1) and is consistent with a female mitotype (Breton et al., 2006). Complete annotated mitochondrial genomes for all Mytilinae (subfamily) to date (11 species; Table A.4) were collected. Concatenated alignments constructed from all mitochondrial shared CDS sequences were used to construct a phylogenetic tree (Figure 2.3B). This phylogenetic tree is consistent with the species relationships observed in previous studies (Lee et al., 2019).

2.4.5 Detecting whole genome duplications

To assess the paleo-history of the Mytilinae (subfamily), we performed a comparative genomic investigation. A total of 2,293 gene duplications younger than Ks = 5 were inferred across the total data set of 16,291 assembled unigene clusters in Mytilinae (*M. coruscus*, *M. edulis* and *M. galloprovincialis*). The histograms of duplication ages for each species analysed demonstrated evidence of two large-scale duplications (Figure 2.4A). Mixture model analyse of Ks distributions (Figure 2.4A and Table A.5) to identify ancient whole genome duplications (Shi et al., 2010; Tiley et al., 2018) were

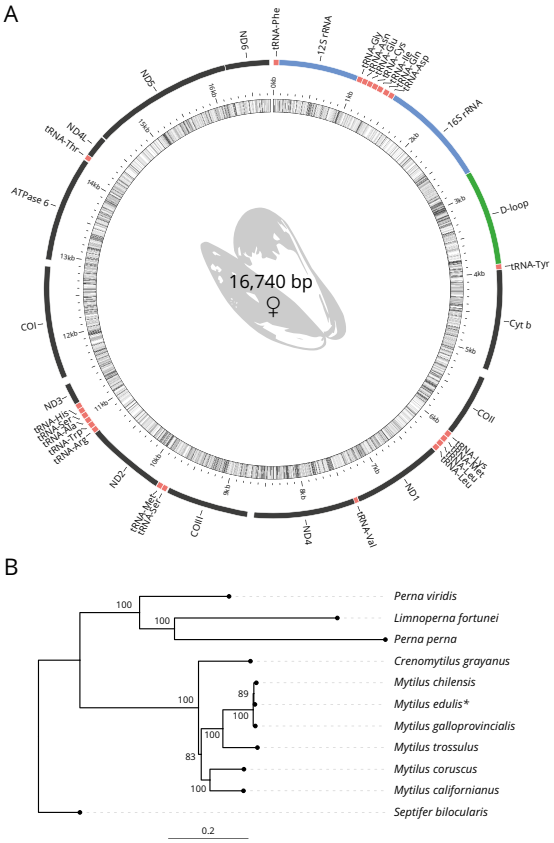

FIGURE 2.3: Genome assembly. (**A**) *M. edulis* annotated mitochondrial genome; (**B**) Phylogenetic tree inferred from the mitochondrial gene.

consistent with the two consecutive whole genome duplication events (α-WGD and β-WGD). The duplication distributions each contained evidence of two peaks of similar synonymous divergences. For example, in *M. edulis* these peaks are located at median Ks of 0.6132 and 1.8196 (Table A.5). We extended the analysis to all Bivalvia (class). Out of the 46 whole genomes available, only 7 (including the three Mytilinae) have gene models allowing further analyses (Table A.6). All exhibit evidence of α-WGD and β-WGD (Table A.5). The median value, for Ks peaks, is compatible with a shared WGD event (compatible age) indicating that these taxa diverged after their most recent WGDs.

2.4.6 Identification and Functional Analysis of Positively Selected Genes

Figure 2.5 shows the mean Ka/Ks ratios for each orthologs, unigene cluster, in *M. edulis* and *M. galloprovincialis* (Figure 2.5A); *M. edulis* and *M. coruscus* (Figure 2.5B) and *M. coruscus* and *M. galloprovincialis* (Figure 2.5C). In each figure, genes clusters are colour coded to indicate groups of orthologs positively selected in both species (blue),

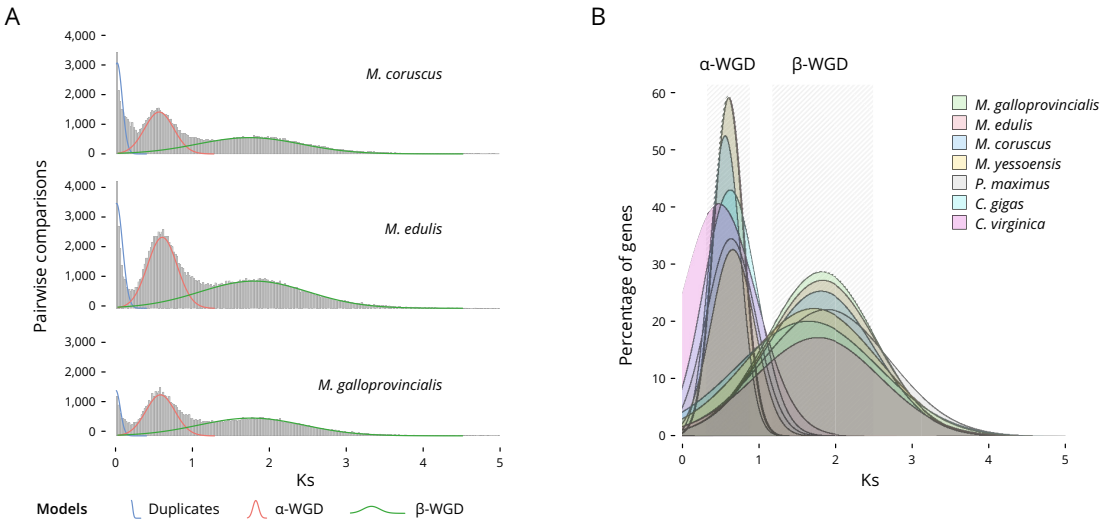

FIGURE 2.4: Ka and Ks analysis. (**A**) Distribution of the Ks values of the duplicate pairs in *M. coruscus*, *M. edulis* and *M. galloprovincialis*; (**B**) Bivalvia (class) ortholog Ks distribution and multiple WGDs. Combined Ks plot of the gene age distributions of seven species (see Tables A.5 & A.6). The median peaks for these plots are highlighted. Analyses of ortholog divergence indicated that these taxa diverged after their most recent WGDs.

species-specific positively selected orthologs (red), and groups of orthologs of interest (green) involved in immunity, stress response and shell formation. Collectively, the data in Figure 2.5 provide a new insight into positive selection occurring in the three *Mytilus* species object of this study.

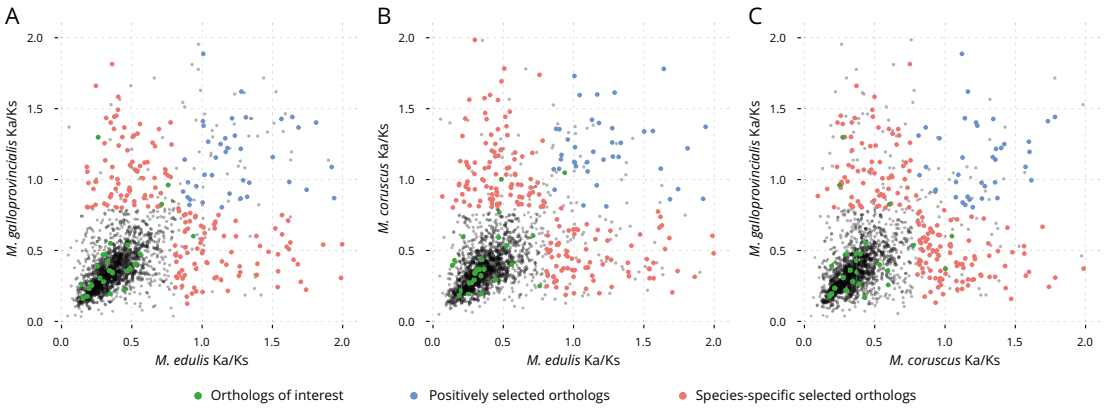

FIGURE 2.5: Mean Ka/Ks ratios for each orthologs cluster in the (**A**) *M. edulis* and *M. galloprovincialis*; (**B**) *M. edulis* and *M. coruscus*; (**C**) *M. coruscus* and *M. galloprovincialis*.

The functional analysis of positively selected orthologs has also allowed for the identification of gene duplications within assembled unigene clusters involved in key physiological processes. Here, we provide an overview of the identified functions of genes

under positive selection. When *M. edulis* and *M. galloprovincialis* Ka/Ks ratios are plotted (Figure 2.5A), several gene clusters can be observed to be under positive selection in both species (in blue). Of these, four were identified as contributing to immunity, stress response or shell formation: the WNT Inhibitory Factor 1 (WIF1), the nucleotide exchange factor SIL1, the kelch-like protein and the midline (MID1) protein. WIF1 contributes to several immune response functions (Capt et al., 2020), and presented a Ka/Ks value of 1.4 and 1.8 for *M. galloprovincialis* and *M. edulis* respectively. SIL1 is a protein that interacts with Hsp70 during stress responses (Fu et al., 2014), and showed a Ka/Ks value of 1.4 and 1.6 for *M. galloprovincialis* and *M. edulis*, respectively. The kelch-like protein facilitates protein binding and dimerisation (Shi et al., 2019), and presented a Ka/Ks value of 1.4 and 1.7 for *M. galloprovincialis* and *M. edulis*, respectively. Finally, the midline (MID1) protein, presenting E3 ubiquitin ligase activity (Zanchetta & Meroni, 2019), showed a Ka/Ks value of 1.4 and 1.6 for *M. galloprovincialis* and *M. edulis*, respectively.

Many of the gene clusters were also found to be under intense positive selection in *M. galloprovincialis*, but under intense purifying selection in *M. edulis* or vice versa (in red). These genes are of particular interest as they indicate relatively rapid divergence between the two species. In Figure 2.5, the genes with highly divergent selection are: the Glycolipid transfer protein, a ubiquitous protein characterised by their ability to accelerate the intermembrane transfer of glycolipids (Brown & Mattjus, 2007); The SGNH Hydrolase-Like Protein for which no function has been identified (Le et al., 2019); The vitellogenic carboxypeptidase-like protein, involved in key developmental processes (Sui et al., 2009). Furthermore, two unknown proteins with Ka/Ks values of 1.7 and 1.5 for *M. galloprovincialis* and Ka/Ks value of 0.2 and 0.4 for *M. edulis* have also been identified. On the other hand, gene clusters with high Ka/Ks values for *M. edulis* but low for *M. galloprovincialis* included: Mucolipin, which promotes calcium homeostasis and is involved in stress response functions (Zhang et al., 2019). The KAT8 regulatory NSL complex with developmental and cellular homeostasis function (Radzisheuskaya et al., 2021). The TolB-like protein, involved in a tol-dependent translocation system (Carr et al., 2000). The RING finger protein 170, which mediates the ubiquitination and degradation of inositol 1,4,5-trisphosphate receptors, and it is

involved in immune response functions (Song et al., 2019) and finally, Fibropellin, a cell adhesion protein (Nie et al., 2020). Only a limited number of gene clusters appear to be under positive selection amongst those commonly used in immunity, stress response and shell formation comparative studies (in green), and the majority of the genes clusters showed negative selection with the exception of four, which resulted to be all involved in immune response pathways (Gerdol & Venier, 2015). Of these, three were positively selected in *M. galloprovincialis* and conserved in *M. edulis* (membrane-bound C-type lectin, Galectin 3, MAP kinase 4-like) and one was positively selected in *M. edulis* and conserved in *M. galloprovincialis* (TNF ligand-like 2).

The comparison of Ka/Ks values between *M. coruscus* and *M. edulis* (Figure 2.5B) shows a similar picture to that of *M. edulis* and *M. galloprovincialis* (Figure 2.5A). Positively selected orthologs in both species (with Ka/Ks values ranging from 1.3 to 1.9) include: the nucleotide exchange factor SIL1; the archease-like protein, related to stress response functions (Auxilien et al., 2007); the midline (MID1) protein and the thiosulfate/3-mercaptopyruvate sulfotransferase protein involved in developmental and stress response functions (Mao et al., 2011). The ortholog clusters positively selected in *M. coruscus* but conserved in *M. edulis* (Ka/Ks values from 1.6 to 2.0 and from 0.3 to 0.7 respectively) include: the purine-nucleoside phosphorylase, which encodes an enzyme which reversibly catalyses the phosphorolysis of purine nucleosides (Stoeckler et al., 1997); the palmitoyl-protein thioesterase, which facilitates the morphological development of neurons and synaptic function in mature cells (Koster, 2019), and the ADAR protein which is an RNA-binding protein and has antiviral immunity in marine molluscs (Green et al., 2015). Two further proteins with unknown associated functions and with Ka/Ks values of 1.8 and 1.7 for *M. coruscus* and of 0.3 and 0.5 for *M. edulis* were also identified. Similarly, orthologs positively selected in *M. edulis* but conserved in *M. coruscus* were functionally characterised and resolved to be the same as those described for *M. edulis* and *M. galloprovincialis* (Figure 2.5A). Only two gene clusters showed positive selection amongst those of physiological interest (Figure 2.5B, in green). C-type lectin 7 (immunity) was positively selected in *M. coruscus* with a Ka/Ks value of 1.0 and 0.4 for *M. coruscus* and *M. edulis*, respectively; while TNF ligand-like 2 (immunity) is presenting positive selection in both species,

with Ka/Ks values of 1.0 and 0.9 for *M. coruscus* and *M. edulis*, respectively.

In the *M. galloprovincialis* and *M. coruscus* comparison (Figure 2.5C), positively selected proteins in both species (in blue) include: the nucleotide exchange factor SIL1, the importin-7 protein, involved in nuclear import of histones and the homeobox protein cut-like (CUTL), involved in cell-cell adhesion interactions that are required for normal development (Pérez-Parallé et al., 2005). The ortholog clusters positively selected in *M. galloprovincialis* (in red) but conserved in *M. coruscus* (Ka/Ks values from 1.4 to 1.8 and from 0.2 to 0.7 respectively) are the same as described in *M. edulis* and *M. galloprovincialis* (Figure 2.5A). The ortholog clusters positively selected in *M. coruscus* (in red) but conserved in *M. galloprovincialis* (Ka/Ks values from 1.6 to 1.9 and 0.3 to 0.4 respectively) are again the same as described in *M. coruscus* and *M. edulis* (Figure 2.5B). The proteins positively selected for *M. galloprovincialis* and conserved for *M. coruscus* (Figure 2.5C, in green) are: the membrane-bound C-type lectin (immunity) with a Ka/Ks value of 1.3 and 0.3 for *M. galloprovincialis* and *M. coruscus*, respectively. The galectin 3 protein (immunity) with a Ka/Ks value of 1.0 and 0.3 for *M. galloprovincialis* and *M. coruscus*, respectively. And the MAP kinase 4-like protein (immunity) with a Ka/Ks value of 0.8 and 0.6 for *M. galloprovincialis* and *M. coruscus*, respectively. Finally, two genes showed positive selection among those of physiological interest (in green) in favour of *M. coruscus*, and conserved for *M. galloprovincialis*. The TNF ligand-like 2 (immunity) with a Ka/Ks value of 1.0 and 0.6 for *M. coruscus* and *M. galloprovincialis*, respectively. And C-type lectin 7 (immunity) with a Ka/Ks value of 1.0 and 0.4 for *M. coruscus* and *M. galloprovincialis*, respectively.

The vast majority of our orthologs of interest (in green) selected from the literature have not shown a substantial number of proteins under positive selection for genes related to immunity, stress response, and shell formation.

For completeness, all genes involved in immunity, stress response and shell formation under positive selection in any of the three species examined here, were identified and grouped by species (Table 2.2, Table 2.3). For *M. galloprovincialis*, 6, 10 and 3 genes related to immunity, stress response and shell formation, respectively were detected. For *M. edulis*, 13, 6 and 4 genes related to immunity, stress response and shell formation, respectively were detected, and for *M. coruscus*, 10, 5 and 2 genes related to immunity,

stress response and shell formation, respectively were detected.

TABLE 2.2: Genes involved in immunity (Imm), stress response (Stress) and shell formation (Shell) under positive selection in *M. galloprovincialis*, *M. edulis* and *M. coruscus*.

Species	Category	ClusterID	Gene Names / Function (Ortholog cluster)	Ka/Ks	Ortholog reference
M. galloprovincialis	Imm	9813	NIT1 Potential tumour suppressor in tumour progression	2.5	Lin et al., 2018
M. galloprovincialis	Stress	6307	Arylesterase / Paraoxonase Confer resistance to organophosphate toxicity	1.4	Bonacci et al., 2004
M. galloprovincialis	Stress	1976	Serine/threonine kinase 17 Cellular processes, proliferation, apoptosis, and differentiation. Abiotic stress	1.4	Zorina et al., 2014
M. galloprovincialis	Stress	8246	Peptidase inhibitor 16 Cardiac stress response	1.4	Regn et al., 2016
M. galloprovincialis	Shell	18160	EF-hand Ca2+-binding domain 6 CaLP has two Ca2+-binding EF hand domains.Growth of nacre-prismatic layer	1.2	Feng et al., 2017
M. galloprovincialis	Imm	19933	Signal transducer-activator of transcription 5B Mediate the signaling of cytokines and a number of growth factors	1.1	Yu et al., 2019
M. galloprovincialis	Stress	1594	Pyruvate dehydrogenase E1 alpha subunit Reduces OXPHOS and oxygen consumption	1.1	Zimmer et al., 2016
M. galloprovincialis	Stress	973	DNA mismatch repair protein MSH6 Responsive to oxidative stress and protection against ROS and DNA damage	1.1	Pinheiro et al., 2021
M. galloprovincialis	Stress	25447	zinc finger MYM-type protein 2-like Significant correlations with salinity, temperature, As, Cd or lindane	1.1	Baillon et al., 2015
M. galloprovincialis	Shell	12897	Ca2+ transporting ATPase, plasma membrane Catalyse the hydrolysis of ATP coupled with the transport of calcium	1	Sillanpää et al., 2018
M. galloprovincialis	Stress	14603	Inositol polyphosphate 1-phosphatase Mg2+ dependent of inositol monophosphatase-like domain	1	Bialojan and Takai, 1988
M. galloprovincialis	Imm	14873	Galectin-4 Regulators of immune cell homeostasis	1	Vasta et al., 2015
M. galloprovincialis	Imm	3134	PRRT1 Proline-rich transmembrane protein 1	1	Marin et al., 2000
M. galloprovincialis	Shell	14056	Metalloproteinase inhibitor 3 Ligament-specific protein	0.9	Kubota et al., 2017
M. galloprovincialis	Stress	1893	Solute carrier family 12 Transport endogenous-exogenous substances. Potassium/chloride transporters	0.9	Xun et al., 2020
M. galloprovincialis	Imm	7356	Leucine-rich repeat domain superfamily Development, growth, and responses to abiotic and biotic stresses	0.9	Wang et al., 2017
M. galloprovincialis	Stress	3258	Heat shock protein 22 HSPB8 Protecting cells, folding of nascent peptides, and responding to stress	0.9	Zhang et al., 2010
M. galloprovincialis	Stress	5345	Mitogen-activated protein kinase 6 MAPK involved in the regulation of Hsp expression in blue mussels	0.9	Anestis et al., 2007
M. galloprovincialis	Imm	5490	LRP1B Low-densitylipoproteinreceptor-related protein	0.9	Liu et al., 2014b
M. edulis	Imm	1359	E3 ubiquitin-protein ligase mind-bomb (MIB2) Antiviral immunity	2.4	Chen et al., 2015
M. edulis	Stress	5390	Mucolipin, Polycystin cation channel Calcium homeostasis	2	Jiao et al., 2019
M. edulis	Imm	13969	RNF170, RING finger protein 170 Ubiquitination and degradation of inositol 1,4,5-trisphosphate receptors	1.7	Song et al., 2019
M. edulis	Stress	9109	Fibropellin-1 Hypoxia responsive gene	1.7	Nie et al., 2020
M. edulis	Stress	10101	Carbohydrate-binding WSC Plasma membrane sensor for surface stress	1.5	Oide et al., 2019
M. edulis	Imm	4324	Lactamase_B Drug resistance among gram-negative bacteria	1.3	Singh et al., 2019
M. edulis	Stress	4307	polycystin Calcium homeostasis	1.3	Wang et al., 2020
M. edulis	Imm	9741	Papain-like cysteine peptidase superfamily Prevent unwanted protein degradation	1.2	Liu et al., 2018
M. edulis	Imm	7225	Peptidase M12B Cell adhesion, signaling, cell-cell fusion, and cell-cell interactions	1.2	Rubin et al., 2014
M. edulis	Stress	7276	ATP-dependent metalloprotease (YME1L) Stress-sensitive mitochondrial protease	1.2	Rainbolt et al., 2015
M. edulis	Imm	14877	P-loop - nucleoside triphosphate hydrolase This domain shows a high specificity for pathogens and parasites	1.1	Arivalagan et al., 2017
M. edulis	Shell	15346	EF-hand Ca2+-binding domain CaLP has two Ca2+-binding EF hand domains(Growth of nacre-prismatic layer)	1.1	Feng et al., 2017
M. edulis	Imm	2105	C-type lectin superfamily 17 member A Mediate crucial cellular functions during immunity and homeostasis	1	Kerscher et al., 2013

TABLE 2.3: Cont. Genes involved in immunity (Imm), stress response (Stress) and shell formation (Shell) under positive selection in *M. galloprovincialis*, *M. edulis* and *M. coruscus*.

M. edulis	Imm	10990	Fibrinogen-like protein A Immunepattern-recognition receptors.	1	Gorbushin and Iakovleva, 2011
M. edulis	Imm	20412	FYVE, RhoGEF and PH domain Signal transduction	0.9	Perrier et al., 2020
M. edulis	Shell	5702	Perlucin-like protein Ca2+-dependent carbohydrate binding activity	0.9	Blank et al., 2003
M. edulis	Imm	22962	C1q-related factor Pattern recognition receptors. Activates innate immune response	0.9	Jiang et al., 2020
M. edulis	Imm	11253	nicotinic acetylcholine receptor alpha-7 Regulates immune response through the neuroendocrine-immune system	0.9	Jiao et al., 2019
M. edulis	Imm	19695	TRIM56, tripartite motif-containing protein 56 virus-inducible E3 ubiquitin ligase that restricts pestivirus infection	0.9	Liu et al., 2014a
M. edulis	Imm	11784	HMCN, hemicentin Immune recognition, signaling and regulation.insulin peptide receptor	0.9	Wang et al., 2016
M. edulis	Shell	12528	COL6A, collagen, type VI Adhesome molecules	0.9	Dyachuk, 2018
M. edulis	Shell	8193	mucin-13-like Molluscan calcification	0.9	Marin et al., 2000
M. edulis	Stress	6739	serine-protein kinase ATM DNA damage sensor	0.8	Matsuoka and Igisu, 2001
M. coruscus	Shell	8395	Hemicentin (HMCN) Extracellular ion-binding proteins in the biomineral matrix	2	Luo et al., 2015
M. coruscus	Stress	2491	O-mannosyltransferase (TMTC) Ca2+regulation and protein folding	2	Larsen et al., 2017
M. coruscus	Imm	5530	ADAR, adenosine deaminase DNA binding. antiviral effectors	1.6	Green et al., 2015
M. coruscus	Imm	9626	Caveolin-1,Caveolin-3 Regulating neutrophil functional responses that underpin innate immunity	1.2	Zemans and Downey, 2008
M. coruscus	Imm	8044	Apoptosis regulator BAX Apoptosis regulator	1.1	Leprêtre et al., 2020
M. coruscus	Stress	17439	2-hydroxyglutaryl-CoA dehydratase (hgdC) Iron-sulfur cluster binding	1.1	Locher et al., 2001
M. coruscus	Imm	302	Mannose receptor, C type (MRC) Pathogen recognition receptor	1	Chen et al., 2015
M. coruscus	Imm	6467	Inhibitor of growth protein 1 (ING1) Tumor suppressor gene	1	Garkavtsev et al., 1998
M. coruscus	Imm	2098	BIRC2_3 Physiological role in growth, immunity, and apoptosis	1	Wilson et al., 2016
M. coruscus	Imm	8921	Filamin Recognition of pathogens	1	Maldonado-Aguayo et al., 2015
M. coruscus	Imm	13594	Proteasome regulatory (PSMD7) Recognition of polyubiquitin chains and cleavage of ubiquitin from degraded proteins	0.9	Smits et al., 2020
M. coruscus	Shell	4996	NOTCH1 Calcium signalling pathway and shell pigmentation	0.9	Auffret et al., 2020
M. coruscus	Stress	3709	Heat shock protein 90kDa beta (HSP90B) Heat shock protein	0.9	Cao et al., 2018
M. coruscus	Imm	8284	Cell division control protein 42 Roles in host defense	0.9	Xu et al., 2017
M. coruscus	Stress	5847	Ammonium transporter (amt) Ammonium transporter	0.9	Bu et al., 2019
M. coruscus	Imm	12429	RING finger protein 145 Ubiquitination	0.8	Cook et al., 2017
M. coruscus	Stress	12587	Lysine methyltransferase 4 (EEF1AKMT4) Enhances the function of heat shock factor 1 during the heat shock response	0.8	Vera et al., 2014

2.5 Discussion

2.5.1 Reference Genome and Whole Genome Duplication

Mussels are also known as poor man's shellfish as they are inexpensive and abundant. These features have perhaps contributed to a relative neglect in the investigation of this species' genomic structural variation, and whether such structural changes can play a significant role in their evolution and ecological adaptations (Davison & Neiman, 2021). In the wild, mussels thrive on rocks and stones along the coast, but the majority of mussels consumed are farmed in coastal waters providing food security and employment opportunities to a multitude of fragile coastal communities

worldwide (Smaal et al., 2019). Similarly, to several other molluscs classes, genomic research into *M. edulis* has been hampered by the lack of a reference genome. This bottleneck is historically linked with the technical difficulties in extracting high molecular weight genomic DNA from Molluscan tissues and thus, allow for long reads sequencing techniques to be successfully applied (Davison & Neiman, 2021). In addition, the relatively large genome size and a high level of heterozygosity further complicates the assembly of high-quality reference genomes in the phylum.

With the aim of shedding new light onto the genomic structure and evolution of the class Bivalvia, we sequenced the blue mussel genome and we introduced the first evidence of WGD events in the Mytilidae family and in Bivalvia more generally. Finally, we identify genes within key physiological pathways under significant positive selection. Taken together, our results provide new insights into the Mytilidae family genome structure and introduce new genomic resources for the investigation of Bivalves evolution, population genetics and for future selective breeding activities. The genome was assembled into 3,339 scaffolds with a total length of 1.83 Gb, a GC content of 32.17% and a scaffold N_{50} of 1.10 Mb. In addition, we found 1.03 Gb (56.33% of the assembly) of repeat content, 69,246 protein-coding genes, 132 rRNAs and a heterozygosity of 0.48% (Table 1). The results are equivalent with the other *Mytilus* genomes: Genome size between 1.90 Gb and 1.28 Gb, and repetitive sequences between 52.83% and 58.56% (Gerdol et al., 2020; Li et al., 2020; Murgarella et al., 2016; Yang et al., 2021). In addition, transcriptomic data and the derived gene models are comparable with the other available Mytilidae transcriptomes. Phylogeny of the *Mytilus* (based on the mitochondrial genomes) confirms the position of *M. edulis* in the genus, with the *M. edulis* and *M. galloprovincialis* (sympatric species) separate from *M. trossulus* and *M. coruscus* (which group with *M. californianus*; Figure 2.3B).

Our analysis provides, for the first time, genomic evidence for paleopolyploidy in the class Bivalvia. Combining our gene age distribution and phylogenomic analyses, we found evidence for two significant, episodic bursts of gene duplication. While some of these duplication events may be caused by other processes of gene duplication, they are compatible with WGDs observed using similar methods in plants (Badouin et al., 2017) and animals (Berthelot et al., 2014; Li et al., 2018). Ks analysis showed that an

ancient WGD event and a more modern WGD event occurred before the divergence of the Bivalvia. This explains why bivalves, and molluscs more generally, present large genomes (Davison & Neiman, 2021). The genomic information for *M. edulis* presented here, will help clarify the evolutionary processes in Bivalvia species and contribute to improving the understanding of the physiological and morphological diversity of Bivalvia species. Our discovery of WGDs in the ancestry of Bivalvia raises questions about the role of gene and genome duplication in their evolution. After duplication, the most likely fate of duplicated genes is the loss of one of the duplicates through non-functionalisation that occurs by accumulation of deleterious mutations (Nei, 1973; Takahata & Maruyama, 1979). While common after WGD, gene loss could however play a key role in speciation (Lynch, 2000), through a process known as divergent resolution (Taylor et al., 2001). In addition, duplicated genes may also be retained in two copies (Ohno, 1970) and either specialise by the partitioning of ancestral gene functions (*i.e.* sub-functionalisation) or by the acquisition of a novel function (*i.e.* neo-functionalisation).

Incomplete genetic data (draft genomes and transcriptomes), as well as reduced datasets (Enzymes, RAD, or EST), made it impossible to correctly detect WGDs and duplicated genes in Bivalvia, before now. In the absence of complete genomes and the full picture of WGD events, duplicated sequences are often overlooked or wrongly interpreted. This can lead to artefacts such as high heterozygosity (Vendrami et al., 2020), pseudogenes, and a rapid rate of gene acquisition and loss (Gerdol et al., 2020). The discovery of several events of WGD in the Bivalvia phylogeny suggests the prospect that large-scale duplications are consistent with the evolution of novelty and diversity in the physiology of mass spawners like Bivalvia. However, dating of such events remains difficult due to the lack of annotated genomes deeper in the phylogeny, which still is a priority to fully elucidate molluscan evolution.

2.5.2 Identification and Functional Analysis of Positively Selected Genes

The functional analysis of positively selected orthologs has allowed us to compare our results with studies related to the identification of gene involved in key physiological processes. When identifying gene clusters under positive selection in both

species (blue dots in Figure 2.5; *M. galloprovincialis-M. edulis*, *M. coruscus-M. edulis*, and *M. galloprovincialis-M. coruscus*), we find the predominant functions for those genes are mainly related to immunity, stress responses and developmental processes. Our results agree with past studies, and confirms that genes related to immunity are under selection in multiple lineages, likely via adaptive evolution mechanisms linked to host-pathogens co-evolution (Oliver et al., 2010). The stress response genes presenting positive selection are related or are interacting with Hsp proteins (SIL1 and the archease-like protein). Since the marine environment has considerable concentration of bacteria and viruses, molluscs depend on cellular and molecular mediated immune responses that help them to survive under challenging conditions (Pourmozaffar et al., 2020). That is why filter-feeding animals such as bivalves rely on the intervention of shock proteins which synthesis depends on environmental stressful conditions such as temperature, salinity, hypoxia, heavy metal, and infectious pathogens (Wan et al., 2012). Genes presenting intense positive selection in one species but intense purifying selection in the others are of interest because they indicate rapid divergence between species. Once again, the three species have their maximum Ka/Ks values in genes related to developmental processes, immunity, and stress response. Overall, genes identified as being under positive selection in this study, are consistent with the defence system of bivalves depending on the innate immune response against stressful conditions such as environmental stressors, pollution and disease outbreaks.

The identification of all the genes involved in immunity, stress response and shell formation under positive selection in any of the three species (Table 2.2, 2.3) has provided us with relevant information that could be used in future studies to identify markers for future comparative physiology and evolution studies. Our results for *M. galloprovincialis* have shown a considerable amount of stress response proteins (10 proteins) under positive selection compared to the other two species. A significant amount of those stress response genes have documented roles in heat tolerance or direct associations to heat-stress responses, *e.g.* zinc finger MYM-type protein 2-like (ZMYM2), mitogen-activated protein kinase 6 (MAPK6), heat shock protein 22 (HSPB8). This is also supported by past studies (Saarman et al., 2017; Popovic & Riginos, 2020) were

genomic functions previously linked to divergent temperature adaptation reflected accelerated molecular divergence between warm-adapted *M. galloprovincialis* and cold-adapted congeners, such as *M. edulis*. Molecular divergence of *M. galloprovincialis* is consistent with warm-temperature adaptation demonstrated by physiological studies. *M. galloprovincialis* also has more positive selection in stress response proteins related to heavy metal detection, transport and metal binding (*e.g.* arylesterase / paraoxonase, pyruvate dehydrogenase E1 component alpha subunit, inositol polyphosphate 1-phosphatase, Solute carrier family 12), than *M. edulis* and *M. coruscus*. *M. edulis* and *M. galloprovincialis* presented positively selected shell formation proteins, in the EF-hand domains, which appears to be evolving faster in the two species, albeit in different gene clusters: EF-hand domain protein, EF-Hand, calcium-binding site for *M. edulis* and EF-hand calcium-binding domain-containing protein for *M. galloprovincialis*. In bivalves, the Ca^{2+} binding EF hand domains include a Calmodulin-like protein (CaLP), a multifunctional calcium sensor that belongs to a new member of the CaM (cell adhesion molecules) superfamily, localised in the organic layer sandwiched between nacre and prismatic (aragonite) layer (calcite) in *Pinctada fucata* (Yan et al., 2007). Studies have shown that CalP might be involved in the growth of nacre layer and prismatic layer (Feng et al., 2017). Our results suggest and support past studies indicating that closely related bivalves use different secretory repertoires to construct their shell (Peterson et al., 2008) which might lead to positive selection at a gene level as reflected in our results. Also, shell dissolution and decreased shell growth caused by ocean acidification have been described in marine bivalves (Melzner et al., 2011) forcing the need for a fast environmental adaptation. Taking in account current alterations in precipitation patterns as well as stronger and more frequent heat waves and fluctuating sea surface salinities (Steeves et al., 2018), our results suggest that *M. galloprovincialis* appears to be better equipped than *M. edulis* and *M. coruscus* to adapt to higher temperatures, aquatic toxicity, and contamination.

In light of the challenges presented by climate change and the steady increase in temperatures, the hybridisation between *M. edulis* and *M. galloprovincialis* in northern latitudes might provide evolutive advantages. This natural process has the potential to

introduce genes from *M. galloprovincialis* into *M. edulis* populations, which could confer to hybrids an increased ability to adapt to progressively warmer conditions. By assimilating genetic traits from a species with a proven abililty to cope with higher temperatures, *M. edulis* populations showing introgression of *M. galloprovincialis* genetic material could exhibit increased resilience when facing thermal stress and changing environmental conditions.

2.5.3 Genetic resources available

At the time of conducting this experiment, within the genus *Mytilus* genome assemblies were available only for *M.coruscus* (Li et al., 2020) and *M. galloprovincialis* (Murgarella et al., 2016; Gerdol et al., 2020); however none of these showed resolution to thechromosome level. Subsequently, additional datasets for *M. coruscus* (Yang et al., 2021), *Mytilus chilensis* (Gallardo-Escarate et al., 2023) and *M. edulis* (Regan et al., 2022) were published, providing a more comprehensive genomic landscape mapped to chromosome level. The new chromosome level assemblies will enable future studies investigating large- scale evolutionary patterns (macroevolution) by examining the synteny across different species, allowing to assess some of the conclusions from this study, such as the proposed whole genome duplication events, using an independent approach.

Advances in DNA sequencing technologies led to rapid additional resource development for Bivalve species, including extensive transcriptome datasets (Jingxiao Zhang & Han, 2019), linkage maps using microsatellite and single nucleotide polymorphism (SNP) markers (Lallias et al., 2007; Wilson et al., 2018; Simon et al., 2021), and medium-density SNP arrays (Nascimento-Schulze et al., 2023). These tools have become valuable genomic resources to enhance genetic improvement of production traits, such as growth and disease resistance, in selective breeding programmes (Potts et al., 2021; Nascimento-Schulze et al., 2021).

Nevertheless, a key resource for enabling genetics and genomic research in a given species is a high-quality reference genome. In summary, the obtention of a reference genome will enhance the genomic toolkit available for further investigations. This research, enables a deeper understanding of the genetic differences and similarities

among *Mytilus* species, shedding light on their evolutionary relationships and molecular intricacies.

2.5.4 The importance of a reference *Mytilus edulis* genome for the West Coast of Scotland

The availability of a high quality *M. edulis* genome holds significant importance for the West Coast of Scotland. This endeavour contributes to various aspects crucial for the region's marine environment and the broader community. The West Coast of Scotland boasts rich marine biodiversity (Hawkins et al., 2009; Tsiamis et al., 2020), making a detailed understanding of the genetic makeup of key species like *M. edulis* is essential for effective environmental monitoring. The reference genome can serve as a reference point for assessing environmental health and understanding the impacts of stressors on marine ecosystems. Moreover, given its significant economic importance, the newly developed genome can enhance selective breeding programs, aiding in the improvement of traits such as growth rate, disease resistance, and overall productivity in aquaculture settings. The genome's availability can contribute to ecosystem management strategies, ensuring the sustainable use of marine resources along the West Coast of Scotland.

In the face of climate change impacts, the *M. edulis* reference genome becomes a valuable tool for studying the species' adaptive responses to environmental changes. This knowledge can contribute to broader climate change impact assessments specific to the West Coast region. Conservation efforts also benefit from a detailed understanding of the genetic diversity and population structure of *M. edulis*.

Beyond practical applications, the genome serves as a resource for ongoing research and education initiatives. Scientists, students, and conservationists can leverage this genomic information to explore various aspects of *M. edulis*' biology, ecology, and evolution, contributing to a deeper understanding of the marine ecosystems in the West Coast of Scotland. In summary, the development of the *M. edulis* genome through de novo assembly is a multifaceted initiative with implications for environmental monitoring, aquaculture, ecosystem management, climate change studies, conservation efforts, and scientific research and education along the West Coast of Scotland.

2.6 Conclusions

The recruitment, settlement, and grow-out phase of bivalve aquaculture and more precisely, in *Mytilus* spp. is strongly dependant with the environmental conditions. Therefore, the implications of climate change are not restricted to wild populations. Strong changes in local environmental conditions may limit production and force the relocation of grow-out sites to suitable areas. Thinner and weaker shells will facilitate their rupture during transportation and increase losses due to predation. Genomic selection studies and identification of molecular markers can favour the development of genetically improved lines for multiple traits and facilitate the management of genetic variability. The development of high-quality assembled genomes, as provided by the current research, will favour the identification of genomic regions linked to traits responsible for environmental resilience, which will support the long-term sustainable management and exploitation of the species.

Chapter 3

Predictive biophysical models of bivalve larvae dispersal

This chapter has been adapted from the research paper published by the Scientific Journal 'Frontiers in Marine Science' as:

Corrochano-Fraile, A., Adams, T. P., Aleynik, D., Bekaert, M., and Carboni, S. (2022). Predictive biophysical models of bivalve larvae dispersal in Scotland. *Frontiers in Marine Science*, **9**:985748. [doi:10.3389/fmars.2022.985748]

I was involved in sourcing and co-developing the conceptual idea, carrying the particle tracking simulations, conducting all the analysis, and writing the manuscript

3.1 Abstract

In Scotland, bivalves are widely distributed. However, their larvae dispersion is still largely unknown and difficult to assess *in situ*. And, while *Mytilus* spp. dominate shellfish production, it is mostly dependent on natural spat recruitment from wild populations. Understanding the larval distribution pattern would safeguard natural resources while also ensuring sustainable farming practises. The feasibility of a model that simulates biophysical interactions between larval behaviour and ocean motions was investigated. We employed an unstructured tri-dimensional hydrodynamic model (finite volume coastal ocean model) to drive a particle tracking model,

where prediction of larval movement and dispersal at defined locations might aid in population monitoring and spat recruitment.

Our findings reveal a strong link between larval distribution and meteorological factors such as wind forces and currents velocity. The model, also, depicts a fast and considerable larval movement, resulting in a substantial mix of plankton and bivalve larvae, forming a large connection between the southern and northern regions of Scotland's West coast. This enables us to forecast the breeding grounds of any area of interest, potentially charting connectivity between cultivated and wild populations.

These results have significant implications for the dynamics of ecologically and economically important species, such as population growth and loss, harvesting and agricultural management in the context of climate change, and sustainable shellfish fisheries management. Furthermore, the observations on Scottish water flow suggest that tracking particles with similar behaviour to bivalve larvae, such as other pelagic larval stages of keystone species and potential pathogens such as sea lice, may have policy and farming implications, as well as disease control amid global warming issues.

3.2 Introduction

Bivalves first appeared in the middle Cambrian (over 500 million years ago) and predate the dinosaurs by about 300 million years (Woods, 1999). Bivalves are an extremely successful class of invertebrates found in aquatic environments all over the world. Bivalve aquaculture, namely oyster, clam, scallop, and mussel cultivation, appears to have a low environmental impact when compared to other aquaculture species (Yaghubi et al., 2022). The selection of sites for bivalve culture depends on components of a generic and site-specific nature (*i.e.*, hydrodynamic stability and the carrying capacity of the system), culture areas must meet water quality standard, and are subject to spatial regulation (Smaal, 2002). But as most of the bivalve aquaculture source their seeds from wild recruitment, connectivity between farmed and wild populations has a significant influence on production outputs, *i.e.*, with the emerging issues of climate change, invasive species, and connectivity between bodies of water

(*e.g.*, translocation of seed), vectors for harmful algal blooms and pathogens are increasing (Wijsman et al., 2019b). A lack of spawning or fluctuation in environmental conditions influencing larval dispersion or survival would result in production losses and food insecurity. As a result, other considerations for improving and protect culture conditions must also be addressed.

In bivalves, larval development occurs shortly after fertilisation and consists of two motile stages, the non-feeding trochophore and feeding veliger, as well as one partially motile stage, the pediveliger. The whole pelagic larval stage lasts three to four weeks, during which time the principal organs (foot, digestive gland, and gills) begin to develop (Gosling, 2003; Helm et al., 2004), and under certain conditions it can be extended to three months (Widdows, 1991). However, it is known in most marine benthic species that the pelagic larval stage is capable of much greater dispersal than juveniles and adults, making the fate of larvae a key determinant of marine population connectivity (Pineda et al., 2007; Cowen & Sponaugle, 2009). After three to four weeks, veliger larvae are fully developed pediveligers ready to settle; a reversible stage of bivalves' lifecycle that precedes metamorphosis (Helm et al., 2004). Dispersal of bivalve's larvae remains largely unresolved; Marine dispersal distances are notoriously difficult to directly measure (Pineda et al., 2007). Quantifying the magnitude and pattern of exchange between populations of marine organisms is hindered by the difficulty of tracking the trajectory and fate of their offspring (Shanks, 2009). The match between larval transport by currents and genetically inferred connectivity is often poor (Hellberg, 2009) and rapidly improving (Jahnke & Jonsson, 2022).

Advanced hydrodynamic models that can describe regional scale dispersal in complex coastline and topography are now available. Geometry based on triangular prism components has been incorporated in hydrodynamic models, allowing them to predict the values in grid cells (Willis, 2011). This accommodates for the topographical intricacy of complex coastlines, islands, and narrow bays. Developments of those irregular mesh models have improved the flow details in complex areas (Chen et al., 2006), boosting our capacity to deploy these models in complex areas such Scottish coastal waters (Adams et al., 2014; Aleynik et al., 2016; De Dominicis et al., 2018).

Because patterns of dispersal remain poorly understood for many marine species, it is highly important to develop and use proper tools to understand the ecological processes linked to dispersion and to inform conservation and management decisions (Weersing & Toonen, 2009). Thus, the need to incorporate biological and physical elements (Cowen & Sponaugle, 2009); biological in the sense of processes influencing offspring production, growth, development, and survival; physical in the sense of advection and diffusion properties of water circulation; and elements influencing interactions between certain larval traits (*e.g.*, vertical swimming behaviour) and physical properties of the environment that operate at various scales, *e.g.*, coastal topography, tidal forces, surface waves, turbulence (Gawarkiewicz et al., 2007; Clark et al., 2021).

The goal of this study was to employ an unstructured tri-dimensional hydrodynamic model to understand patterns of larval movement on the West coast of Scotland, quantify variability in connectivity between regions, and identify the most likely sources of larvae for key bivalve productions locations in dynamic places such as the West coast of Scotland.

3.3 Material and Methods

3.3.1 Study domain

The study domain encompasses the majority of Scotland's West coast, extending from the Isle of Man to 50 miles north of Cape Wrath and westward to the Outer Hebrides archipelago (Figure 3.1). It builds on previous research that used smaller domains in the same area (Adams et al., 2014, 2016; Aleynik et al., 2016) where most of the Scottish bivalves production is located. Complexity of the Scottish coastline and regional topography, exposed to high winds, strong tides and mild seasonal cycles at mid-latitude require unique modelling for accurate reproduction.

3.3.2 Biophysical model

The biophysical model was comprised of a hydrodynamic model, a particle tracking model, and a post-processing module to determine the source location of the bivalve larvae. The post-process of data has been made using MATLAB R.2019b. The count

of accumulated particles in each area and subsequent count of particles in each target location has been made with the *inpolygon* function. The data used (Table B.1) for the ANOVA test is the obtained after counting the accumulation of particles and the statistical test has been done using the *anovan* function. Finally, the representation of physical variables such as wind roses and current velocity has been done using the *WindRose* and the *cquiver* functions, respectively.

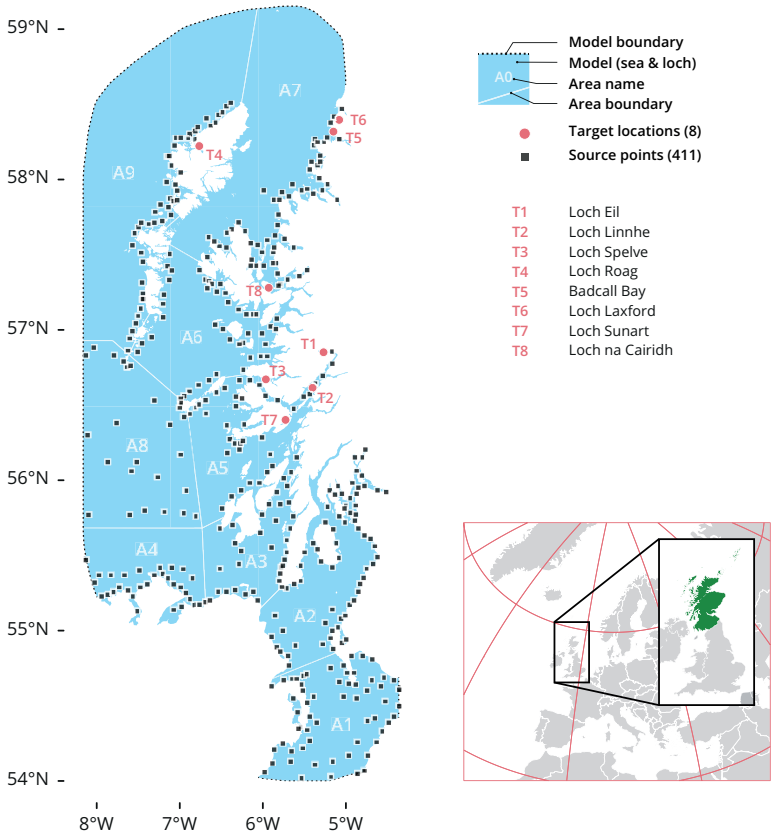

FIGURE 3.1: The Model's source and target locations. A total of 411 source points (black squares) were used and 8 target sites (red dots): T1 Loch Eil, T2 Loch Linnhe, T3 Loch Sunart, T4 Loch Roag, T5 Badcall Bay, T6 Loch Laxford, T7 Loch Spelve and T8 Loch na Cairidh. The model also spliced the region in 9 geographical area divisions: A1. Northern Irish Sea and Solway Firth; A2. North Channel and Firth of Clyde; A3. Sound of Jura; A4. Malin Head or Northern coast of Ireland; A5. Firth of Lorne (and southern part of Inner Hebrides Sea); A6. South Minch and Small Isles; A7. North Minch; A8. Atlantic and South Hebrides; and A9. West Outer Hebrides.

3.3.3 Hydrodynamic model

This study's hydrodynamic model was based on the Finite Volume Coastal Ocean Model (FVCOM; Chen et al., 2006). The WeStCOMS v1 model domain (Aleynik et al., 2016) was expanded in v2 and became operational in April 2019 for hindcast/forecast

use (Davidson et al., 2021). Triangular elements in WeStCOMS-FVCOM allowed for variation in element size and were capable of resolving the flow along the complex coastline and bathymetry in fjordic coastal environments such as the West coast of Scotland. The open lateral boundaries of the model were forced (nested) with the output of a high resolution (2 km regular grid) North-East Atlantic ROMS operational model (Dabrowski et al., 2016) supplied by the Marine Institute, Ireland. The layer depths were determined using the uneven terrain-following sigma layer proportions. Tides at the boundaries were calculated using the inverse barotropic tidal solution developed by Oregon State University (Egbert & Erofeeva, 2002). Fresh-water discharge estimates based on precipitation over 228 river catchment basins, as well as fluxes across the air-sea-surface interface, were derived from the regional implementation of Weather Research Forecasting (WRF v4; Skamarock & Klemp, 2008) and run at SAMS. Integration stability of 2D (external) and 3D (internal) momentum equations in mod-splitting hydrostatic FVCOM model is predetermined by the smallest horizontal length-scales (up to 80 m near-shore) and short external time-step 0.3 seconds. Wetting and drying scheme was activated, however, to prevent 'drying' all the shoreline nodes assigned fixed value -5 m, assuming that the width of littoral (intertidal zone) along the western Scottish coasts is usually shorter than the nearest model element side. WeStCOMS-FVCOM outputs contain one-hourly snapshots of 3D temperature, salinity, velocity and turbulence fields as well as its surface meteo-forcing 2D time-series. Initial WeStCOMS (v1) simulations cover the period between June 2013 and June 2019 and switched to extended domain (v2) since April 2019 to run operationally onward. Surface elevation, velocity, temperature, salinity, and turbulence intensity were among the model outputs. The hydrodynamic model's accuracy had been tested using multiple oceanic observations (Aleynik et al., 2016; Davidson et al., 2021). The International Hydrographic Office provided the sites data for tidal analyses and comparison. Temperature, salinity, and current data were obtained from conductivity, temperature and depth (CTD) transects and thermistor loggers (Inall et al., 2009), as well as an 18-year time series of currents and subsurface CTD readings (Fehling et al., 2006) and recently deployed several sea-gliders missions in south-western model segment. Comprehensive description of the model's skills validation against observational data near shore was given in (Aleynik et al., 2016) and recently published (Davidson et al., 2021).

The hydro-files containing weather data implemented for WeStCOMS-FVCOM can be found at https://thredds.sams.ac.uk/thredds/catalog/scoats-westcoms2/catalog.html (folders covering 2017 to 2021).

3.3.4 Particle tracking model

Particle tracking was conducted using the model of Adams et al. (2014, 2016). This was originally developed to predict dispersal of larvae and linked physical processes such as water movements with biological processes such as maturation and mortality. The particle tracking builds upon an established larvae simulation model, offering universality by simulating the larvae phase for benthic species. The movement of larvae incorporated advection due to local currents and horizontal diffusion equal to 0.1 m^2 s^{-1}. Particle depths below the water surface were fixed for the duration of each simulation (meaning trajectories were effectively 2D). Particle movement vectors were set to zero when they would have taken the particle onto land.

Maturation and mortality were omitted from the particle definition, however we made use of a 'settlement window' for the 'tidal release' simulations. Details relating to particle numbers, source sites and release schedule are given in the subsections 'Single day release simulations' and 'Tidal cycle release simulations'.

Velocities at particle locations were interpolated horizontally and vertically from WeStCOMS-FVCOM irregularly grid current output, and the model is integrated using a fourth-order Runge Kutta scheme.

The particle tracking code can be found at https://github.com/tomadams1982/WestLice (commit 9fbf1bb). The software used to run the particle tracking model was NetBeans v11.3i, an integrated development environment for Java.

3.3.5 Post process

The domain area (West coast of Scotland) was partitioned into 9 different geographical areas (Figure 3.1) for reporting analysis purposes (A1 to A9). Each geographical area had several source points from which the larvae were released during the simulations. Finally, within the domain area, 8 aquaculture sites with bivalve recruitment operations were chosen as target locations for larval settlement (T1 to T8).

Boundaries between A1 and A9 reflected island chains and other natural geographical features, and as such are consistent with the complicated coastlines and flow patterns of the studied area. The following polygons were used to fill the different geographical areas (Figure 3.1): A1, The Northern Irish Sea and the Solway Firth. A2, The North Channel and the Firth of Clyde. A3, The Sound of Jura. A4, Malin Head to Ireland's Northern Coast. A5, The Firth of Lorne (including the southern half of the Inner Hebrides Sea). A6, The South Minch and Small Isles. A7, The North Minch. A8, The Atlantic and South Hebrides. And A9, The West Outer Hebrides. The division of the areas help us to identify the release-source coordinates for the particle simulation.

Settlement sites ('target locations') included: Loch Eil (T1; Latitude 56.85°N, Longitude 5.27°W), Loch Linnhe (T2; 56.61°N, 5.40°W), Loch Sunart (T4; 56.67°N, 5.96°W), Loch Roag (T4; 58.22°N, 6.77°W), Badcall Bay (T5; 58.31°N, 5.15°W), Loch Laxford (T6; 58.40°N, 5.08°W), Loch Spelve (T7; 56.40°N, 5.73°W) and Loch na Cairidh (T8; 57.28°N, 5.93°W).

3.3.6 Single day release simulations

Single-day release simulations were run to evaluate the particles' circulation through the mesh from emitter point ('source points'). We assumed that all bivalves from each source point spawned at the same time, representing a mass-spawning events, which are common in the spring, rather than trickle spawning events, which are more common later in the season (Fernández et al., 2015).

A range of particle tracking simulations were carried out in order to assess variability in predicted dispersal patterns. Like most benthic organisms, bivalves spend their early life stage within the water column, which lasts from three to four weeks (Bayne, 1965; Pineda et al., 2007). We adjusted the simulation start time (1st March, 1st April, 1st May), the release year (2017-2021), dispersal period (30 or 45 days; Helm et al., 2004; Pineda et al., 2007; Demmer et al., 2022), and the particle depth (2 m, 6 m or 10 m below sea level) following literature that indicates mussel pediveliger larvae are found primarily in near-surface waters (Baker & Mann, 2008; Demmer et al., 2022). The total number of released particles was 8,877,600 (20 particles $\times$ 411 number of sites $\times$ 24 first hours $\times$ 3 different months $\times$ 5 years $\times$ 3 depths).

On average, the ocean currents around Scotland flow in a clockwise direction along the coast (De Dominicis et al., 2018). Since the water flow within the study region has a dominant northward movement and considering our target locations, we limited the possible source points to areas A1 to A6. During the first 24 hours of each simulation, twenty particles were released per hour from each source points in each region, at a given depth, month, and year. This initial test enabled us to validate the model and to a broad identification of the larvae trajectory and proximity to the selected target locations.

3.3.7 Tidal cycle release simulations

Tidal cycle release simulations were run to determine the source points seeding receiver zones ('target locations'). After a broad scale identification of the larvae trajectory, we pursued a more realistic scenario, releasing particles continuously for the first 14 days to cover a spring/neap cycle. The following simulations were run from 1st April to 17th June 2021. Ten particles were released each hour for the first 14 days from the source points located in A1 to A9 at 6 m below the sea level (total number of release particles was 6,904,800). For each particle, the coordinates for each release point (source point), settlement site (target location) and arrival time in hours were recorded.

We established a 20 km $\times$ 20 km square centred on each target site to aggregate particles ending up in proximity to each target location and its surroundings, counting particles moving within these zones during the last 7 days of our period of interest (27th April to 3rd May 2021). Each particle had a unique ID and coordinate, making it possible to locate their initial release coordinate (source point). Particle dispersal accumulation has been converted into density values by dividing particle counts within mesh elements by the element area (particles/m^3).

3.4 Results

3.4.1 Particle trajectory

To set up the model, we explored different methods, including diffusion and non-diffusion, as well as releasing particles forwards and backwards. Diffusion is a physical process where particles experience random motion due to the movement of water molecules or other factors. In addition to diffusion, we also tested a non-diffusion approach. Non-diffusion models can be useful when the behaviour of particles is influenced by specific physical or biological factors, which for the limited information on mussel larvae dispersion this last approach was deficient. We decided to add diffusion into our model to simulate the random motion of particles in the marine environment. By including diffusion, we were able to capture the complex transport processes that occur in the environment and obtain more realistic results.

We also used a reversal seeding technique in our model, where we released particles backwards from a final location to locate the starting point. The idea behind this method was to identify the starting point of the particles by finding the location where the particles accumulate the most. However, we found that this method did not always result in particles returning to the original starting point, which limited its usefulness for our model.

More specifically, particles were released in a forwards direction from a start point (mussel farms) and then particles were released from an end point; after thirty days running the model forwards, the selection of the location with higher density of particles (Figure 3.2) was used as a start point for the backwards release.

Since there are some random elements and interactions with the coastline that interfere with particle trajectory, the particles weren't going back to the mussel farms when doing the backwards run (Figure 3.2). For example, in Figure 3.3D the backwards simulation for Loch Eil shows how the last days corresponding to the trajectories in yellow are settling in areas different as where Loch Eil is located. From here on out in this research, the release of particles was solely accounted in a forwards period of time, considering the random elements and analysing other elements affecting their movement.

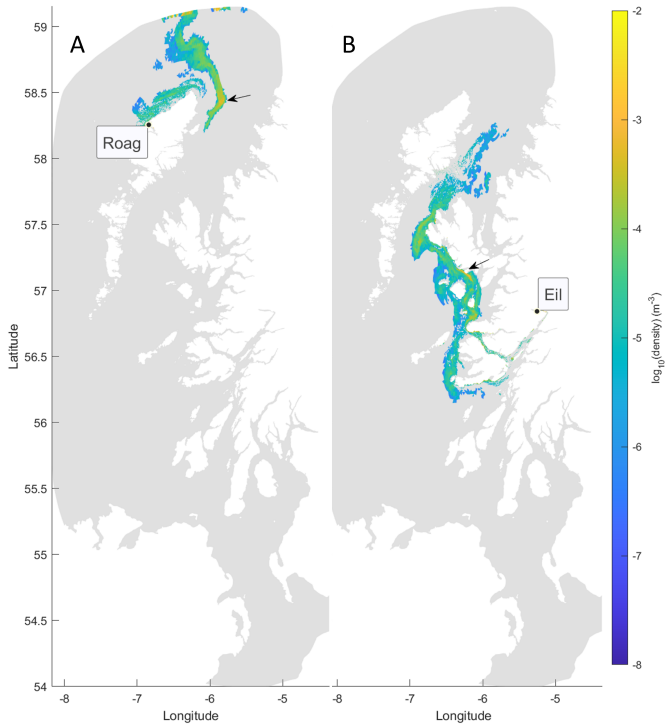

FIGURE 3.2: Example of density accumulation in the mesh to select the start point for the backwards release for the locations in Loch Roag (**A**) and Loch Eil (**B**). Colour blue corresponds to low density areas, and colour orange/yellow to high density areas. The arrow indicates the start point for the backwards simulation.

The use of diffusion and a forward release allowed to capture the random motion of particles due to physical processes, while also identifying areas where particles tend to accumulate. By releasing particles forwards from a starting point and simulating their trajectories using diffusion, we were able to obtain more realistic results that better captured the behaviour of particles in the marine environment.

3.4.2 Physical variables affecting the simulation of particles

Using April 2021 as an example, the wind rose (Figure 3.4A) shows that winds from the North-West sector occur 10% of the time, reaching maximum speeds of 9.6 m/s to 11.0 m/s from those directions. The same is happening from the south, but in this case, winds reach speeds between 7.3 m/s and 9.6 m/s. There is a general wind flow occurring from the North-West. In April 2020 (Figure B.1), the wind rose shows that winds from the east sector occur 9% of the time, reaching maximum speeds of 3.0 m/s to 6.0 m/s. However, the greatest speed is coming from the south sector just 2% of the time, achieving maximum speeds of 12.0 m/s to 15.0 m/s.

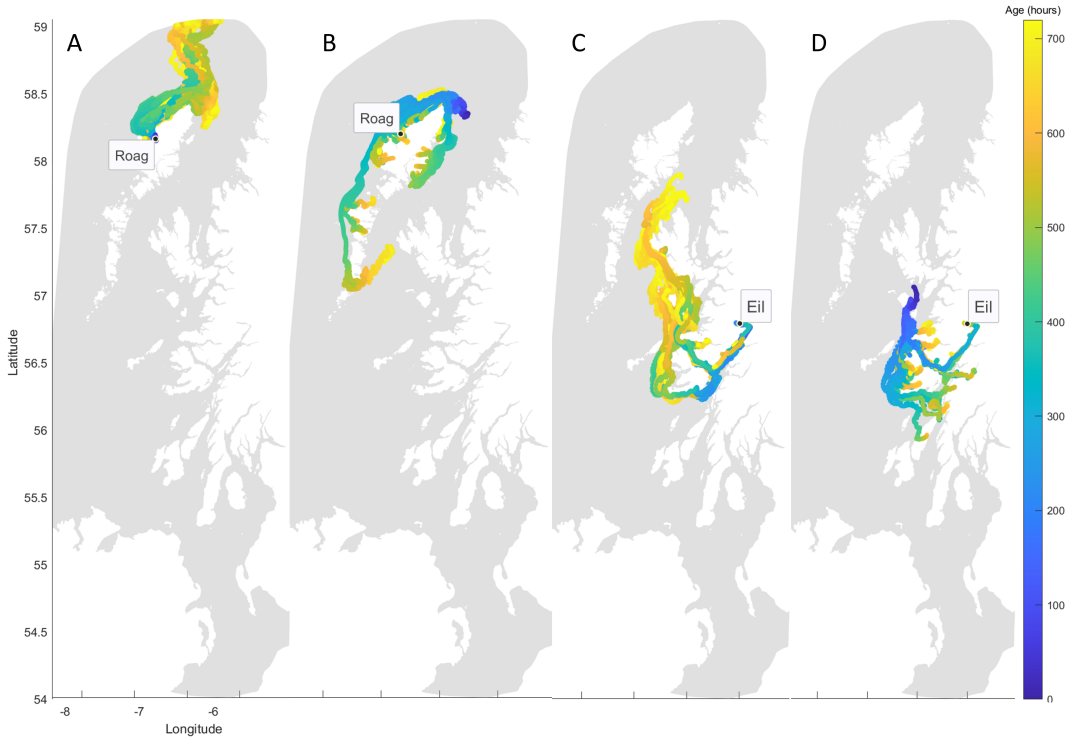

FIGURE 3.3: Example of particles released forwards (**A** & **C**) and backwards (**B** & **D**) in the mesh for the locations in Loch Roag and Loch Eil. Colour blue corresponds to first days particles and colour yellow to last days particles.

The wind is a major driving force for the currents, and the model allows for the estimation of average current speeds throughout Scotland's West coast. Sea-surface currents (0 to 10 m below sea level) suggest a mainly northward flow with velocities of 0.3 m/s to 0.4 m/s dominating in the open areas of the basin in March and April 2021 (Figure 3.4B and Figure B.2). The average speed for complex areas and narrow channels goes up to 0.65 m/s. May 2021 however remains calmer, reaching only maximum speeds in the south channel. On the other hand, the intense current speeds in March 2020, are not present in April 2020, where the sea surface currents show a calmer picture for that year and month; only in the north, between Western Isles and the Highlands, the sea surface currents reach maximum speeds of 0.5 m/s to 0.6 m/s, and May 2020 remains similar as in year 2021. The combination of wind rose data with the sea surface currents, provides an understanding for the observed variability in determined areas of the West coast of Scotland.

3.4.3 Variability analysis

We carried out a regression analysis to identify significant differences within groups in our data set. Being years, release areas, target areas, and months the nominal variables (factors); depth continuous variable (co-variate), and accumulation of particles (in each settlement area) the continuous dependent variable (Table 3.1). Results suggest that there is not a noticeable change in overall structure between the depths, years, and months. The only significant differences are between source areas (settlement sites) and target areas (P-value < 0.001).

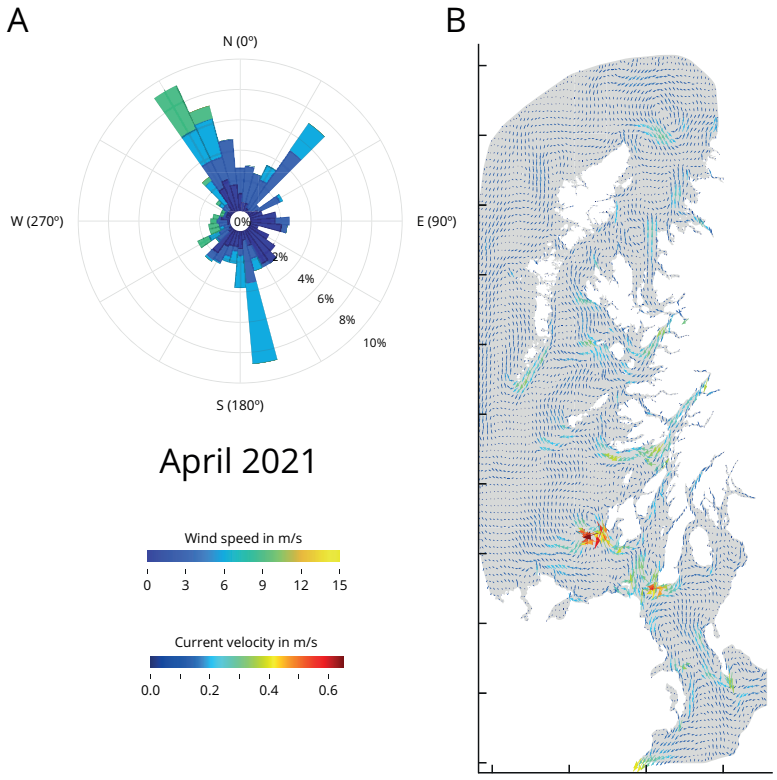

FIGURE 3.4: Wind rose and surface current velocity for April 2021. (**A**) Wind rose. The wind direction determined by where it blows to. The colour scale represents wind speed (m/s), whereas the inner circle represents frequency. The data are from WRF v4. (**B**) Average current velocity between 0 m to 10 m below sea level (m/s). Detailed Wind roses and for average surface current velocity for March, April, and May 2021 and 2020 are available Figures B.1 and B.2 respectively.

TABLE 3.1: Analysis of variance. Sq Square; d.f. degree of freedom.

	Sum sq.	**d.f.**	**Mean sq.**	**F**	**P-value**
Depth	$1.80\ 10^{7}$	1	$1.80\ 10^{7}$	0.00	0.97
Year	$1.27\ 10^{10}$	4	$3.17\ 10^{9}$	0.25	0.91
Month	$1.75\ 10^{9}$	2	$8.74\ 10^{8}$	0.07	0.93
Source	$8.09\ 10^{11}$	5	$1.62\ 10^{11}$	12.80	0.00
Target	$2.65\ 10^{12}$	8	$3.31\ 10^{11}$	26.17	0.00
Error	$3.04\ 10^{13}$	2406	$1.26\ 10^{10}$		
Total	$3.39\ 10^{13}$	2426			

3.4.4 Single day releases

When combined with the climatic pattern, the particle tracking model yielded large-scale patterns of larval distribution that were consistent with expectations. We compared a 30-day simulation period to a 45-day simulation period, with the first days of the simulations being 1st March, 1st April, and 1st May.

When analysing the variability between years 2017-2021 (Figure 3.5) and between depths in one specific year, 2021 (Figure 3.6), climatic conditions need to be considered to understand the annual differences in the particle dispersion. The identification of physical parameters influencing the larvae trajectory between years has been done, producing wind roses and current surface velocity plots for the years 2020-2021 (Figure 3.4 and Figures B.1 & B.2). In general, annual variability is higher since the weather fluctuations are affecting the overall integrated transport of our fixed-depth particles from one year to another due to the fact that model momentum equations include the wind-driven, the density-driven (baroclinic) and tidal (barotropic) components. When looking at the variability between depths, the retention of our fixed-depth particles remains similar at 2 m, 6 m, and 10 m depth for year 2021.

During the 30-day simulation (Figure 3.5A and Table B.2), the release of particles from the source points in A1 in years 2021, 2020 and 2019 show higher retention of particles in that same destination area (A1), but more dispersal towards A2 in years 2018 and 2017. With 81.7%, 72.3% and 63% of particles accumulated in A1 for years 2021, 2020, and 2019. The release of particles from source points in A2 show higher retention of particles in all the years. With 15.2% and 15.5% of particles accumulated in A1, and 45.1%, and 72.7% in A2 for years 2019 and 2018. From the release of particles from source points in A3, A4, and A5 the dispersion of particles across the rest of the areas

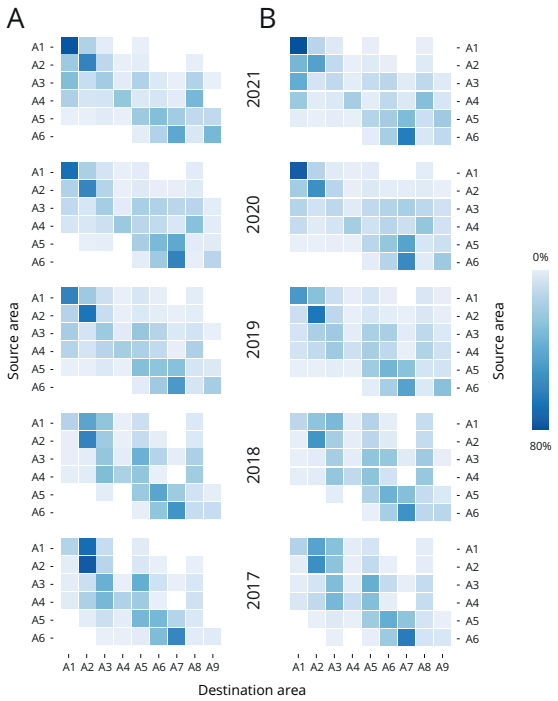

FIGURE 3.5: Heatmaps of the particle connectivity between the source (Y axis) and destination area (X axis). The particle accumulations from Single day release setup, where the accumulation for each source regions have been averaged for March, April, and May at 2 m, 6 m, and 10 m depth for the years 2021 to 2017; (**A**) 30-day release and (**B**) 45-day release with beginning points on 1st March, 1st April, and 1st May 2021. Dark blue boxes corresponding to areas with higher particle accumulation, and light blue to white boxes corresponding to areas with lower particle accumulation in percent. Detailed means and standard deviations are provided in Table B.1.

is more predominant in all the years. Finally, the release of particles from the source points in A6 show dispersion of the particles mainly between the areas in A6 (16.1%, 22.1%, 22.1%, 27.1%. And 32.4% for years 2021, 2020, 2019, 2018, and 2017, respectively), and A7 (43.8%, 62.9%, 50.6%, 53.6%, and 61.1%, for years 2021, 2020, 2019, 2018, and 2017, respectively). During the 45-day simulation period (Figure 3.5B and Table B.3), the release of particles from the source points in A1 to A6 show a similar pattern as the one described for the 30-day simulation period. But since the particles have been circulating for 45 days, slightly more dispersion has been observed. However, the release of particles from the release points in A1 and A2 keep reflecting higher particle retention values. Details of particle dispersal through 2021 for March, April, and May, at 2 m, 6 m and 10 m depth are available in Figure B.3.

3.4.5 Tidal cycle releases

Through the tidal cycle simulations, we have identified possible source points of larvae for every aquaculture site. After analysing 5 years of particle dispersal within three different depths, and since our variability analysis (Figure 3.6) between depths showed not significant changes in the movement of particles for 2021, for our purpose (finding the possible source points of the larvae ending up in our target locations) we decided to focus on April 2021 at 6 m depth. Our results represent a week window, from the 27th of April to the 3rd of May (*i.e.*, four weeks of pediveliger larvae swimming through the water body plus three extra days assuming larvae does not settle exactly the 30th of April). The results are shown in Figure 3.7 and visually, there is a clear particle dispersal separation between particles released from source points located in southern areas (A1 to A4) compared to the particles released from source points located in the central-northern areas (A5 to A9).

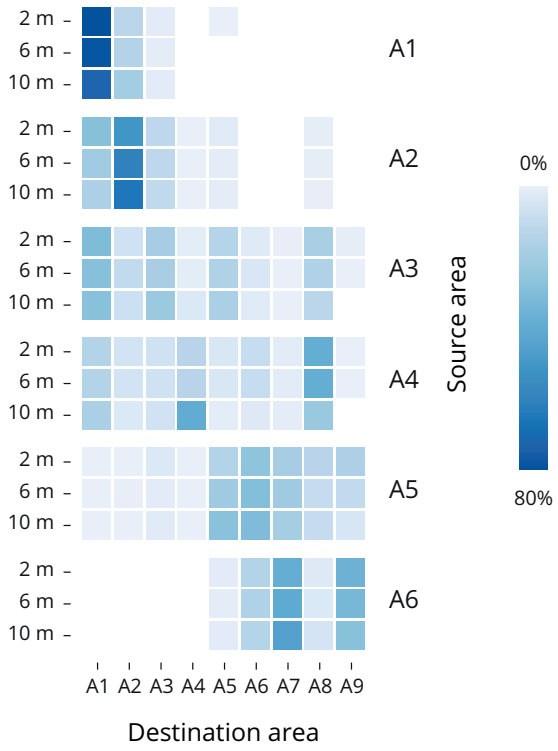

FIGURE 3.6: Heatmaps showing particle connectivity between the source (Y axis) and destination (X axis) areas for the Single day set up. For the years 2021, the accumulation for each source region was averaged for March, April, and May at each depth. Dark blue boxes represent area with high particle accumulation, whereas light blue to white boxes represent areas with lower particle accumulation in percent. Table B.3 has detailed means and standard deviations.

Higher densities values are observed for the releases occurred in source areas corresponding to A5, A6, A7, A8 and A9, with density values of 2.39, 2.70, 3.04, 2.06, and 2.34 particles/m^3, respectively. Whilst the lowest mean density values are coming from the source areas corresponding to A1, A2, A3, and A4, with density values of 0.02, 0.12, 0.38 and 0.10 particles/m^3, respectively.

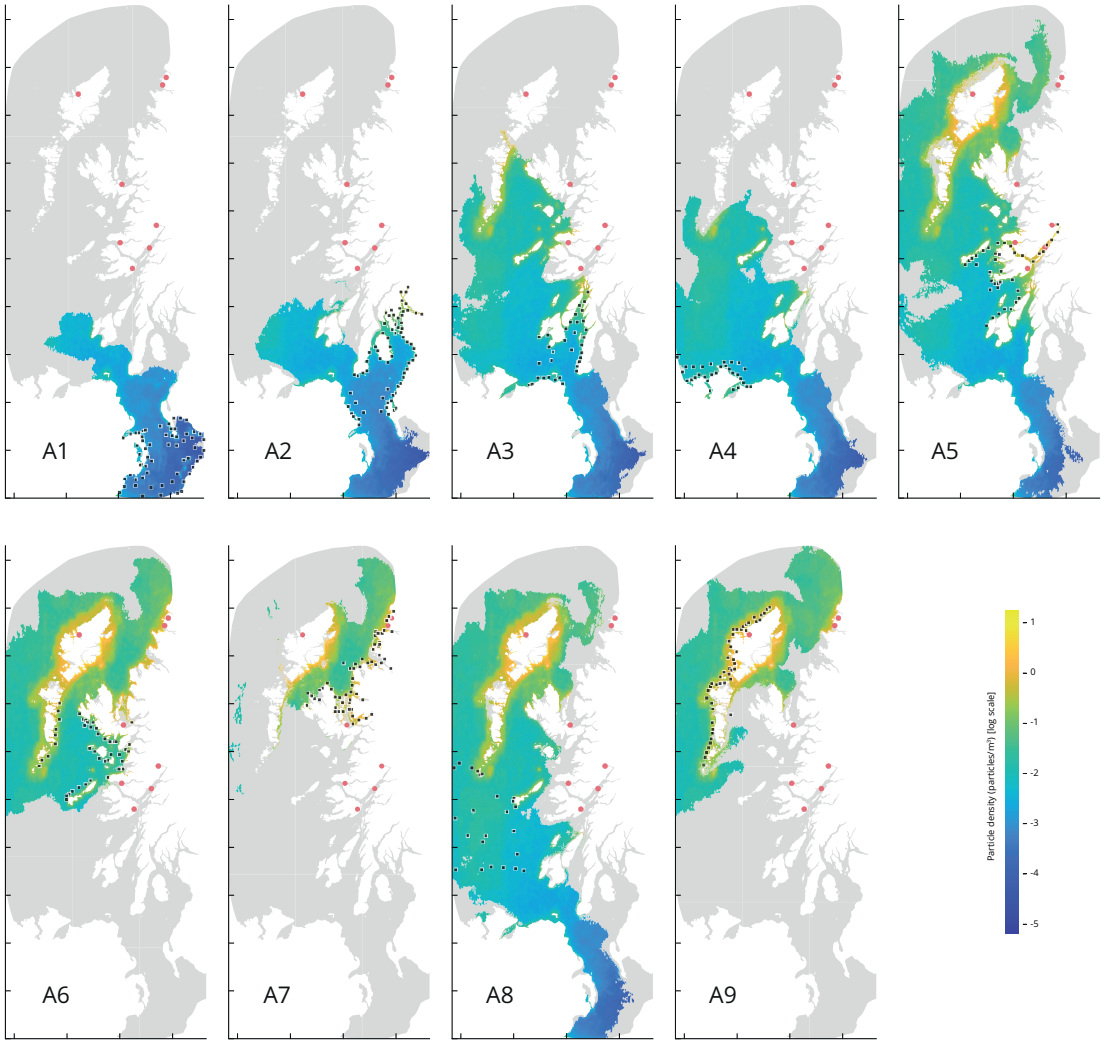

FIGURE 3.7: Particle density (particles/m^3) from particles released from each source area. the map depicts the period from 27th April to 3rd May 2021, at a depth of 6 m. Tidal cycle releases simulation set up (continuous release for the first 14 days). The red dots represent the eight target sites. Each source points from the area A1 through A9 are represented by a black square.

Releases from A1 and A2 (Figure 3.7) show less particle dispersal compared to the rest of the source areas. Although the results for A3 and A4 show more particle dispersal, the maximum density values do not coincide with our target locations. The

higher density accumulation sourced from A5 coincides with four of the target locations: Loch Eil, Loch Linnhe, Loch Spelve and Loch Sunart. The higher density accumulation sourced from A6 coincides with the target locations corresponding to Loch na Cairidh, Loch Roag, Badcall Bay, and Loch Laxford. The higher density accumulation sourced from A7 coincides with the target locations corresponding to Badcall Bay, Loch Laxford, and Loch na Cairidh. The higher density accumulation source from A8 coincides with Loch Roag. And lastly, the higher density accumulation source from A9 coincides with Loch Roag, Badcall Bay, and Loch Laxford.

3.4.6 Source location identification

The particles released from each geographical source area on the last day of our period of interest (tidal cycle releases simulation set up, continuous particle release for the first 14 days), 27th April to 3rd May 2021 at 6 m depth, were quantified in each target location, and found within the 20 km defined area (Table 3.2). The source point closest to the target location is just 6 km away, and the source point being further away is 170 km far from the target location. For example, all the particles observed in Loch Eil originate from the same place, A5-1 (6 km to the target location). The results suggest (Figure B.4) that none of the particles emitted from the sources areas A1 to A4 seed any of the target locations, however particles released from A5 appear to seed the majority of our target locations (5/8 target locations). Furthermore, T4 (Loch Roag) is the target location that receives particles from the majority of the source areas (A5, A6, A8, and A9).

Based on the source point locations, we found three types of larval recruitment dynamics: self-recruiting, self-recruiting with external recruitment influence, and low-self-recruitment with high external recruitment influence (Table 3.2). Figure 3.8 depicts an example of each case: self-recruiting site, A5-1 is the only source points for the target location in T1 (Figure 3.8B); self-recruitment with external recruitment influence, A5-1 and A5-2 are the source points for T2 (and Figure 3.8B) and A5-1, A5-2, and A5-3 are the source points for T7. Finally, Figure 3.8C depicts an example of low-self-recruitment with high external recruitment influence for T4 where source points are in A6, A8, and A9.

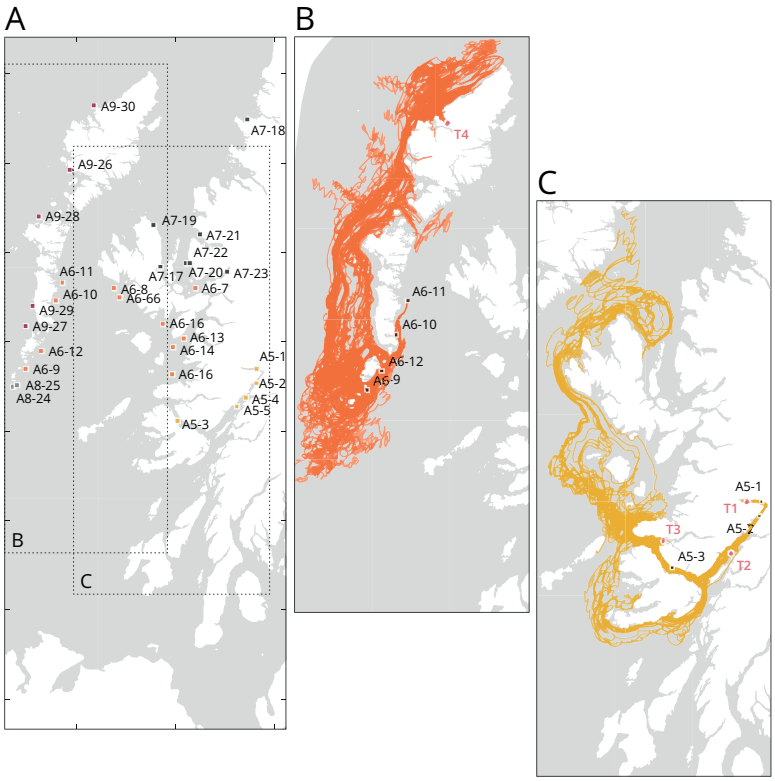

FIGURE 3.8: Details of the source locations significatively contributing to the particle accumulations at each target locations. (**A**) Representation of the source points; (**B**) Cases of self-recruitment, Loch Eil (T1), and self-recruiting and influence of external recruitment for Loch Linnhe (T2) and Loch Sunart (T3); (**C**) Example of external recruiting only: Loch Roag (T4). To avoid visual clutter, only the source points A6-9 to A6-12 are illustrated. Distinct colours represent different geographical areas. Details are available in Table B.4.

3.5 Discussion

Inter-annual variability in larval dispersal and connectivity of bivalve populations in the West coast of Scotland has been investigated through the parameterisation of a particle tracking model exposed to climatic variables. Our models represent two plausible scenarios: the first (single day releases), confirmed the optimum larval movement through the mesh representing the West coast of Scotland; and the second (tidal cycle releases) allowed for the identification of distinct source points for each target location.

As mentioned before, in most marine benthic species the pelagic larval stage is capable of much greater dispersal than juveniles and adults, making the fate of larvae a key determinant of marine population connectivity (Pineda et al., 2007; Cowen & Sponaugle, 2009). Previous studies have shown the importance of circulation patterns on interannual variability of larval recruitment and dispersal (McQuaid & Phillips, 2000;

Largier, 2003), and interactions between larval vertical migration and stratification have been shown to be an important driver of dispersal (Raby et al., 1994). Moreover, the water column in the West coast of Scotland remains well mixed since winter until May, which implies that stratification might not play a role in earlier spring on larval dispersal, as also shown in Figure 3.6; this is in addition supported by Demmer et al. (2022) for the study of mussel dispersion in the northern Irish Sea.

In our model study, virtual larvae distributed at 6 m depth dispersed away from their native bed to a target location by a maximum of 170 km after four weeks, suggesting both local connectivity and general connectivity within the West coast of Scotland. Our results show that there is no significant difference in dispersal patterns between the three depths tested by the model. Indeed, assuming that larvae are distributed throughout the water column, performing only a limited vertical migration and in the absence of stratification, then their dispersal would be primarily controlled by tidal currents (Raby et al., 1994; McQuaid & Phillips, 2000; Demmer et al., 2022). Furthermore, assuming that bivalve larvae are mainly distributed in the near-surface waters, their dispersal would additionally be influenced by wind-driven currents. Currents can be divided into tidal (barotropic) and non-tidal (residual) components. We assumed that residual currents in the higher layers are primarily generated by a combination of two factors: wind stress and pressure variations caused by density gradients. Seasonal thermal vertical stratification is highest in the summer, whereas saline stratification is associated with nearshore sources of freshwater discharge in sea-lochs and along coasts. Larvae released from the Northern Irish Sea and Solway firth (A1); North Channel and Firth of Clyde (A2); Sound of Jura (A3) and Malin Head (A4) present more particles retention and less accumulation after four weeks, meaning that the southern part of the West coast of Scotland is facing a barrier for the larvae to travel northwards. This can be observed in Figure 3.4B with dynamic velocity currents facing maximum values in the south channel. On the other hand, the larvae released from Firth of Lorne (A5); South Minch and Small Isles (A6); North Minch (A7); Atlantic and South Hebrides (A8); and West Outer Hebrides (A9) all present a higher dispersion of the particles as well as greater particle accumulation after four

weeks. This initial observation holds significant implications for aquaculture practices, as it suggests the potential existence of distinct genetic structures among mussel larvae from the southern (A1 to A4) and northern (A5 to A9) regions of the West coast of Scotland. Such genetic differentiation could give rise to variations in mussel traits and adaptations between these regions. It is noteworthy that unless a physical barrier, such as ocean currents, hinders genetic exchange between the southern areas and northern England, there is a possibility of genetic flow. This insight carries substantial value for both mussel aquaculture practices and conservation efforts, as it offers vital information for making informed decisions regarding seed selection, implementing breeding programs, and devising effective management strategies.

Looking closely to the source points seeding in T1, T2, T3 and T7, the particles are coming from the same area (A5), being T1 an example of self-recruitment, and T2, T3 (in a lesser extent; Table 3.2) and T7 receiving seeds from T1. The source points seeding T5 and T6, have their origin in the south and north of Skye peninsula; at the same time, T8 location (north of the Skye peninsula) is in the same area where multiple source points are seeding T5 and T6, suggesting these three target locations might be connected. Finally, the remainder target sites located in A9 (T4) is an example of robust external seed recruitment and to a lesser extent self-recruitment from nearby areas. From A5 to A9 a rapid dispersion of the particles simulating the bivalve larvae is predominant. The fast flow on Scottish waters carrying particles with similar behaviour, e.g., other larvae and sea lice, entail implications for policy and farming practices, disease control and global warming issues.

While our developed particle simulation model effectively tracks particle movements along Scotland's West coast, it's crucial to acknowledge its significant limitations. These limitations must be carefully addressed in future research. The selection of appropriate methods for simulating particle behaviour is pivotal in creating a precise and dependable tracking model. Considering specific physical and biological factors impacting particle behaviour, along with the unique characteristics of the studied system, is imperative during the method selection process, provided such information is available. This approach ensures the development of more resilient and accurate particle tracking models, thereby enabling the exploration of diverse oceanic physical

and biological processes. These limitations encompass several critical aspects: Vertical Migration, our particle tracking model does not currently incorporate vertical migration patterns of particles through the various layers of the FVCOM mesh. The omission is primarily due to a dearth of available data that would enable us to accurately model this phenomenon. Vertical migration is a complex ecological process that often depends on various factors such as temperature, light, and food availability, and its exclusion can affect the comprehensiveness of our simulations. Additionally, our model lacks considerations for biological parameters such as mortality. This absence restricts our ability to account for mortality rates, a crucial factor in understanding and predicting particle dynamics accurately. The absence of reliable data on mortality rates for bivalves during their larval phase is a significant gap. Mortality among larvae can result from multitude of factors, including predation, diseases, and environmental conditions such as temperature and salinity, all of which can impact biological aspects. The lack of information on these parameters hampers our ability to accurately portray them in simulations.

Notably, elevated temperatures, can exert diverse effects on bivalve larvae. These effects encompass changes in biological parameters like larval duration, increased susceptibility to diseases, heightened larval stress leading to mortality, and potential alterations in the timing and intensity of spawning events (Jones et al., 2014; Seuront et al., 2019). These intricacies emphasize the necessity of considering broader environmental factors when interpreting our model's outcomes. They also underscore the urgency of comprehensive data collection and further research to mitigate these limitations in future studies.

The consequences of climate change on the marine environment are predicted to include shifts in sea surface salinity, temperature, and ocean chemistry, notably ocean acidification, along with alterations in precipitation patterns and more frequent heat waves (Mechler et al., 2020). Specifically, higher sea surface temperatures, particularly during summer months, might pose challenges for species with lower thermal tolerance (Steeves et al., 2018). Fluctuating sea surface salinities can negatively impact shell growth (Riisgård et al., 2014), and when combined with increased temperature or hypercapnia (elevated CO2), it can elevate mortality rates and reduce shell hardness

and resistance (Dickinson et al., 2013; Rybovich et al., 2016).

Moreover, it is crucial to consider the intricate interplay between environmental factors and hybridisation interactions. These interactions can significantly impact the biology and larval survival of mussel populations. Additionally, low gene flow poses a threat to the population's ability to adapt to changing environmental conditions, thereby diminishing the quality of spat produced. For instance, Bierne et al. (2003a) conducted a study on the genetic structure of the mosaic hybrid zone formed by *M. edulis* and *M. galloprovincialis* along the Atlantic coast of Europe. Their analysis, utilizing three length-polymorphic PCR loci as neutral and diagnostic markers in 32 samples, revealed dynamic changes in the frequency of alleles typical of *M. galloprovincialis* in different areas. Notably, within the hybrid zone, distinct genetic patches resembling *M. edulis* and *M. galloprovincialis* exhibited differentiated allele frequencies compared to external reference populations. Furthermore, these patches displayed partial introgression of alleles from the other species, indicating the presence of strong genetic barriers in transition zones. Maintaining gene flow is vital for preserving genetic diversity and ensuring the long-term survival of mussel populations. Therefore, understanding and managing factors affecting gene flow, such as hydrodynamics and environmental changes like temperature and salinity, are essential for the sustainability and success of mussel farming practices (Stuckas et al., 2009).

Moreover, climate change is expected to affect phytoplankton communities (Käse & Geuer, 2018). Shifts in species abundance and composition may subsequently impact nutrient uptake in marine bivalves, thereby limiting their physiological and biological processes. Furthermore, the effects of climate change may lead to decreased immune responses in bivalves (Mackenzie et al., 2014) and alter host-pathogen interactions, potentially increasing susceptibility to diseases (Asplund et al., 2014).

Including parameters such as salinity and temperature in larvae simulation models becomes crucial for predicting the impact of climate change on marine ecosystems. These parameters offer insights into how changes in temperature and salinity affect the growth, survival, and overall physiological responses of larvae. By incorporating these variables into simulation models, scientists can forecast how potential alterations in temperature and salinity levels, as predicted by climate change models,

might influence the development, distribution, and survival of marine organisms like bivalves. This predictive understanding is vital for assessing and managing the potential impacts of climate change on marine biodiversity and ecosystems.

In this study, we show how a biophysical model can help in the understanding of system dynamics and the identification of breeding grounds and settlement areas, which can be utilised to rationalise bivalve farming activities. This approach could help the understanding of bivalve populations in a dynamic maritime environment such as the West coast of Scotland. Furthermore, our observations on the Scottish waters can help to develop new particle simulations with similar characteristics, e.g., other pelagic larval stages of keystone species and potential pathogens like sea lice. With the corresponding policy, farming, and disease control implications in the face of global warming concerns.

TABLE 3.2: Identification of the major source points contributor for each target locations.

Target location	Source point (ID)	Received particles	Source point Latitude	Source point Longitude	Geodesic distance
Loch Eil T1	A5-1	625	56.85°N	5.17°W	6.0 km
Loch Linnhe T2	A5-1	127	56.85°N	5.17°W	29.9 km
	A5-2	108	56.77°N	5.17°W	22.5 km
Loch Sunart T3	A5-2	352	56.77°N	5.17°W	49.8 km
	A5-1	234	56.85°N	5.17°W	52.4 km
	A5-3	112	56.56°N	5.97°W	12.0 km
Loch Spelve T7	A5-4	83	56.69°N	5.28°W	45.5 km
	A5-5	61	56.64°N	5.37°W	34.6 km
	A5-2	35	56.77°N	5.17°W	53.5 km
	A5-1	22	56.85°N	5.17°W	60.6 km
Badcall Bay T5	A6-6	202	57.25°N	6.56°W	145.0 km
	A6-7	186	57.30°N	5.79°W	119.2 km
	A7-17	154	57.42°N	6.15°W	115.8 km
	A7-18	77	58.24°N	5.27°W	10.8 km
	A6-8	75	57.30°N	6.61°W	142.3 km
	A7-20	41	57.44°N	5.88°W	106.6 km
	A7-19	32	57.65°N	6.22°W	97.0 km
Loch Laxford T6	A6-8	160	57.30°N	6.61°W	151.7 km
	A7-18	47	58.24°N	5.27°W	20.2 km
	A7-20	46	57.44°N	5.88°W	116.2 km
	A6-6	40	57.25°N	6.56°W	154.3 km
	A7-21	40	57.60°N	5.74°W	96.5 km
	A6-7	23	57.30°N	5.79°W	128.7 km
	A7-22	19	57.44°N	5.85°W	115.3 km
Loch Roag T4	A8-25	504	56.76°N	7.60°W	169.7 km
	A6-9	336	56.85°N	7.51°W	158.7 km
	A6-10	299	57.23°N	7.20°W	113.1 km
	A9-26	294	57.96°N	7.06°W	33.7 km
	A9-27	275	57.09°N	7.51°W	133.1 km
	A6-11	168	57.33°N	7.14°W	101.4 km
	A6-12	168	56.95°N	7.35°W	145.4 km
	A8-24	168	56.75°N	7.64°W	171.6 km
	A9-28	168	57.70°N	7.38°W	68.0 km
	A9-29	152	57.20°N	7.44°W	120.2 km
	A9-30	136	58.32°N	6.82°W	11.4 km
Loch na Cairidh T8	A6-15	52	56.82°N	6.03°W	51.2 km
	A6-13	43	57.02°N	5.91°W	28.7 km
	A6-14	20	56.97°N	6.02°W	34.6 km
	A6-16	19	57.10°N	6.12°W	22.8 km
	A7-23	11	57.39°N	5.47°W	30.5 km

Chapter 4

Estimating the connectivity and settlement capacities

This chapter has been adapted from the research paper published by the Scientific Journal 'Communications Biology' as:

Corrochano-Fraile, A., Carboni, S., Green, D.M. et al. Estimating blue mussel *(Mytilus edulis)* connectivity and settlement capacity in mid-latitude fjord regions. *Commun Biol*, **7**, 166 (2024). [doi:10.1038/s42003-023-05498-3]

I was involved in sourcing and preparing the biological materials, DNA extraction and library preparation, conceptualisation, methodology, formal analysis, and writing the manuscript.

4.1 Abstract

The mussel industry faces challenges such as low and inconsistent levels of larvae settlement and poor-quality spat, leading to variable production. However, mussel farming remains a vital sustainable and environmentally responsible method for producing protein, fostering ecological responsibility in the aquaculture sector.

We investigated the population connectivity and larval dispersion of blue mussels (*Mytilus edulis*) in Scottish waters, as a case study, using a multidisciplinary approach

that combined genetic data and particle modelling. This research allowed us to develop a thorough understanding of blue mussel population dynamics in mid-latitude fjord regions, infer gene-flow patterns, and estimate population divergence.

Our findings reveal a primary south-to-north particle transport direction and the presence of five genetic clusters. We discovered a significant and continuous genetic material exchange among populations within the study area, with our biophysical model's outcomes aligning with our genetic observations. Additionally, our model revealed a robust connection between the southwest coast and the rest of the west coast. This study will guide the preservation of mussel farming regions, ensuring sustainable populations that contribute to marine ecosystem health and resilience.

4.2 Introduction

Over the past two decades, the blue mussel industry in mid-latitude fjord environments has faced significant challenges marked by fluctuations in production (Munro et al., 2022) caused by low levels of spat settlement and poor quality spat. Certain regions along the western continental coasts, like Scotland, have also encountered production losses due to issues such as fragile shells and low-quality meat (Carboni et al., 2021; Gubbins et al., 2012; Seuront et al., 2019). However, despite these obstacles, the industry has shown remarkable growth with the tonnage of mussels produced surging by an impressive 52% from 5,661 tonnes in 2020 to a record breaking 8,590 tonnes in 2021 (Munro et al., 2022). This remarkable increase in production not only signifies the highest level of mussel production ever recorded in Scotland but also underscores the expanding nature of the mussel farming sector in the region. Mussel farming in Scotland is primarily conducted through an off-bottom approach, where ropes are utilised to cultivate the mussels (McKindsey et al., 2011b). The main producers are strategically situated along the west coast of Scotland, near the shore, in dynamic areas. The recruitment process for mussels involves both natural spawning from wild populations and seeding from existing mussel farms (Cockrell et al., 2015; Stirling & brahim Okumus, 1995).

Addressing fluctuations in mussel production is crucial because mussels are a vital

component of aquatic ecosystems, serving as keystone species and providing numerous ecosystem services such as water filtration, habitat creation and coastal defence, carbon sequestration, and nutrient cycling (Olivier et al., 2020; Rönnbäck et al., 2007). Moreover, blue mussel aquaculture is both an eco-friendly protein source and a crucial component of the seafood industry in Western Europe and Scotland, with the added benefit of being used as sustainable feed for farmed fish, thereby promoting environmental responsibility within the aquaculture sector (Munro et al., 2022; Regan et al., 2021). Mussels are a key source of nutrition, rich in protein, omega-3 fatty acids, and essential vitamins and minerals, making them a healthy food choice (Carboni et al., 2019).

Marine population connectivity plays a crucial role in distribution, recruitment, and stability of marine species, affecting the fishery industry and aquatic wildlife (Cowen & Sponaugle, 2009). The lifecycle of many marine organisms incorporates a pelagic larval stage where the connectivity only occurs via dispersal of larvae in the planktonic stages, as adults are sessile. The pelagic stage is relevant to understanding population dispersal patterns, as well as the timing and magnitude of recruitment (Swearer et al., 2019). Mussels have a complex life history, making it important to understand their dispersal and settlement patterns to manage and conserve wild and farmed populations. Nevertheless, barriers to gene flow in the marine pelagic environment are often not clearly identified (Cowen et al., 2006) and must be studied in relation to hydrology and hydrography (Bradbury et al., 2008; Kaiser et al., 2021).

Larval development of the blue mussels begins shortly after fertilisation, and consists of two motile stages, the non-feeding trochophore and feeding veliger, then one partially motile stage, the pediveliger. The whole pelagic larval stage lasts three to four weeks, depending on temperature and food availability, during which time the principal organs (foot, digestive gland, and gills) begin to develop (Gosling, 2003; Helm et al., 2004). After three to four weeks, veliger larvae are fully developed pediveligers, ready to settle and metamorphose: an irreversible stage of bivalves' lifecycle. As the pelagic larval stage alone is capable of greater dispersal, the fate of larvae is a key determinant of population connectivity (Cowen & Sponaugle, 2009; Pineda et al., 2007).

Management of bivalve populations for sustainable food production goes hand in

hand with adequate conservation of spawning populations in the wild. In the case of mussels, this means gaining a clear understanding of how larval connectivity, habitat locations and environmental conditions allow the population to maintain a stable connected network. In turn, this requires an understanding of both the genetic structure of populations and the biological and physical processes which generate this (Callaway, 2022; Xuereb et al., 2009).

Biophysical models are a useful tool to study larvae dispersal and connectivity in marine environments. These models replicate both the physical and biological mechanisms that shape larval transport within the ocean, encompassing vital factors such as ocean currents, turbulence, and larval behaviour (Lavelle & Mohn, 2010). Comprising three fundamental constituents, such models consist of a hydrodynamic module for the emulation of ocean currents and transport phenomena, a particle tracking module that reproduces larval movement and responsiveness to environmental cues, and a biological component designed to replicate processes such as growth, mortality, and settlement (Adams et al., 2016, 2014). Furthermore, biophysical models can provide insights into the processes driving population structure. However, in isolation they do not describe the realised connectivity, and require verification by empirical methods. Depending on the species of interest, a range of approaches are available for this, from otolith microchemistry (Hogan et al., 2014) to a wide range of genetic methods.

Genetic methods are a powerful tool for understanding population structure, particularly in species with high levels of genetic diversity and complex life histories (Hedgecock et al., 2007). These methods can help researchers identify distinct populations, estimate gene flow between them, and assess the degree of genetic diversity within populations. Population genetic studies have advanced dramatically over the last twenty years, enabling much more refined distinction between local populations, e.g. sea lice population genetic studies (Jacobs et al., 2018; Todd et al., 2004). Additionally, population genetic studies can be used in combination with other approaches, such as biophysical models, to gain more comprehensive understanding of population structure and connectivity in marine environments (Harrison et al., 2017). For example, genetic methods can be used to validate biophysical models predictions of connectivity between populations or to identify sources of recruits to populations that are

experiencing declines (Pastor et al., 2021).

In this study, we employed a multidisciplinary approach to investigate patterns of population connectivity and larval dispersion of blue mussels (*Mytilus edulis*) in Scottish waters. Genetic data enabled us to estimate population divergence, genetic connection, and infer gene-flow patterns; particle modelling allowed prediction of larval migration throughout the pelagic phase. Combining these two methods generated a comprehensive understanding of the population dynamics of blue mussels in Scottish waters and the factors influencing dispersal and population connectivity. The results of this research contribute to the conservation and management of this economically important species by providing insights into its population structure and dynamics.

4.3 Materials and Methods

4.3.1 Study samples

The study was undertaken on the western coast of Scotland, where the primary mussel producers strategically position themselves in dynamic shoreline regions characterised by oceanographic conditions that promote a continuously changing environment. We collected a total of 520 blue mussels from 13 different locations along this coastal stretch, averaging approximately 40 samples per site. These samples were obtained from natural mussel beds located along the coast in Portree, Applecross Bay, Loch Torridon, Loch na Cairidih, and Bo Sligachan, as well as from mussel farms in Loch Eil, Loch Linnhe, Bàgh a Tuath, Loch Sunart, Loch Spelve, Loch Roag, Loch Laxford, and Badcall Bay (Figure 4.1A, Table 4.1). We then took tissue samples, including gills from the adult mussels and all body tissues from the spats, and stored them in 99% ethanol at a temperature of -20 °C.

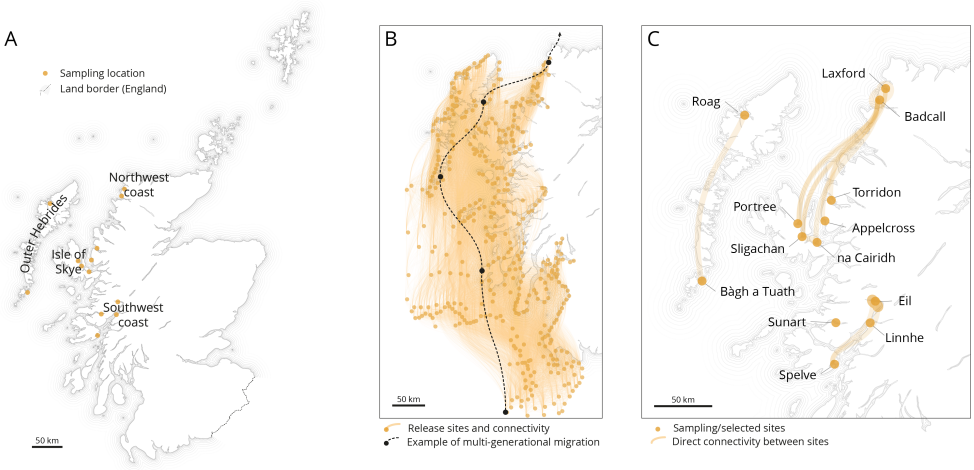

FIGURE 4.1: Hydrodynamic modelling and particle tracking. (**A**) General study location (west coast of Scotland), sampling sites are located in orange. (**B**). Connectivity network between all the 440 sites, in 2021 (light orange tracks). All south-west sites are along of Northern-Irish coastline (not depicted for simplicity). An example of multi-generational larval dispersal is illustrated by the black dotted line, a black circle identifies each settlement site. (**C**) Connectivity network between sampling sites only (over a simulated period, April 5th to May 8th, 2021).

TABLE 4.1: Blue mussel sampling information

Sampling site	Date	Latitude	Longitude	Stages	Remarks
Loch Eil	Oct 2020	5.21933°W	56.85365°N	Adults & Spats	Farm site
Loch Linnhe	Mar 2021	5.14962°W	56.80510°N	Adults & Spats	Farm site
Bàgh a Tuath	May 2022	7.38155°W	56.99230°N	Adults	Oyster farm facilities
Portree Bay	May 2022	6.14700°W	57.42000°N	Adults	Coastal sampling
Applecross Bay	May 2022	5.84500°W	57.44000°N	Adults	Coastal sampling
Loch Torridon	May 2022	5.74300°W	57.60000°N	Adults	Coastal sampling
Loch Sunart	Oct 2020	5.62250°W	56.67897°N	Adults & Spats	Farmed from Loch Eil
Loch Spelve	Sep 2021	5.72428°W	56.40262°N	Adults & Spats	Farm site
Loch Roag	Jun 2021	6.84620°W	58.20171°N	Adults & Spats	Farm site
Badcall Bay	Mar 2021	5.15477°W	58.31498°N	Adults & Spats	Decommissioned fish farm
Loch Laxford	Mar 2021	5.05577°W	58.39468°N	Adults & Spats	Farm site
Loch na Cairidh	Sep 2021	5.93147°W	57.27781°N	Spats	Coastal sampling
Bo Sligachan	May 2022	6.09293°W	57.32606°N	Adults	Coastal sampling

4.3.2 Particle modelling

The particle-tracking method developed in our previous study (Chapter 3) was applied. It used 3D current fields, derived from the Finite Volume Coastal Ocean Model (FVCOM) hydrodynamic model (Chen et al., 2006). The latest regional implementation of coupled atmospheric and ocean WeStCOMS v2 model system became operational in April 2019 for producing flow hindcast and forecast (Davidson et al., 2021; Aleynik et al., 2022). Particle tracking was conducted using the BioTracker model (Adams et al., 2014, 2016). This predicts the dispersal of particles as a result of the interaction of physical processes such as tidally and wind-driven water movements, with biological processes such as maturation and mortality. In this study, maturation and mortality were omitted from the particle definition, but instead a settlement window for calculation of successful dispersal was applied for the simulations. The particle-tracking code can be found at
https://github.com/tomadams1982/BioTracker (commit 9fbf1bb).

For the simulations, particles were released from 440 randomly selected distinct locations, and from the specific sampling sites from where mussels were collected at 6 meters below the sea level as per results in **Chapter 3** showing no significant differences when releasing the particles from three different depths. This approach was chosen to ensure a comprehensive and unbiased representation of particle dispersal patterns in the region. By employing random release sites, we aimed to minimise potential biases that could arise from selecting specific locations based on preconceived notions or prior assumption. It is important to note that certain source locations in real life might not contribute to larvae release at all. The 440 selected locations ranged from the Isle of Man in the south to Cape Wrath in the north, and westward to the Outer Hebrides archipelago. Twenty particles were released per hour at each location. Particle tracking simulations lasted 34 days, of which particles were released for the first 14 days every hour, and accumulations were counted for the last 7 days within a 2 km radius of each sampling point to estimate settlement, particles did not stop moving allowing us the use of a seven-day window to account for settlement/accumulation of particles. To account for the asynchronous aspect of the spawning between locations, the simulations were run every week, between 1st February and 2nd/3rd May

for 2020 and 2021 (years of the larval dispersal of the samples), respectively, and wind fields collected for the same period. Connectivity matrices ((Chapter 3; Watson et al., 2010; Adams et al., 2012) obtained from the particle tracking model simulations were analysed to identify source and sink locations.

4.3.3 Sample preparation

Using an adapted version of a previously published protocol (Brown et al., 2016), the genotyping data of 520 individuals was obtained through double digest restriction-site associated DNA (ddRAD) sequencing. Briefly, genomic DNA was extracted using a salt-extraction method. All samples were tested for *M. edulis* species identity against the Wilson et al. (2018) SNP panel, before further processing. The DNA of each sample was digested with restriction enzymes *Sbf*I and *Sph*I, which recognise the sequence CCTGCA^GG and GCATG^C, respectively. After adding a unique index to each sample, 550-650 bp DNA fragments were amplified and sequenced on the Illumina NextSeq platform, resulting in 150-nt paired-end reads (Edinburgh Clinical Research Facility, UK).

4.3.4 Genotyping and Variant calling

Using fastp v0.20.1 (Chen et al., 2018), we filtered the raw reads for quality (QC $\geq$ 25), length (150 nt), the absence of primers/adaptors, and complexity (entropy over 15). We then used BWA v0.7.17 (Li & Durbin, 2009) to map the clean reads to the *M. edulis* reference genome (MEDL1; GCA_905397895.1; Chapter 2). The resulting SAM files were converted into BAM files and sorted using SAMtools v1.16.1 (Danecek et al., 2021). We used *gstacks* from Stacks v2.62 (Rochette et al., 2019) to identify SNPs, and called all bi-allelic SNPs that were common to at least 50% of the individuals using *populations* (Stacks v2.62). We filtered these SNPs using PLINK v2.00a3.7LM, keeping only those with a minor allele frequency over 0.05 and not deviating from the expected Hardy-Weinberg equilibrium (P-value of χ^2 test $\geq 10^{-8}$). The threshold for the P-value cut-off was determined empirically, by examining the spread of P-values from the Hardy-Weinberg test in the data and selecting a threshold under which there are a greater number of variants than expected by chance (with small data sets, this is

typically around P = 10^{-8}). Finally, we used Beagle v5.4-22Jul22.46e (Browning et al., 2021) with the GT parameter to infer missing data.

4.3.5 Connectivity and population structure

Dimension reduction and visualisation was carried out using a t-distributed stochastic neighbour embedding (t-SNE) analysis (van der Maaten & Hinton, 2008), as implemented in the Rtsne v0.16 package (Krijthe, 2015). Additionally, we used FastSTRUCTURE v1.0 (Raj et al., 2014), with the logistic prior admixture model, to evaluate the population structure and admixture. The optimal number of populations, or K, was determined by maximising the marginal likelihood. To examine the amount of genetic differentiation among sampling localities and years, pairwise F_{st} (Weir & Cockerham, 1984) with 1000 bootstrap replicates and P-values were calculated between sites using the HIERFSTAT v0.5-11 package (Goudet, 2005). Community and network analysis were done using the function *cluster_walktrap* (Pons & Latapy, 2005) from the igraph v1.3.4 package (Csardi & Nepusz, 2006). Two different clustering methods were used to analyse mussel populations: the t-SNE clustering method (genetic data) and the community and network clustering method (particle tracking simulations). The comparison of the clusters generated by the two methods allowed for a deeper understanding of the relationships between the mussel populations.

4.3.6 Statistics analysis and visualisation

All statistical analysis and visualisation were performed using R v4.2.2 (R Core Team, 2022), and map visualisation was achieved using the Database of Global Administrative Areas (GADM) v4.1. All scripts and workflows used at available at https://github.com/pseudogene/Corrochano-Fraile_et_al_2023.

4.4 Results

4.4.1 Spatial structure

Particle modelling using the WeStCOMS v2 hydrodynamic model indicated a predominant directional transport of particles from south to north, following major coastal currents along the mainland (Chapter 3). This was observed through the release of particles at 440 sites along the north Irish and western Scottish coastlines (including sites with mussel farming activities and wild mussel populations, and randomly selected sites located homogenously along the west coast). There are strong connections northward between sites (Figures 4.1B & 4.1C, and Table 4.1).

This movement of particles is consistent with the estimated patterns of larval dispersal across multiple generations (Chapter 3). The particles, which originate from the southern limit of the model/region, move northward before settling and require several spring-neap tidal cycles to reach the northern limit of the model, with an average of three dispersal events between "stepping stones" being necessary. Figure 4.1B (black dotted line) illustrates an example where five such dispersal events were required. The extensive network of connectivity between sites is a result of the strong currents and surface winds in the region, creating a rapidly moving environment (Figure 4.1B). The connectivity on Figure 4.1B (and supplementary Data S1) shows Eil, Linnhe, and Sunart as self-recruitment locations; Eil and Linnhe located in proximity within each other are receiving particles from the same source locations; Eil and S196 (source location near Eil). Sunart's only source of particles is the source location in Sunart. The rest of the sampling locations are receiving particles from more than 5 other source locations.

In contrast, short-term connectivity (single year/"stepping stone") is extensive (large number of particles) but limited in distance, with only sites in the vicinity connected (Figure 4.1C). In fact, each site receives a large number of particles from multiple sources and distributes particles to various locations in turn (Figures 4.1B). There are a few notable examples of this trend: the Outer Hebrides, where Bàgh a Tuath (off the Isle of Barra) only connects to Loch Roag; the sites off the Isle of Skye export particles only to sites on the northern west coast; and the cluster of sites on the southern west

coast. Additionally, Loch Spelve, Loch Eil and Loch Linnhe are net donors, with Loch Eil being particularly connected to numerous sites despite its inland location (receiving almost no particles but being a significant particle source).

4.4.2 Genetic distances and population structure

We collected 520 blue mussels from 13 locations along the west coast of Scotland (Table 4.1) and used ddRAD sequencing to genotype them (EBI Project accession PRJEB52177). A total of 970,858,642 raw paired-end reads were sequences. After cleaning, 713,556,146 (73.5%) were mapped to the *M. edulis* genome. We identified 284,984 distinct RAD-loci and narrowed them down to 552 informative SNP markers for further analysis (Supplementary Data S2).

A t-SNE analysis (Figure 4.2A) revealed the presence of five genetic clusters, each of which corresponded broadly to a geographic region: (1) the northwest coast/Outer Hebrides, (2) the southwest part of the coast, (3) the Isle of Skye, and (4) Loch Spelve. This population structure was further supported by membership assignment probability (Figure 4.2B), which suggested gene flow between these regions and clarify the complex northern part of the west coast by slitting it in two broad clusters: (1a) the north part of the west coast (Loch Roag and Loch Laxford) and (1b) the central part of the west coast (and Badcall Bay). The maximum likelihood model for this data (with K = 5 populations) separated the southern individuals into a unique cluster, specifically isolating the samples from Loch Spelve.

The genetic distances (F_{st} values) acting as genetic differentiation indices, calculated to further explore the relationships between populations. F_{st} values calculated on the 552 informative markers (Figure 4.2C) between all pairs of populations were statistically significant, except for three higher-latitude locations (Loch Roag in the Outer Hebrides, Badcall Bay, and Loch Laxford on the northwest coast) and two locations in the southern west coast (Loch Eil and Loch Sunart). Notably, the highest Fst values were observed in adult samples collected from Loch Spelve when compared to the other locations: Loch Spelve appears to be genetically more isolated. These findings suggest a strong and ongoing exchange of genetic material between populations from year to year, shaping the evolution of these populations from one year to another. It

is worth noting that when considering all 284,984 loci, the F_{st} value is substantially lower at 0.01.

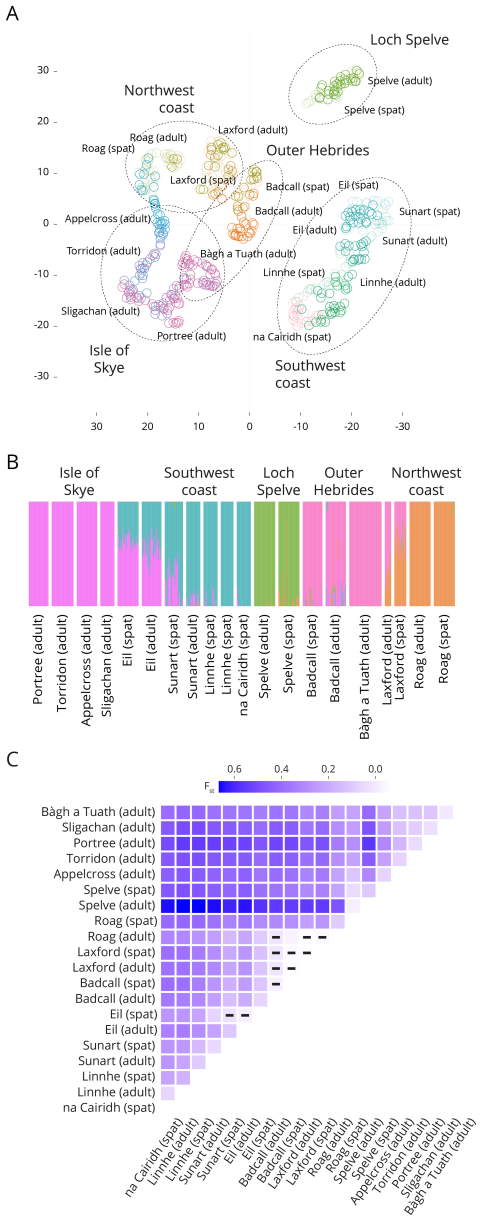

FIGURE 4.2: Blue mussel genetic analysis. (**A**) -SNE. Individuals broadly grouped geographically: Northern part of the west coast, southern part of the west coast, the Outer Hebrides, The Isle of Skye area, and Loch Spelve. The colour representations signify distinct locations and cohorts. Notably, the northwest coast, Outer Hebrides, and Isle of Skye form three distinct clusters. However, there are instances where specific cohorts share similarities with other clusters, such as Laxford-Badcall, Bacall-Bagh a Tuath, and Applecross-Roag. These clusters are defined using 552 informative SNP markers . (**B**) Genetic clustering analysis, each colour represents a different genetic cluster (K = 5 populations) of potential populations. (**C**) Genetic distance between each population and time points. The F_{st} values are reported by the colour shades. Non-significant distances, where the P value exceeds 0.05, are denoted by a black dash (–).

4.4.3 Model comparison with genetic data

From the biophysical model, we located the source of the mussel populations at the 13 mussel sampling sites along the west coast of Scotland. By examining the connectivity matrix used for Figure 4.1B (Supplementary Data S1), we were able to identify a total of 229 locations out of 440 as the sources for these mussel populations (April 5th to May 8th, 2021; Figure 4.3). The results align with our genetic findings, revealing a strong connection between the upper southern coast and the rest of the west coast. Mussel locations such as Badcall Bay, Bàgh a Tuath, Loch Roag and Loch Laxford receive larvae from 119, 98, 93 and 42 different source locations, respectively (Figure 4.3A-D).

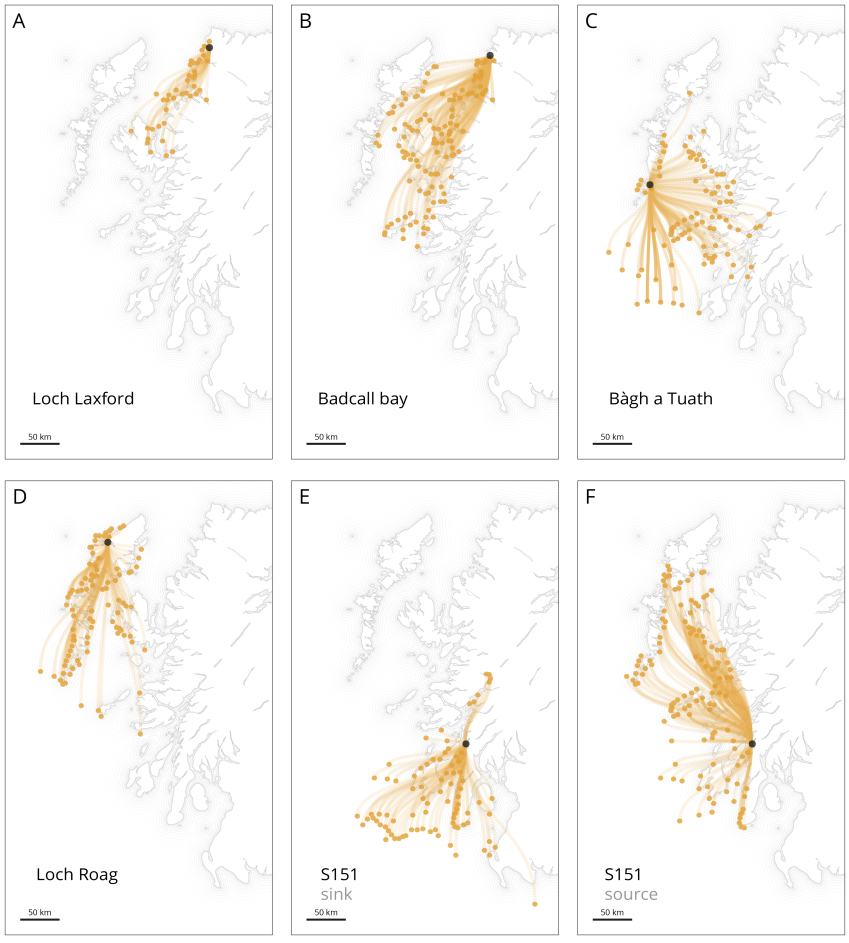

FIGURE 4.3: Larvae sources and sinks. Locations acting as source for the several mussel locations of interest. (**A**-**F**) Locations acting as source for the several mussel locations of interest. In total 229 locations out of 440 are sending samples to the mussel farms of interest. S151, acting as a sink (**E**) and source (**F**) for samples in higher latitudes.

To further understand the dispersion patterns of these mussels, from the 229 locations previously identified, we selected a "stepping stone" source location in the southern west coast. As shown in Figure 4.3, the source location S151 (southwest coast) receives larvae from 87 different locations and in turn, disperse larvae to 127 other locations (Figure 4.3F), including the location of Bàgh a Tuath (Outer Hebrides). This behaviour is further supported by our population structure and genetic results, which identifies southern mussel farms as a major primary source for the rest of the locations. Specifically, we observe that the mussel population at Spelve receives particles primarily from its own location and just nine other source locations, with Loch Eil and Linnhe being the most substantial contributors. Our genetic results concur with this pattern, as Loch Spelve exhibits higher levels of genetic differentiation (Figure 4.2C).

Accounting for the asynchronous nature of the spawning events occurring across a range of locations, we conducted simulations for 34-days starting every week between February and June of both 2020 and 2021 (Figure 4.4, Figure 4.5 and Figure 4.6). Figure 4.4 displays the cumulative networks for these respective years. The clusters, denoted by coloured nodes and links, signify densely connected sub-graphs formed through random walks. Figure 4.5 and Figure 4.6 illustrate simulations spanning 34 days from February to May in 2020 (Figure 4.5) and 2021 (Figure 4.6). In these figures, orange lines indicate observed connectivity between locations for each simulation, while the dark grey lines represent connectivity aligning with results derived from the admixture analysis. This allowed us to explore the possible connection between the population genetic structure and the particle migration. Our analysis revealed sampling locations grouped in two main clusters (southwest coast with Outer Hebrides, and Isle of Skye with northwest coast). Simulations from both 2020 and 2021 demonstrated similar temporal trajectories for the particles, indicating a connection between genetic population structure and particle movement.

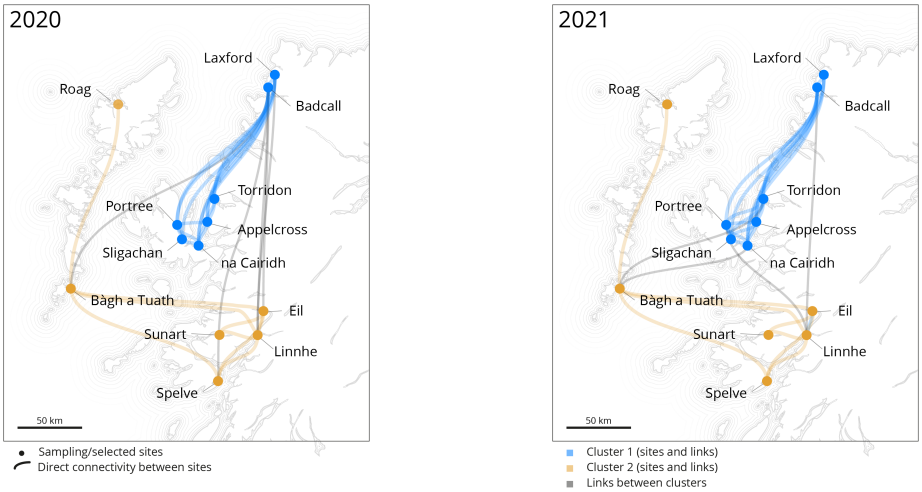

FIGURE 4.4: Cumulative networks for 2020 and 2021. Considering the asynchronous nature of the spawning events occurring across a range of locations, we conducted simulations for 34-days starting every week between February and June of both 2020 and 2021. The cumulative results are reported. The clusters (coloured nodes and links) are the densely connected sub-graphs via random walks.

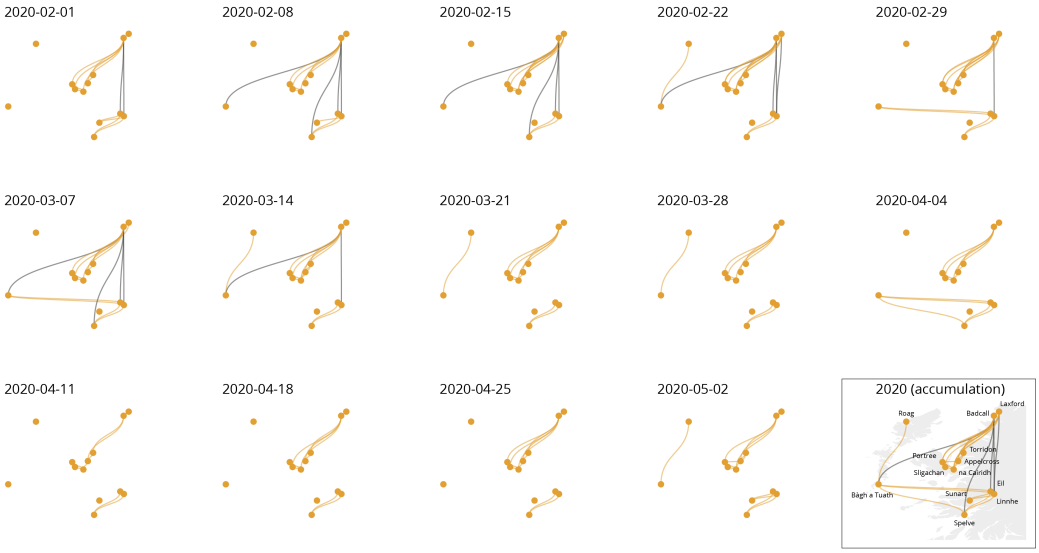

FIGURE 4.5: All 34-days simulations between February and May 2020. The start date of each simulation is reported. The spat accumulations of the last 7 days were used, and their origin reported. The orange lines represent the modelled connectivity between locations for each simulation, while the dark grey lines illustrate the observed connectivity that corresponds with the results obtained from the admixture analysis.

There is one notable disagreement between the genetic data and the WeStCOMS v2 hydrodynamic model transport in regard to the Sligachan location: The genetic data indicates a strong connection with the southern west coast cluster, whereas the model

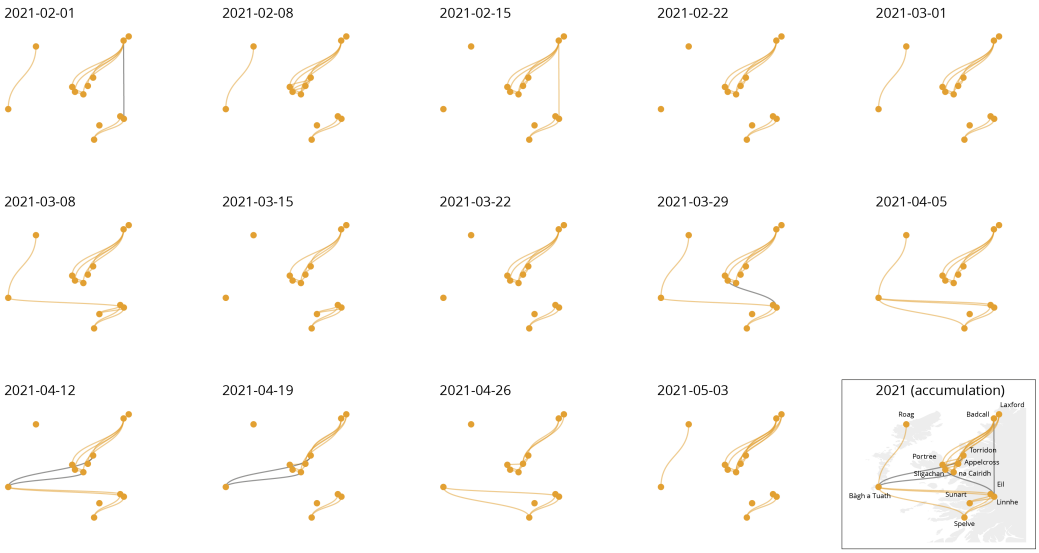

FIGURE 4.6: All 34-days simulations between February and May 2021. The start date of each simulation is reported. The spat accumulations of the last 7 days were used, and their origin reported. The orange lines represent the modelled connectivity between locations for each simulation, while the dark grey lines illustrate the observed connectivity that corresponds with the results obtained from the admixture analysis.

does not show a direct link between the two locations, restricted by very shallow region under the Skye Bridge with periodic exposure of seabed under low tides.

4.4.4 Model predictions

The particle accumulation of larvae (particles/m^3) predicted by the model (Figure 4.7) predicts those areas with the likely highest density of blue mussel larvae for both 2020 and 2021, assuming that all modelled sites have equal larval output. Simulations of the 440 release sites (Figure 4.1B), highlight three main areas of particle accumulation: the northern section of the Outer Hebrides; the northern mainland (from Skye to the northern limit of the model); and the southern west coast area, specifically near Loch Eil.

Although Figure 4.7 predicts higher accumulation in these three areas, genetic analysis reveals that locations on the north and central west coast are genetically more diverse and influenced by other sampling locations, and, to a lesser extent, the locations in the Isle of Skye area (Figure 4.2). The east side of the Outer Hebrides shows a particularly high accumulation of particles, however, we do not have genetic information from this area.

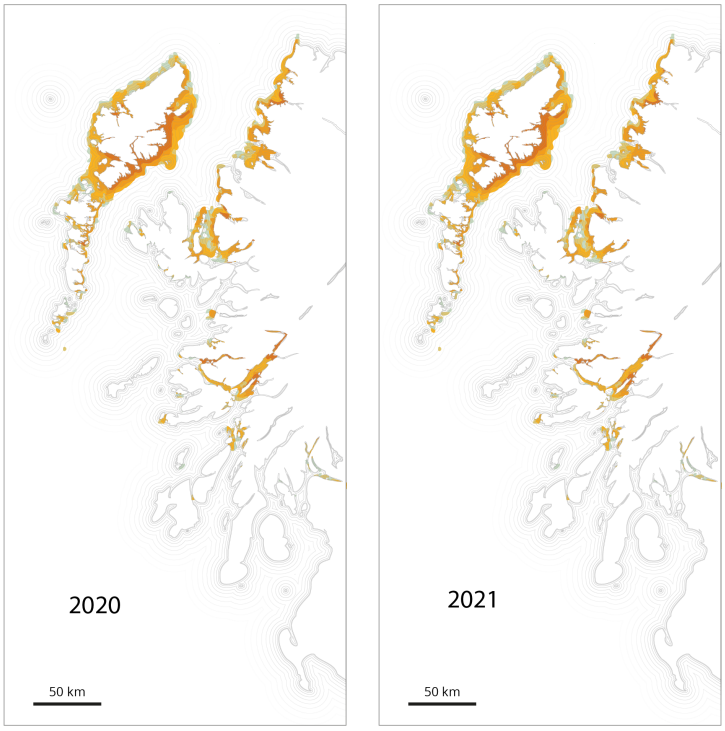

FIGURE 4.7: Predicted areas of accumulation of blue mussel larvae, based on 2020 and 2021 simulation and hydrodynamic data.

4.5 Discussion

This study represents the first integrated study in Scotland that combines population genomics and particle tracking modelling to enhance spatial and temporal understanding of population connectivity for the commercially significant mussel species *M. edulis*. By combining an assessment of population genomics with a hydrodynamic and particle tracking model described previously (Chapter 3), this study reveals the degree of genetic exchange occurring among populations, even those separated by great distances and impacted by oceanographic factors. To date, a few studies have combined population genomics and particle modelling in *M. edulis*. Pastor et al. (2021), Coolen et al. (2020), and Stuckas et al. (2017), have explored *M. edulis* connectivity in the Limfjorden, North Sea, and Baltic Sea, respectively. Other studies have focused solely on hydrodynamic larval particle tracking models. *E.g.*, Stechele et al. (2022), Demmer et al. (2022), and Newell et al. (2010) investigate *M. edulis* connectivity in Belgium, Ireland, and Maine, respectively. Moreover, Alexander et al. (2021), Mathiesen

et al. (2017) and Yund and McCartney (2016) have exclusively examined genetic diversity to explore *M. edulis* connectivity in Wales, the Arctic, and Maine, respectively. Available data on population connectivity of *M. edulis* in Scotland are relatively limited, and we argue that the most effective approach to exploring population connectivity involves combining hydrodynamic larval particle tracking models with *in-situ* spat and adult sequencing. This method provides a more complete understanding of the genetic structure of mussel populations and their dispersal patterns, ultimately allowing for more accurate predictions of future population dynamics.

4.5.1 Mussel connectivity

Overall, the west coast of Scotland shows a strong physical link between southern and northern areas maintained by the regional water circulation. Looking at particle dispersal only, the thirteen areas of interest (sampling locations) appear to be connected with neighbouring sampling sites only (Figure 4.1C). However, the thirteen areas of interest are receiving particles from multiple other locations (Figure 4.3). Looking at the genetic structure only, we found five genetic clusters grouped by geographical regions: going from southern areas to the northern part of the west coast (southwest coast, Loch Spelve, Isle of Skye, Outer Hebrides, and northwest coast). The rapid water movement and larval transportation in Scottish waters result in a genetically diverse population of mussels. If genetic analysis were to be conducted again on new collected samples, the resulting clusters would likely be similar, although the relative positioning of each population would differ. This is due to a continual exchange of genetic variants and shifts in their geographic distribution. This becomes apparent when comparing successive generations ((*i.e.*, spats and adults) of the mussel populations (Figure 4.2A). Genetic mixing is driven by the high levels of connectivity facilitated by water currents, which allow for the exchange of genetic material between different mussel populations. Therefore, it can be inferred that the genetic diversity of mussels in Scotland is constantly evolving, with new genetic clusters forming as populations mix and exchange genes. The disagreement between the genetic data and the WeStCOMS v2 hydrodynamic model transport in regard to the Bo Sligachan location could be explained either by the presence of an indirect connection that is not easily

captured by the genetic data, or by the inaccuracy of the simulating for the unique conditions of the Bo Sligachan location. Another suggestion is that the northern entrance to the Sound of Raasay is sufficiently wide to allow aperiodic influx of the waters from the Minch Sound between Skye and Outer Hebrides, occasionally delivering here the particles (larvae) from the southwestern coast cluster.

The connectivity among mussel populations on the west coast of Scotland suggests that some areas act as net sources or sinks. Specifically, our analysis indicates that the mussel farm in Loch Eil, which belongs to the southern west coast cluster, serves as a source of larvae, while areas in the Outer Hebrides and northern west coast, such as the mussel farms in Loch Roag and Badcall Bay, act as sinks, receiving larvae from other locations. The extent of larval supply to these sink stocks is likely influenced by the hydrodynamic regime and the intermediate blue mussel population involved in the transfer process. Despite potential genetic differences, this exchange seems to be sufficient to support connectivity among the areas and create a well-mixed genetic pool. Sink stocks, like those found in Loch Roag and Badcall Bay, play an important role in maintaining genetic diversity within a population. This diversity can provide benefits such as increased resilience to environmental stresses, improved adaptability to changing conditions, and greater fitness, ultimately promoting the long-term survival of the population (Sgrò et al., 2011). However, the survival of sink stocks depends on the availability of larvae from source stocks with sufficiently large effective population sizes and self-recruitment rates to maintain themselves, such as those found in Loch Eil and S151 (Figure 4.7). Overexploitation of source stocks, pollution, and climate change can have negative impacts on the viability of the entire population, including decreased recruitment, productivity, and persistence of associated sink stocks (Kaiser et al., 2021; Carroll et al., 2020). Therefore, the importance of maintaining source stocks with large effective population sizes and high self-recruitment rates cannot be overstated. This highlights the importance of studying population connectivity to better understand the genetic structure of mussel populations and how they may be impacted by factors affecting the accuracy of genetic analyses and the conclusions drawn about mussel connectivity (Becker et al., 2007; Pineda et al., 2007; Pastor et al., 2021).

The settlement and genetic composition of mussel populations are subject to intricate environmental influences, particularly concerning larval transfer and post-settlement selection. There is a notable possibility of spat importation into farming areas from other locations, potentially leading to distinct genetic differences between natural mussel beds and rope cultures (Gurney-Smith et al., 2017). This divergence becomes particularly pronounced in regions where hybridisation among mussel species—such as *M. edulis, M. galloprovincialis,* and *M. trossulus*—has been observed, notably in locales like the Loch Eil region (Dias et al., 2011; Michalek et al., 2016). Past studies, notably (Comesaña & Sanjuan, 1997; Wilhelm & Hilbish, 1998; Katolikova et al., 2016), affirm the existence of recruitment selection at small spatial scales in zones where different mussel lineages coexist. This process is intricately linked to various factors like local environmental conditions and genetic disparities, potentially impacting settlement and genetic exchange among populations. Hybridisation, however, introduces new genetic traits, posing concerns regarding local adaptation and genetic integrity. Understanding population connectivity and genetic structure becomes challenging when studying factors like larval transport and water circulation patterns, pivotal in genetic exchange between locations. Sessile organisms face challenges post-settlement, wherein phenotypic mismatches due to selective mortality hinder connectivity (Marshall et al., 2010). The delineation of intra- and inter-species boundaries heavily depends on conflicting environmental factors influencing different life stages. The natural geographical constraints of species offer a unique setting to explore the intricate interplay between environment and genetics. In our study, samples from distinct locations, despite exhibiting identical genetic patterns due to shared farming operations (Loch Eil and Loch Sunart), raised intriguing questions. For instance, although genetic similarity was observed between Loch Eil and Loch Sunart samples, the biophysical modelling results failed to demonstrate direct connectivity between these areas (Figure 4.1C). This suggests that other influential factors—possibly larval transport or water re-circulation patterns, like those around Loch Spelve in the Isle of Mull— are capable only occasionally or even may not facilitate significantly genetic exchange between these two locations on a local scale. Although the mussel farm in Loch Spelve is situated in an isolated area, strategically avoiding exposure to open

waters, it is important not to dismiss the possibility of receiving larvae from southern areas when the current flows permit access to the region. Conducting additional population studies in the southern areas and comparing them to Spelve would provide valuable insights to determine whether speciation is occurring in this isolated location

4.5.2 Mussel farming implications

The west coast of Scotland is a well-connected system for mussel dispersal and recruitment. Identifying the key source and sink areas for mussel larvae is important for decision-making related to mussel farming, and crucial for the ecosystem as a whole (Mackenzie et al., 2018). For example, Loch Eil, in the southern west coast has been identified as a key source area for mussel larvae, it may be important to protect that area to ensure a sustainable supply of mussel larvae for other areas. Similarly, Loch Roag and Badcall Bay have been identified as key sink areas, it may be important to focus mussel farming efforts in that area to ensure a healthy and sustainable population of mussels. Mussels play an essential role in supporting other marine organisms and maintaining water quality. By ensuring sustainable populations of mussels through responsible farming practices and protecting key source areas, we can contribute to the overall health and resilience of marine ecosystems in Scotland.

Recently, the Marine Scotland Science Scottish Shellfish Farm Production Survey 2021 have reported presence of the parasitic haplosporidian *Bonamia ostreae* mainly in the southern west coast region and more specifically in Loch Sunart, implementing movement restrictions in those areas. In the past, Scottish farmers have also reported fluctuations in spat recruitment and settlement (Mayes, 2012; Gubbins et al., 2012; Seuront et al., 2019), which supports the idea that stopping mussel production in important source areas could reduce the supply of mussel larvae to other regions, affecting the overall productivity and profitability of the mussel farming industry in Scotland. Therefore, it is crucial to carefully consider the potential consequences of stopping production in areas where the source of larvae is abundant and supplies multiple other regions. Any decisions related to the management and protection of these areas should be based on a thorough understanding of the ecological and economic factors

involved, and aim to balance the needs of the industry with the need to protect and maintain the health of the broader marine ecosystem.

There are alternatives to completely stopping production in key source areas, like mussel farms in Loch Eil. Simple and effective ways for farmers to maintain the genetic integrity of mussel populations include establishing buffer zones (Di Franco et al., 2016) around the farming area where no mussels are cultivated. This reduces the chances of hybridisation occurring between farmed and wild populations and ensures a sustainable supply of mussel larvae for other areas. Additionally, buffer zones can help to protect the long-term health and productivity of the broader marine ecosystem (Jamieson & Chew, 2002). Monitoring and controlling mussel populations, regularly tracking, and removing individuals showing signs of hybridisation, and conducting regular genetic testing of mussels are other options (Potasman et al., 2002; Naish et al., 2007). In northern areas where mussel farms receive seeds from multiple source locations, reports of hybridization events or disease spread have not been frequent. Farmers can obtain mussel seeds from multiple sources to reduce the risk of introducing hybrids into their farming area, maintaining genetic diversity, and improving the overall health and resilience of the local mussel population.

Alternatives are important to consider in the context of mussel farming and stock recruitment. Identification of key source and sink areas for mussel larvae is crucial for decision-making related to mussel farming in Scotland. Connectivity patterns are important ecological features for stock recruitment because they help determine which local stocks rely on larval retention and self-recruitment and/or migration (Gilroy & Edwards, 2017). Understanding mussel recruitment processes can also help to address climate change concerns in specific areas. By implementing strategies such as hatchery production monitoring, and controlling mussel populations, establishing buffer zones, conducting regular genetic testing, and diversifying seed sources, farmers can help to maintain the genetic purity of local mussel populations, reduce the risk of hybridisation, and contribute to the overall health and resilience of marine ecosystems in Scotland.

Changes in mussel connectivity, whether due to natural variability or as a result of human activities, can have significant impacts on the dispersal and recruitment of

mussel larvae.

In this research, the initial hypothesis underlying the study posited that each target location would have one or two source locations, and the sampling strategy was designed accordingly, *i.e.*, sampling adult and spat mussels from nearby areas in each mussel farm. However, a more nuanced and intricate picture emerged from the findings, which were further refined by the advanced biophysical model. The results suggesting that each site consists of a complex assemblage of self-recruitment and a vast network of source, which creates a mesh-like pattern that cannot be readily tested through conventional biological means. Indeed, meaningful validation of the fine details would require a rigorous and protracted sampling effort, involving weekly collections from each of the 440 source locations. And even though, we represented a simulation of particles (bivalve larvae) moving along the West coast of Scotland, our model has significant limitations that will need to be addressed in future studies. For example, the particle tracking model does not account for vertical migration of particles through the different layers of the FVCOM mesh, environmental parameters such as temperature and salinity as little data are available to model the phenomenon and biological parameters as no mortality was considered because there is insufficient information on mortality rates for bivalves during the larval phase.

Lastly, the ddRAD analysis did not yield the expected number of markers, which could be attributed to the genome duplication events in *Mytilus* evolution (Chapter 2). These events, creating four copies of each chromosome, lead to an excess of duplicated genes, known as paralogs. The abundance of paralogs complicates distinguishing between homologous and non-homologous loci, potentially resulting in fewer reliably identifiable markers, or inadequate ddRAD library preparation and sequencing issues (Kondrashov et al., 2002). Another contributing factor to the lower-than-expected marker count in the ddRAD analysis could be the challenge of distinguishing highly similar genes, such as paralogues arising from a recent WGD event, as distinct loci. Failure to recognize and correctly assign these sequences to individual loci (assuming they are allelic variants) could lead to an underestimation of marker numbers and an overestimation of genetic variability at those loci, impacting metrics such as

heterozygosity. Alternatively, various factors can contribute to a low number of markers during ddRAD library preparation, including DNA quality and quantity, library preparation protocol, and PCR amplification bias, all of which may affect the number of markers generated (Peterson et al., 2012).

4.5.3 Future perspectives

For future improved studies, mapping mussel populations to areas beyond the west coast of Scotland could give a more complete understanding of larger-scale patterns of mussel dispersal. Furthermore, to assess migration rates efficiently, it is recommended to collect the samples from the same year/season to minimise any potential biases due to temporal variability. In addition to monitoring the changes in mussel populations, investigating the effects of climate change on mussel connectivity is also crucial. Climate change is expected to disrupt larval dispersal through changes in temperature, salinity levels, and current patterns, potentially leading to declines in mussel populations and shifts in their distribution (Lett et al., 2010; Andrello et al., 2015; Bani et al., 2021). Understanding how these changes may impact mussel populations is important for developing effective management strategies to maintain their health and sustainability. Overall, continued research into mussel connectivity and the factors that influence it is essential for the long-term conservation of these valuable resources. By better understanding how ocean and coastal circulation patterns affect mussel populations and developing strategies to adapt to changes, we can ensure the continued productivity and resilience of marine ecosystems in Scotland and beyond.

4.5.4 Conclusion

This research emphasises the importance of understanding the genetic dynamics and connectivity patterns of mussel populations in Scotland. The findings show a continuous mixing of genetic material between locations, leading to rapid evolution and changes in local populations. This highlights the need for effective management strategies to maintain genetic integrity, reduce the risk of hybridisation, and ensure a sustainable supply of mussel larvae for other areas. These findings can inform decisionmaking for mussel farming and conservation efforts in Scotland. Future work

should focus on developing and implementing management strategies that account for the genetic connectivity patterns of mussel populations and concomitant ecological impacts.

Chapter 5

General discussion

In recent years, the mussel farming sector has faced significant challenges due to poor spat settlement, highlighting the urgent need to understand the underlying causes of the issue for the sustainable growth of the Scottish shellfish industry. Accordingly, Avdelas et al., 2021, emphasises the importance of identifying the root causes of the decline in mussel production and implementing effective management strategies to overcome these challenges.

Despite extensive research on blue mussel larval behaviour, significant knowledge gaps remain, particularly concerning their specific swimming patterns and performance during the planktonic phase. Moreover, the extent to which different populations of *M. edulis* are genetically connected remains poorly understood, which can have implications for conservation and management efforts. Additionally, environmental factors affecting mussel production, such as nutrient availability, diseases, and waste build-up in coastal areas, must be addressed to consider potential mitigation strategies. Furthermore, there is limited information on market demand and potential market growth for *M. edulis* in Scotland and other regions, which is relevant for aquaculture producers aiming to build marketing strategy and ultimately increase production (Azra et al., 2021).

The overarching objective of this Ph.D. thesis was to advance current studies, methods, and the development of tools for the identification of spat sources in mussel farming. These goals have been achieved through the combination of biophysical models

with genome-wide sequencing, facilitating a deeper understanding of larval dispersal patterns and population connectivity. To accomplish this, the following three aims have been pursued:

- Generation of a whole genome assembly of the blue mussel that will allow accurately identifying molecular markers essential for evolutionary, population genetics, and conservation studies.
- Analysis of larval movement patterns along the West coast of Scotland using an unstructured 3D hydrodynamic model. The model allowed the quantification of particle dispersion variability and connectivity between regions, facilitating the identification of probable larval sources.
- Assessment of the genetic structure of blue mussel populations using the genotyping-by-sequencing approach ddRAD-seq. This allowed to validate the hydrodynamic model in crucial bivalve production locations, particularly in dynamic areas such as the West coast of Scotland.

5.1 Summary of key findings

5.1.1 Genome duplication's and positively selected genes

The aim of Chapter 2 was to generate a whole genome assembly of the blue mussel to correctly identify molecular markers for evolutionary, population genetics, and conservation studies. In chapter 2, we presented genomic evidence for paleopolyploidy in *M. edulis*. By combining gene age distribution and phylogenomic analyses, we uncovered two significant and episodic bursts of gene duplication. Our analyses demonstrate that an ancient whole-genome duplication (WGD) event and a more recent WGD event occurred prior to the divergence of the Bivalvia. This finding provides evidence that could lead us to explain the large genomes observed in bivalves and molluscs more broadly (Davison & Neiman, 2021).. Incomplete genetic data (draft genomes and transcriptomes), as well as reduced datasets (Enzymes, RAD, or EST), made it impossible to correctly detect WGDs and duplicated genes in Bivalvia, before now. The absence of complete genomes and the full picture of WGD events has led to

the oversight or misinterpretation of duplicated sequences, resulting in potential artefacts such as an overestimation of the degree of heterozygosity (Vendrami et al., 2020), numer of pseudogenes, and rates of gene acquisition and loss (Gerdol et al., 2020).

Our study overcame these limitations by accurately establishing the genome of Bivalvia while accounting for genome duplications. This provided a robust scaffold for the alignment of reads generated from ddRAD-seq. This step significantly enhanced the accuracy of our subsequent analyses. The ddRAD-seq data, complemented by the genome reference, facilitated the identification of SNPSs within the bivalve populations, enabling the research into the genetic diversity, connectivity, and population structure of the blue mussel. We found that the discovery of several WGD events in the Bivalvia suggests that large-scale duplications events can lead to increased genetic diversity, and the presence of additional copies of genes may provide raw material for new functions to emerge, facilitating the evolution of novel traits. This process can help bivalve species adapt to changing environmental conditions, including those brought about by anthropogenic pressures such as climate change and pollution. By understanding the importance of WGD events in the evolution of bivalves, we can gain insights into how these species have evolved and adapted over time, and potentially apply this knowledge to improve their management and conservation (Berthelot et al., 2014).

In the study, a functional analysis of positively selected genes was also conducted in three species of *Mytilus*, namely *M. edulis*, *M. galloprovincialis* and *M. coruscus*, in order to identify genes that are associated with unique traits or adaptations in response to specific environmental or biological pressures. Our results reveal that genes in *M. edulis* and other *Mytilus* species primarily associated with immunity, stress responses, and developmental processes are positively selected. Furthermore, a comparison between the two mussel species most commonly cultivated in Europe, *M. edulis* and *M. galloprovincialis*, shows that the latter appears to be adapted to higher temperatures, and may have a higher tolerance to potential water pollutants, as apparent from the presence of stress response proteins under positive selection in *M. galloprovincialis*.

Given the challenges posed by climate change and rising temperatures, the hybridisation between *M. edulis* and *M. galloprovincialis* in higher latitudes has the potential to introduce genes from *M. galloprovincialis* that could confer thermal tolerance to *M. edulis* populations. This could enhance their capacity to withstand increasingly warmer conditions.

5.1.2 Larval dispersal simulations

Chapter 3 aimed to use an unstructured 3D hydrodynamic model to examine patterns of larval movement on the West Coast of Scotland. Allowing the quantification of variability and connectivity between regions and the identification of the most probable sources of larvae. The investigation of bivalve larval movement in Scotland waters is an ongoing process, and model scenarios were developed based on literature observations of interannual variability of seed recruitment and, to a lesser extent, shellfish farmer expertise. Previous research has indicated that circulation patterns play a crucial role in the inter-annual variability of larval recruitment and dispersal (McQuaid & Phillips, 2000; Largier, 2003), while interactions between larval vertical migration and stratification significantly impact dispersal (Raby et al., 1994). Nevertheless, the water column in the West coast of Scotland remains well mixed from winter to May, suggesting that stratification may not influence early spring larval dispersal citepAdams2016.

In chapter 3, we adapted the particle-tracking model to simulate the movement of bivalve larvae under various scenarios exposed to environmental climate variability. Our findings revealed that virtual larvae dispersed from their native bed to a target location by a maximum distance of 170 km after four weeks, indicating both local and general connectivity within the West coast of Scotland. This is relevant, since the identification of source points for specific locations can have implications for aquaculture practices and disease control.

Our study found no significant difference in the dispersal patterns of bivalve larvae between different depths, indicating that tidal currents play a critical role in larval dispersal (Raby et al., 1994; McQuaid & Phillips, 2000; Demmer et al., 2022). Larvae were assumed to be distributed throughout the water column and exhibit limited vertical migration in the absence of stratification, further reinforcing the importance of

tidal currents in their dispersal. Additionally, wind-driven currents may have a considerable impact on the dispersal of bivalve larvae, particularly those distributed in near-surface waters.

Although our model has limitations, this study shows how biophysical models can help in the understanding of system dynamics and identifying breeding and settlement areas, which can be leveraged to rationalise bivalve farming activities. This biophysical approach could enhance our understanding of bivalve populations in dynamic maritime environments such as the West coast of Scotland.

5.1.3 Population structure

Chapter 4 aimed to assess the genetic structure of blue mussel populations and use the obtained genetic data to validate the hydrodynamic model for key bivalve production locations along the West Coast of Scotland. Research on population connectivity of *M. edulis* in Scotland is limited, and a comprehensive understanding of genetic structure and dispersal patterns is crucial for accurate predictions of future population dynamics. In this study, chapter 4, combined hydrodynamic larval particle tracking models with *in-situ* spat and adult DNA analysis to investigate population genomics and particle modelling of *M. edulis*. This method provides a more complete understanding of the genetic structure of mussel populations and their dispersal patterns, ultimately allowing for more accurate predictions of future population dynamics.

Our results revealed that *M. edulis* populations along the West coast of Scotland exhibit predominant directional flow of particles from south to north, consistent with estimated patterns of larval dispersal. Genetic analysis of *M. edulis* show the presence of five genetic clusters, corresponding to geographic regions. The connectivity among mussel populations on the west coast of Scotland suggests that some areas act as net sources or sinks, as supported by both genetic and hydrodynamic modelling approaches. The extent of larval supply to these sink stocks is likely influenced by the hydrodynamic regime and the intermediate blue mussel population involved in the transfer process. Despite potential genetic differences, this exchange seems to be sufficient to support connectivity among the areas and create a well-mixed genetic pool. Sink stocks, such as those found in Loch Roag and Badcall Bay, play a critical role in

maintaining genetic diversity within a population, which can provide benefits such as increased resilience to environmental stresses, improved adaptability to changing conditions, and greater fitness, ultimately promoting the long-term survival of the population (Sgrò et al., 2011).

However, the survival of sink stocks depends on the availability of larvae from source stocks with sufficiently large effective population sizes and self-recruitment rates to maintain themselves (Kaiser et al., 2021; Carroll et al., 2020). In other words, sink populations to survive need to receive enough larvae from source populations that have sufficiently large effective population sizes and self-recruitment rates. If source populations have small effective population sizes or low self-recruitment rates, they may not produce enough larvae to sustain themselves, let alone supply sink populations. In this case, sink populations may not receive enough larvae to maintain their numbers, which could ultimately lead to their decline or even extinction. Therefore, the survival of sink populations is dependent on the availability of larvae from source populations with robust reproductive potential.

Our findings underscore the importance of understanding the genetic dynamics and connectivity patterns of mussel populations in Scotland. The results show that there is a continuous mixing of genetic material between locations, leading to rapid evolution and changes in local populations. Our study suggests that there is an extensive ongoing exchange of genetic material between populations from one year to another, shaping the genetic evolution of these populations.

5.1.4 Conclusions of the study

This Ph.D. project has successfully achieved the first of three aims by generating a chromosome level whole genome assembly of the blue mussel that was functionally annotated to reveal gene orthologs, and allowed comparative genomic and evolutionary studies. Additionally, the second aim of this Ph.D. was achieved through the application of an unstructured 3D hydrodynamic model, which. The was adapted to examine patterns of larval movement on the West Coast of Scotland, enabling the quantification of variability and connectivity between regions and the identification of most probable sources of larvae. Furthermore, the third aim of the Ph.D. was achieved

through the study of the genetic structure of blue mussel populations . An output of this study were inferred patterns of genetic connectivity between populations, allowing to validate empirically the predictions of the hydrodynamic model for key bivalve production locations. Overall, the overarching objective of the thesis was to advance current knowledge in mussel larval dispersal to advise mussel farming planning, conservation, and management practices. This Ph.D. achieves this objective by providing valuable insights into the population genetics and ecology of blue mussels, and contributes to the broader understanding of bivalve species and their importance in marine ecosystems.

5.2 Limitations and future perspectives

The simulation of particle movements along the West coast of Scotland presented in chapters 3 & 4 provided meaningful results; however, the biophysical model used has limitations that must be addressed in future studies. Firstly, the vertical migration of particles was not accounted for, which could have significant implications for the survival and dispersal of bivalve larvae, as vertical migration can play a crucial role in their movement and survival (Knights et al., 2006). Although there is little information available on this phenomenon, future studies should aim to incorporate it to ensure more accurate predictions of larval movement in relation to changes in depth. Secondly, the mortality rate of bivalve larvae during the larval phase was not considered due to a lack of information on mortality rates for this life stage. However, this is an essential factor in understanding population dynamics and ecology (Lupo et al., 2021), and future studies should also aim to incorporate this into their models.

The population genetic study presented in chapters 4 also has its limitations. The ddRAD analysis did not yield the expected number of markers, which can be attributed to the genome duplication events or inadequate ddRAD library preparation and sequencing issues. These WGD events result in four copies of each chromosome, leading to numerous duplicated genes (paralogs). The presence of many paralogs makes it challenging to distinguish between homologous and non-homologous loci, leading to a lower number of markers being reliably identified (Kondrashov et al., 2002). Alternatively, there are several factors that can contribute to obtaining a low

number of markers during ddRAD library preparation. These include the choice of restriction enzyme, the size selection range, sequencing depth, and the bioinformatic pipeline used for data analysis. In addition, factors such as DNA quality and quantity, library preparation protocol, and PCR amplification bias can also have an impact on the number of markers generated (Peterson et al., 2012).

Additionally, temporal variability within the sampling sites (samples collected between 2019 and 2021) could have reflected temporal variation in the population genetic structure. This could have led to potential differences in population connectivity between years, and the introduction of additional sources of variation that could have made it more challenging to validate the biophysical model. The initial hypothesis underlying the study posited that each target location would have one or two source locations, and the sampling strategy was designed accordingly. However, a more nuanced and intricate picture emerged from the findings presented in chapter 3, which were further refined by advanced modelling techniques in chapter 4. The results suggest that each site consists of a complex assemblage of self-recruitment and a vast network of sources, which creates a mesh-like pattern that cannot be readily tested through conventional biological means. Indeed, meaningful validation of the fine details would require a rigorous and protracted sampling effort, involving weekly collections from each of the 400 sites over a period of two years. Nevertheless, the overarching conclusion drawn from the data remains robust and defensible.

5.2.1 Expanding the study area and climate change integration

The FVCOM model is a popular numerical model used to simulate coastal processes. It has special features like an adaptive unstructured grid, allowing for better representation of complex coastlines (Chen et al., 2006, 2011). In this study, we used a modified version to simulate coastal circulation, tides, and wave dynamics in Scotland (Aleynik et al., 2016). Expanding the model to other regions could have significant benefits for understanding and managing other coastal ecosystems. However, the first step should be the compilation of substantial data requirements, including bathymetry, meteorological, and biological data specific to the target areas (Dabrowski et al., 2016). In addition, careful calibration and validation would be necessary to ensure that the model

accurately captures the physical and biological processes of the new areas. Successful implementation in new regions could offer significant benefits for our understanding and management of coastal ecosystems beyond Scotland.

The target regions could include other dynamic coastal areas such as the entire UK, Norway, or France. For example, extending the model to cover the entire UK coastline could provide insights into a range of important coastal processes. The model could be used to simulate the transport and fate of pollutants in the waters around major ports and industrial centres, or to predict the spread of harmful algal blooms that can have negative impacts on fisheries and other marine resources (Adams et al., 2014; Aleynik et al., 2016; Davidson et al., 2021; Aleynik et al., 2022). In Norway with long and complex coastlines, many fjords and other coastal features that can affect ocean circulation and the transport of nutrients and other materials (Nesje, 2010), the bio-physical model covering its coastal waters could help to improve understanding of how these features impact coastal dynamics and ecosystem health. The model could also be used to study the impacts of climate change on Norway's coastal ecosystem, which are expected to be particularly vulnerable to warming temperatures and ocean acidification (Hindar et al., 2004).

France, like Norway, has a diverse coastline with a variety of coastal features that could influence ocean circulation and ecological processes. The model could be used to study transport of pollutants and nutrients in the waters around major port cities like Marseilles and Le Havre (Guerrero, 2014), and to investigate the connectivity of different populations of economically important species like oysters or mussels (Briant et al., 2017).

The model could also be used to assess the impacts of climate change on coastal ecosystems, which are expected to experience increasing temperatures, sea level rise, and changes in ocean circulation patterns in the coming decades (Robert & Schleyer-Lindenmann, 2021).

To fully comprehend the impacts of climate change on blue mussel connectivity, it

would be crucial to identify potential effects on predation rates, disease, and competition, which can significantly affect mussel survival and physiology (Cowen & Sponaugle, 2009). Changes in temperature and other environmental variables can have significant effects on the growth, behaviour, and physiology of mussels and their predators, competitors, and pathogens (Lockwood et al., 2015). Conducting experiments to assess the effects of rising sea temperatures on blue mussel physiology, growth, and reproduction could be a valuable approach. For example, studies on the bivalve Spectaclecase *Cumberlandia monodonta* have used genetic population structure analysis and ecological niche modelling to predict suitable habitat while considering temperature variations, and biophysical modelling could complement experiments by simulating the effects of different temperature scenarios on the bivalves (Inoue & Berg, 2017).

Moreover, monitoring mussel populations in the field over time, along with genetic analysis and biophysical modelling, can provide a more comprehensive understanding of how bivalve populations respond to climate change. This approach can help identify areas where mussel populations may be particularly vulnerable to climate change, and further research may be needed to understand the underlying genetic mechanisms. Ultimately, models need to be developed to accurately represent the species' distribution and dispersal patterns between different regions, accounting for natural connectivity mechanisms such as larval migration and potential human intervention. By capturing these mechanisms, a better understanding of how the resilience of bivalves may be affected by changes in their environment and how they may adapt to future challenges can be achieved.

5.3 General conclusion

Understanding the behaviour and recruitment process of mussel larvae is critical for sustainable management of mussel populations and adapting to climate change, as it is the least understood demographic process in marine waters (Swearer et al., 2019). A healthy recruitment process is crucial for maintaining viable populations and ensuring the long-term sustainability of mussel fisheries.

This research project facilitates the identification of mussel populations that are particularly vulnerable to stressors by identifying potential areas acting as a source of larvae, areas acting as both source and sink, and areas acting mainly as receivers of larvae. This information can guide conservation efforts and inform management decisions, ensuring the sustainability of mussel populations. Additionally, this research can help identify areas crucial for larval settlement and connectivity within the ecosystem by predicting areas of higher particle accumulation, validated by genetic analysis.

This research provides valuable insights into the connectivity of blue mussel populations in Scottish waters, making a significant contribution to sustainable management of these species and supporting the adaptation of management practices to changing environmental conditions. It highlights the importance of taking a multidisciplinary approach to complex problems related to aquaculture and environmental management, recognising that these issues cannot be solved by a single discipline alone.

Appendix A

Mytilus edulis whole genome sequencing

TABLE A.1: Sequencing data, summary statistics. $*$ Estimation based on *M. galloprovincialis* and *M. coruscus* genomes size.

Category	Number/length
Total number of long reads	15,945,130
Total number of bases	111,654,433,463
Mean length	7,002 nt
Maximum read length	672,255 nt
Coverage*	64x
Total number of PE short reads	652,465,784
Total number of bases	195,739,735,200
Read length	150 nt
Coverage*	113x
Total number of RNA-seq PE short reads	50,593,080
Total number of bases	15,177,924,000
Read length	150 nt
Coverage*	9x

TABLE A.2: RepeatMasker statistics. ∗ repeats fragmented by insertions or deletions have been counted as one element. † LTR_Finder results: 255,413 LTR pairs over 4,932 regions and 151,703,353 bp.

Element	Number of elements*	Length occupied	Percentage of sequence
SINEs	10,261	2,030,467 bp	0.11%
ALUs	0	0 bp	0.00%
MIRs	0	0 bp	0.00%
LINEs	343,643	130,980,160 bp	7.17%
LINE1	11,902	5,537,561 bp	0.30%
LINE2	7,970	3,762,443 bp	0.21%
L3/CR1	6,535	4,167,698 bp	0.23%
LTR elements†	4,932	151,703,353 bp	8.30%
DNA elements	99,267	25,504,172 bp	1.40%
hAT-Charlie	2,603	433,449 bp	0.02%
TcMar-Tigger	0	0 bp	0.00%
Unclassified	2,976,814	707,039,397 bp	38.70%
Small RNA	133	25,717 bp	0.00%
Satellites	2,252	400,532 bp	0.02%
Simple repeats	224,100	10,280,290 bp	0.56%
Low complexity	50,703	2,473,133 bp	0.14%
Total repeats		1,029,206,554 bp	56.33%

TABLE A.3: Summary of annotation results for *M. edulis* gene models using a range of databases. ∗ InterPro covers 12 databases (CDD-3.17,Coils-2.2.1, Gene3D-4.2.0, Hamap-2020_01, MobiDBLite-2.0, PANTHER-14.1, PRINTS-42.0, ProSitePatterns-2019_11, ProSiteProfiles-2019_11, SFLD-4, SMART-7.1, SUPERFAMILY-1.75, TIGRFAM-15.0).

Database	Number annotated
PfamA	61,453
InterPro*	48,772
SwissProt	11,211
KEGG	51,091
GO	31,620
All	9,089
Total	69,246

TABLE A.4: Mytilinae (subfamily) mitochondrial genomes.

Accession	Genome size	Species name	Common name
NC_018362.1	16,014 bp	*Perna viridis*	Asian green mussel
NC_044131.1	16,253 bp	*Septifer bilocularis*	-
NC_044128.1	17,582 bp	*Crenomytilus grayanus*	-
NC_030633.1	16,765 bp	*Mytilus chilensis*	Chilean mussel
NC_028706.1	18,145 bp	*Limnoperna fortunei*	Golden mussel
NC_026288.1	18,415 bp	*Perna perna*	Brown mussel
NC_024733.1	16,642 bp	*Mytilus coruscus*	Hard-shell mussel
NC_006886.2	16,744 bp	*Mytilus galloprovincialis*	Mediterranean mussel
NC_006161.1	16,740 bp	*Mytilus edulis*	Blue mussel
NC_007687.1	18,652 bp	*Mytilus trossulus*	Bay mussel
NC_015993.1	16,730 bp	*Mytilus californianus*	California mussel

TABLE A.5: Bivalvia (class) genome where Ka & Ks estimations were possible: All exhibit evidences of α-WGD and β-WGD. $*$ this study.

Species	Peak/Mean (α-WGD)	Std. dev. (α-WGD)	Peak/Mean (β-WGD)	Std. dev. (β-WGD)
*M. edulis**	0.6132	0.1939	1.8196	0.7103
M. galloprovincialis	0.6010	0.1934	1.8109	0.7011
M. coruscus	0.5635	0.1996	1.7977	0.7082
C. gigas	0.6299	0.3699	1.6350	0.4601
C. virginica	0.4787	0.4921	1.7627	0.3479
M. yessoensis	0.6612	0.2278	1.7064	0.4722
P. maximus	0.6392	0.2759	1.8794	0.4541

TABLE A.6: Bivalvia (class) genome and availability of gene models and annotations. $*$ this study.

Assembly	Reference	Species name	Common name	Gene Models
GCA_014843695.1	ASM1484369v1	*Archivesica marissinica*	Deep-sea clam	-
GCA_004382765.1	QAU_Acon_1.1	*Argopecten irradians* concentricus	Bay scallop	-
GCA_004382745.1	QAU_Airr_1.1	*Argopecten irradians* irradians	Bay scallop	-
GCA_002080005.1	Bpl_v1.0	*Bathymodiolus platifrons*	Cold seep mussel	-
GCA_001632725.1	ASM163272v1	*Corbicula fluminea*	Asian clam	-
GCF_902806645.1	cgigas_uk_roslin_v1	*Crassostrea gigas*	Pacific oyster	yes
GCA_005518195.2	NWPU_Cgig_v2	*Crassostrea gigas*	Pacific oyster	-
GCF_000297895.1	oyster_v9	*Crassostrea gigas*	Pacific oyster	-
GCA_000297895.2	ASM29789v2	*Crassostrea gigas*	Pacific oyster	-
GCA_011032805.1	ASM1103280v1	*Crassostrea gigas*	Pacific oyster	-
GCA_015776775.1	ASM1577677v1	*Crassostrea hongkongensis*	Hong Kong oyster	-
GCA_002022765.1	C_virginica_1.0	*Crassostrea virginica*	Eastern oyster	-
GCA_002022765.3	C_virginica-2.0	*Crassostrea virginica*	Eastern oyster	-
GCF_002022765.2	C_virginica-3.0	*Crassostrea virginic*a	Eastern oyster	yes
GCA_012932295.1	ASM1293229v1	*Cyclina sinensis*	Venus clam	-
GCA_000806325.1	ASM80632v1	*Dreissena polymorph*a	Zebra mussel	-
GCA_007657795.1	UV_Dro_v1.1	*Dreissena rostriformis*	Quagga mussel	-
GCA_003130415.1	ASM313041v1	*Limnoperna fortunei*	Golden mussel	-
GCA_008271625.1	LuRhyn_1.0	*Lutraria rhynchaena*	Snout Otter Clam	-
GCA_016163765.1	CUHK_oyster_2.0	*Magallana hongkongensis*	Hong Kong oyster	-
GCA_015947965.1	ASM1594796v1	*Margaritifera margaritifera*	Freshwater pearlshell mussel	-
GCA_016617855.1	ASM1661785v1	*Megalonaias nervosa*	Washboard	-
GCA_014805675.1	ASM1480567v1	*Mercenaria mercenaria*	Northern quahog	-
GCF_002113885.1	ASM211388v2	*Mizuhopecten yessoensis*	Yesso scallop	yes
GCA_002113885.1	ASM211388v1	*Mizuhopecten yessoensis*	Yesso scallop	-
GCA_002080025.1	Mph_v1.0	*Modiolus philippinarum*	Philippine horse mussel	-
GCA_017311375.1	Mcoruscus_HiC	*Mytilus coruscu*s	Hard-shell mussel	-
GCA_011752425.2	MCOR1.1	*Mytilus coruscus*	Hard-shell mussel	yes
GCA_011752425.1	MCOR1	*Mytilus coruscus*	Hard-shell mussel	-
GCA_905397895.1*	MEDL1	*Mytilus edulis*	Blue mussel	yes
GCA_900618805.1	MGAL_10	*Mytilus galloprovincialis*	Mediterranean mussel	yes
GCA_000715055.1	mussel1.0	*Mytilus galloprovincialis*	Mediterranean mussel	-
GCA_001676915.1	ASM167691v1	*Mytilus galloprovincialis*	Mediterranean mussel	yes
GCA_903981925.1	v081	*Ostrea lurida*	Olympia oyster	-
GCA_902825435.1	PGEN-v1.0	*Panopea generosa*	Pacific geoduck	-
GCF_902652985.1	xPecMax1.1	*Pecten maximus*	King scallop	yes
GCA_002216045.1	PinMar1.0	*Pinctada imbricata*	Akoya pearl oyster	-
GCA_016161895.1	ASM1616189v1	*Pinna nobilis*	Noble penshell	-
GCA_016746295.1	UT_Pstr_1.0	*Potamilus streckersoni*	Brazos heelsplitter	-
GCA_009026015.1	ASM902601v1	*Ruditapes philippinarum*	Manila clam	-
GCA_003671525.1	Sgl1.0	*Saccostrea glomerata*	Sydney rock oyster	-
GCA_007844125.1	ASM784412v1	*Sinonovacula constricta*	Chinese razor clam	-
GCA_009762815.1	ASM976281v1	*Sinonovacula constricta*	Chinese razor clam	-
GCA_013375625.1	ASM1337562v1	*Tegillarca granosa*	Blood clam	-
GCA_003401595.1	ASM340159v1	*Venustaconcha ellipsiformis*	Ellipse	-

Appendix B

Predictive biophysical models of bivalve larvae dispersal

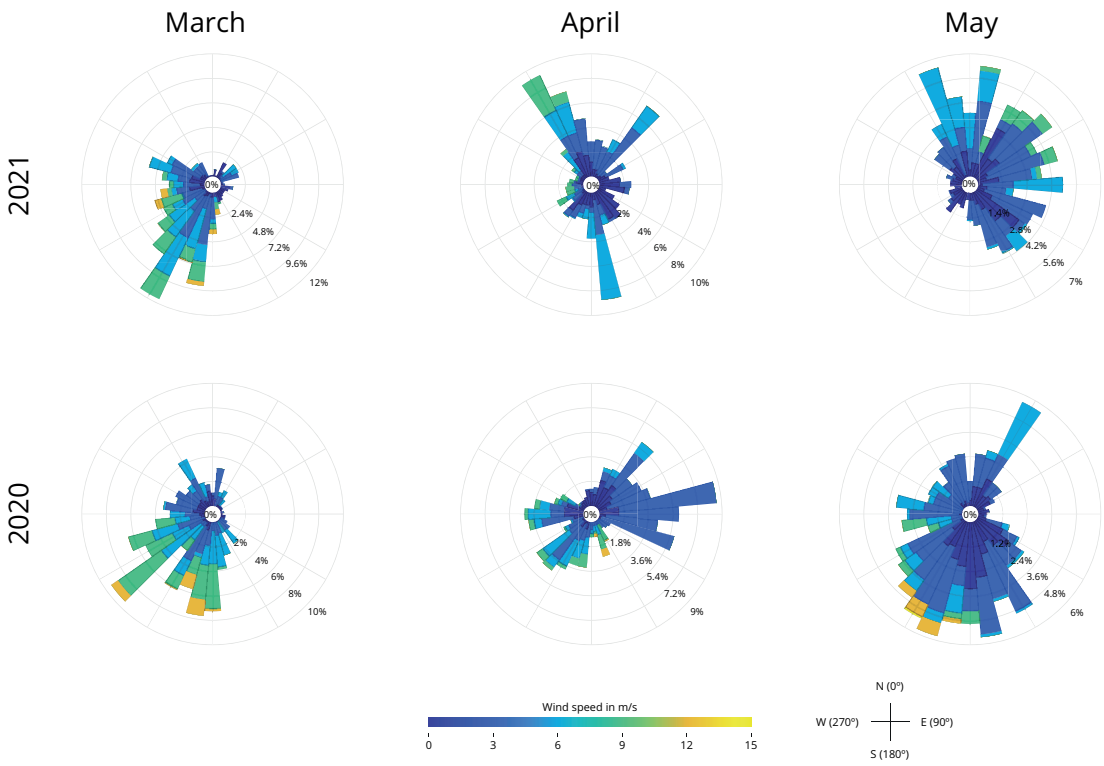

FIGURE B.1: Wind roses for March, April, and May 2021 and 2020. The wind direction determined by where it blows to. The colour scale represents wind speed (m/s), whereas the inner circle represents frequency. The data are from WRF v4.

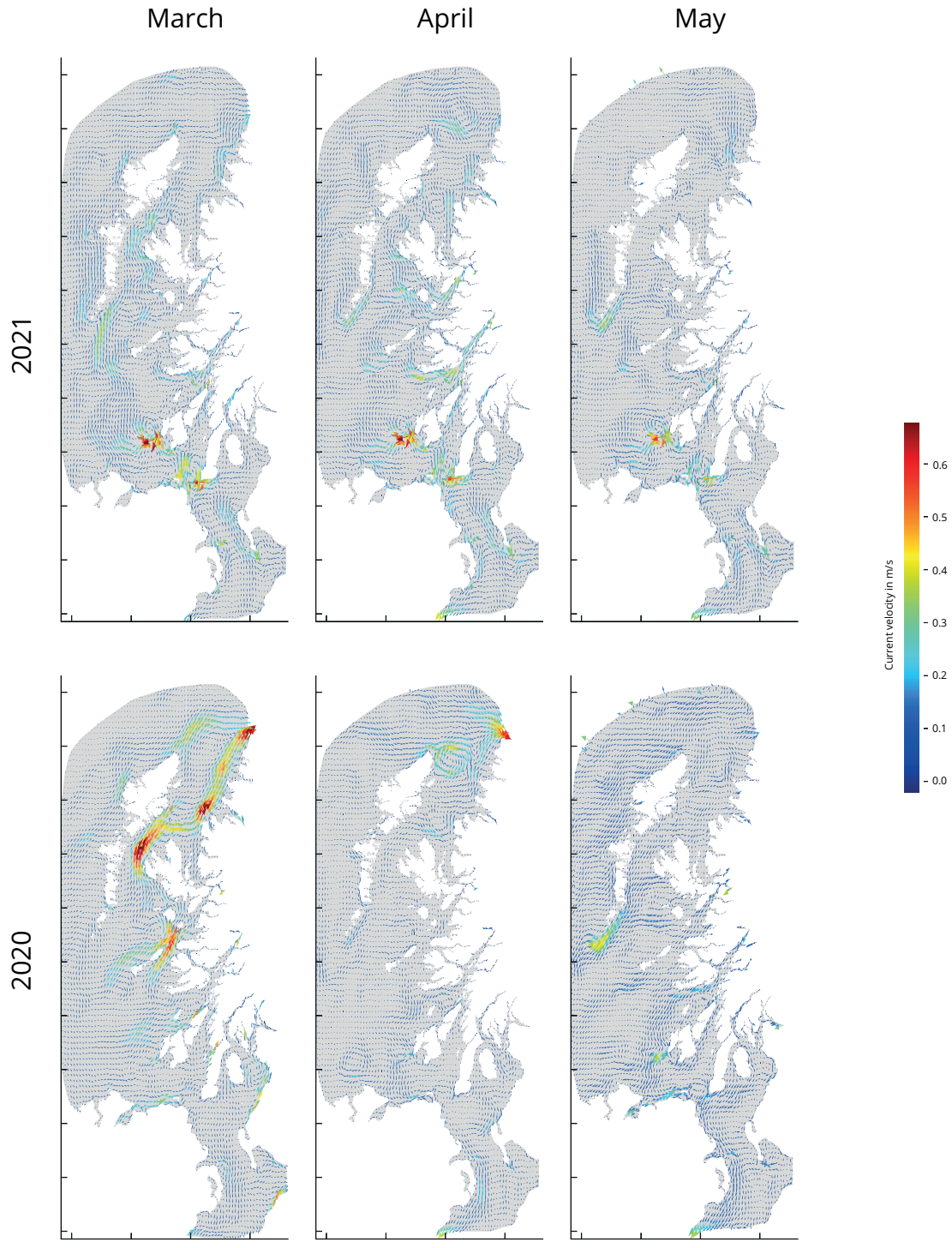

FIGURE B.2: Average current velocity (0 m to 10 m below sea level) for March, April, and May 2021 and 2020. Current velocity in m/s.

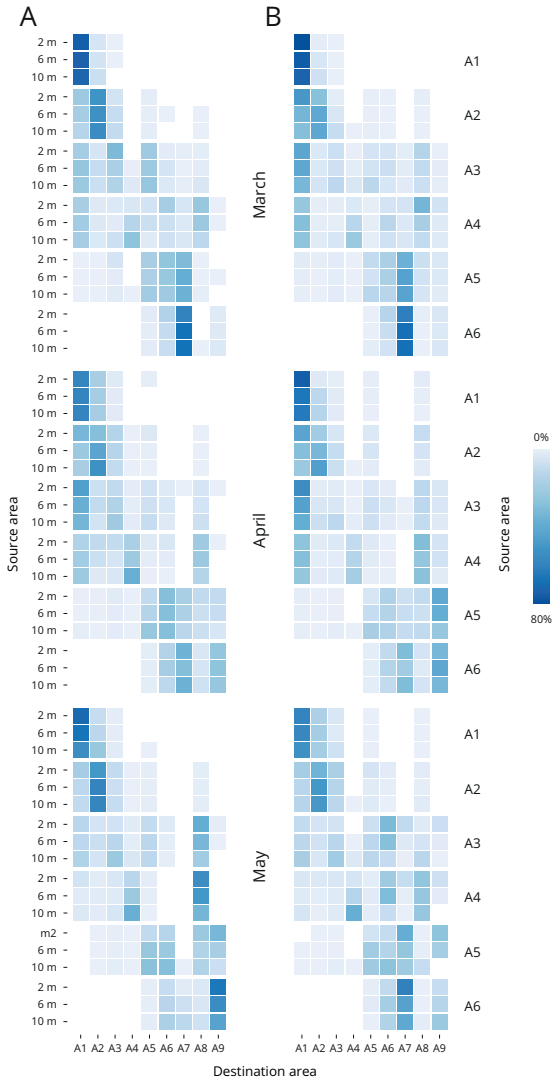

FIGURE B.3: Heatmaps of the particle connectivity between the source (Y axis) and destination area (X axis) for March, April, and May 2021, at 2 m, 6 m and 10 m. The particle accumulations from the Single day release setup, where the accumulation for each source regions have been averaged for (**A**) One month release (March, April, and May); (**B**) 45-day release with beginning points on 1st March, 1st April, and 1st May. Dark blue boxes corresponding to areas with higher particle accumulation, and light blue to white boxes corresponding to areas with lower particle accumulation in percent. Detailed means and standard deviations are provided in Table B.3

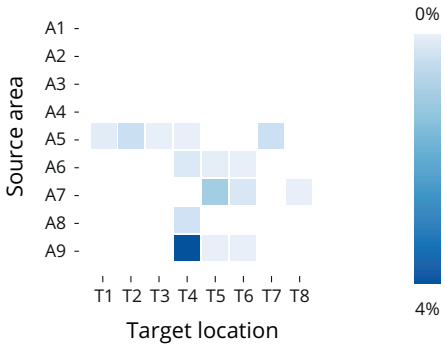

FIGURE B.4: Heatmaps of the particle connectivity between the source (Y axis) and destination area (X axis) through year 2021 for April at 6 m depth. The heatmap depicts the average amount of particles arriving in each target locations. Note the percentage represented in the figure goes up to 4% because we are only considering 10 particles released every hour during 14 days from each source point. Dark blue boxes corresponding to areas with higher particle accumulation, and light blue to white boxes corresponding to areas with lower particle accumulation in percent.

TABLE B.1: The particle accumulations in each target area from the Single day release setup (30-day release). Representing March, April and May, at three different depths in 2021 to 2017.

Month	Depth	Source	A1	A2	A3	A4	A5	A6	A7	A8	A9
April-21	2	A1	503180	154985	26424	0	6207	0	0	188	0
April-21	2	A2	332586	292794	144766	1612	31779	0	0	2133	0
April-21	2	A3	233417	37268	53760	5932	30603	14458	244	24076	714
April-21	2	A4	65650	45806	42572	71578	11406	17450	0	88384	735
April-21	2	A5	2593	2167	12865	989	58008	160417	96237	59719	51957
April-21	2	A6	0	0	0	0	9506	94227	229671	25040	159347
April-21	6	A1	509280	164377	17216	0	233	0	0	36	0
April-21	6	A2	228478	431885	134949	1571	8105	0	0	1241	0
April-21	6	A3	182933	48241	76150	6755	34769	20772	12	28779	49
April-21	6	A4	86316	35478	20842	92721	4915	7848	0	95947	0
April-21	6	A5	1626	2895	9519	1755	80945	158484	89194	45302	48397
April-21	6	A6	0	0	0	0	7711	120259	188191	32021	168058
April-21	10	A1	514527	159556	17102	0	0	0	0	0	0
April-21	10	A2	174249	533529	93715	594	3168	0	0	945	0
April-21	10	A3	168255	29412	102642	8345	43274	12859	0	35629	0
April-21	10	A4	94310	12158	16470	158561	1702	1081	0	61070	0
April-21	10	A5	3293	876	9654	1067	123653	150506	64332	42604	23311
April-21	10	A6	0	0	0	0	9383	74300	230677	43494	154668
March-21	2	A1	645802	43934	1373	0	91	0	0	0	0
March-21	2	A2	219827	515145	58799	0	12284	0	0	327	0
March-21	2	A3	97474	24427	156343	168	105642	8362	4336	6375	0
March-21	2	A4	75031	10808	13961	16602	25604	78917	20229	98906	212
March-21	2	A5	837	1148	30346	0	100303	143264	171679	1676	24
March-21	2	A6	0	0	0	0	13560	100155	386188	3	18489
March-21	6	A1	638698	51853	615	0	34	0	0	0	0
March-21	6	A2	181532	530072	87110	20	6566	448	0	601	0
March-21	6	A3	124593	43869	85567	2511	107340	28219	1566	9416	0
March-21	6	A4	86028	13535	9085	59345	34614	31141	14789	94876	274
March-21	6	A5	1982	1948	10511	101	89229	131034	211749	2005	509
March-21	6	A6	0	0	0	0	6126	61803	432730	10	17608
March-21	10	A1	618840	72312	48	0	0	0	0	0	0
March-21	10	A2	140313	558272	93592	70	11283	5	0	2602	0
March-21	10	A3	113533	38614	76959	12909	120658	15897	4127	20063	0
March-21	10	A4	79388	15389	31753	113804	12542	27547	16960	47085	24
March-21	10	A5	1101	835	12522	366	111112	115196	200597	4827	0
March-21	10	A6	0	0	0	0	10155	53320	432633	377	21550
May-21	2	A1	604303	76832	9044	0	0	0	0	0	0
May-21	2	A2	183512	499792	93047	3963	10943	0		14535	0
May-21	2	A3	62615	25836	29371	11743	47299	14125	0	184590	7333
May-21	2	A4	25018	8320	18362	54881	5239	26	0	232765	105
May-21	2	A5	3	595	3294	1109	56384	74056	0	121318	177714
May-21	2	A6	0	0	0	0	3894	56047	15748	21787	420433
May-21	6	A1	565147	104413	9158	0	51	0	0	0	0
May-21	6	A2	108889	597601	75876	982	8483	0	0	14223	0
May-21	6	A3	54807	40753	64581	7156	58970	6400	0	164089	1370
May-21	6	A4	8722	9353	12927	96507	3321	0	0	214660	0

Cont.

Month	Depth	Source	A1	A2	A3	A4	A5	A6	A7	A8	A9
May-21	6	A5	7	549	4059	894	125167	121074	131	80618	94750
May-21	6	A6	0	0	0	0	5489	81263	46199	26192	357408
May-21	10	A1	460542	196697	20812	0	344	0	0	0	0
May-21	10	A2	108308	589774	87637	738	10482	0	0	9289	
May-21	10	A3	77935	26872	113219	20250	56728	4267	0	101137	0
May-21	10	A4	13494	7609	19548	158703	1377	0	0	144821	0
May-21	10	A5	0	299	7616	956	141525	150223	775	79601	39745
May-21	10	A6	0	0	0	0	6799	106861	76447	43522	282182
April-20	2	A1	531085	83917	24329	401	17615	0	0	32669	0
April-20	2	A2	125317	389131	156022	875	39903	6774	24	79396	0
April-20	2	A3	15790	1503	10925	904	57943	155401	2780	104044	28977
April-20	2	A4	23201	927	2452	16408	17090	64787	0	168171	38759
April-20	2	A5	0	0	10	0	49749	239144	92428	8706	55700
April-20	2	A6	0	0	0	0	648	190660	224904	7137	94986
April-20	6	A1	491524	124271	33126	1585	21019	0	0	18330	0
April-20	6	A2	120206	487627	143469	195	39273	2150	0	13231	
April-20	6	A3	26069	5835	59229	788	127023	121126	1386	50553	1084
April-20	6	A4	15599	5033	29601	77874	33894	71260	0	60051	3719
April-20	6	A5	0	24	693	19	97085	227446	111252	5037	5316
April-20	6	A6	0	0	0	0	5586	148610	288050	3928	71577
April-20	10	A1	499846	157741	12211	233	11392	0	0	9543	0
April-20	10	A2	115198	521763	136619	240	20417	8	0	11035	0
April-20	10	A3	35418	8753	135086	10210	78366	68950	72	57118	513
April-20	10	A4	1874	1416	20738	154585	12764	40607	0	100786	724
April-20	10	A5	0	25	3929	33	134880	169665	120344	8143	6966
April-20	10	A6	0	0	0	0	10797	94432	329824	6863	71680
March-20	2	A1	341793	157576	157104	5039	15823	0	0	12737	0
March-20	2	A2	78493	466804	139761	14190	25331	10054	16434	51941	750
March-20	2	A3	3686	2113	5608	977	24314	75147	201395	76256	8575
March-20	2	A4	12255	4974	15178	9363	31813	53391	60985	138514	8679
March-20	2	A5	0	0	57	0	51448	56883	329027	7734	2933
March-20	2	A6	0	0	0	0	1512	88009	428372	10	495
March-20	6	A1	360051	175784	126813	5563	10253	0	0	11605	0
March-20	6	A2	63225	495501	139096	5140	35992	9965	9499	46538	420
March-20	6	A3	6427	7607	34181	14245	39752	53561	131184	96019	4471
March-20	6	A4	8779	11937	17166	109758	29733	32730	48225	79955	3389
March-20	6	A5	0	0	1309	2	43601	46414	342036	11774	2426
March-20	6	A6	0	0	0	0	1218	68847	446034	1217	843
March-20	10	A1	380229	238205	13129	4871	30427	0	0	24168	0
March-20	10	A2	84985	524590	126170	895	39225	5319	7164	17575	24
March-20	10	A3	23737	8650	74888	23227	72878	30272	113918	36737	883
March-20	10	A4	3049	1997	11292	174264	19400	45678	31752	52235	1557
March-20	10	A5	24	0	4989	72	50854	21591	362465	5676	1522
March-20	10	A6	0	0	0	0	722	48483	447637	2676	18563
May-20	2	A1	643807	28095	0	0	0	0	0	0	0
May-20	2	A2	287980	456125	56450	0	0	0	0	0	0
May-20	2	A3	83207	52675	141308	2894	85999	24541	211	11386	0
May-20	2	A4	27645	34033	56478	33793	108783	125	0	77251	0
May-20	2	A5	99	2937	12468	144	129683	212333	75573	3579	8016
May-20	2	A6	0	0	0	0	14280	120132	301131	260	82583
May-20	6	A1	630594	38539	0	0	0	0	0	0	0
May-20	6	A2	192388	487115	93958	0	523	0	0	451	0
May-20	6	A3	94587	50417	122440	5497	82791	24460	1766	18930	0
May-20	6	A4	28846	45722	37570	32497	93822	1516	0	87651	0
May-20	6	A5	267	3383	5677	643	90264	217113	113507	5757	9103
May-20	6	A6	0	0	0	0	4284	146638	217560	996	148405
May-20	10	A1	558031	110788	0	0	0	0	0	0	0
May-20	10	A2	128328	554568	79187	0	1963	0	0	484	0
May-20	10	A3	96768	35951	138423	5366	82996	17230	468	17701	0
May-20	10	A4	29441	19264	35730	87367	32365	4034	0	106528	0
May-20	10	A5	912	2801	6689	201	94744	152087	164730	10954	13765
May-20	10	A6	0	0	0	0	8143	122254	247743	2990	136600
April-19	2	A1	276979	26259	142544	1666	152206	35892	9	44238	0
April-19	2	A2	0	472904	73897	3699	124747	60559	21578	42966	0
April-19	2	A3	0	0	9495	945	80742	193382	82937	19783	4934
April-19	2	A4	0	24	1605	13711	20749	181599	1925	116062	0

Cont.

Month	Depth	Source	A1	A2	A3	A4	A5	A6	A7	A8	A9
April-19	2	A5	0	0	3757	0	103230	92790	202303	7501	36215
April-19	2	A6	0	0	0	0	8781	71036	319745	1585	116695
April-19	6	A1	275442	130045	159780	3198	97592	0	0	25037	0
April-19	6	A2	0	579044	83555	5847	77754	37641	592	22719	0
April-19	6	A3	0	122	44538	2213	149346	177150	12082	15970	0
April-19	6	A4	0	206	9159	71518	95780	104329	2263	57421	0
April-19	6	A5	0	0	2328	0	85621	165688	184779	2268	7207
April-19	6	A6	0	0	0	0	3187	97275	324659	11419	79432
April-19	10	A1	352937	161591	116522	2888	39145	0	0	18102	0
April-19	10	A2	0	620067	82796	8006	55727	8113	0	31455	0
April-19	10	A3	0	3665	108685	5484	168935	84419	334	28299	0
April-19	10	A4	0	846	22499	120392	96466	19634	0	54902	0
April-19	10	A5	0	24	9729	18	182416	150070	86045	12930	0
April-19	10	A6	0	0	0	0	11422	135397	252434	27231	79737
March-19	2	A1	61207	157596	76	0	0	0	0	0	0
March-19	2	A2	68879	731520	5513	0	199	0	0	208	0
March-19	2	A3	107843	95003	70152	3257	123125	411	188	3220	0
March-19	2	A4	65238	55264	19126	214	48866	6365	35	12250	0
March-19	2	A5	1025	1423	23276	16	130138	106384	186584	415	19
March-19	2	A6	0	0	0	0	745	63513	452707	1391	20
March-19	6	A1	143633	75022	208	0	0	0	0	0	0
March-19	6	A2	217297	574552	14162	0	52	0	0	26	0
March-19	6	A3	233206	12170	60656	2127	71342	6004	9042	8453	0
March-19	6	A4	105819	3069	20999	18366	19731	9114	1200	28112	0
March-19	6	A5	2815	353	37750	10	97575	67655	238332	2896	1443
March-19	6	A6	0	0	0	0	646	38021	460328	186	19194
March-19	10	A1	180633	38137	107	0	0	0	0	0	0
March-19	10	A2	277075	443393	81972	326	2906	0	0	396	0
March-19	10	A3	201633	7329	83432	18509	44653	11403	15381	18885	0
March-19	10	A4	68165	2443	15768	63319	10266	1036	92	45785	0
March-19	10	A5	2992	368	34759	250	65717	51776	281724	9008	237
March-19	10	A6	0	0	0	0	2880	37145	460938	48	17388
May-19	2	A1	654997	21783	14371	0	0	0	0	0	0
May-19	2	A2	252567	412517	114247	143	5091	0	0	2313	0
May-19	2	A3	47890	27929	143355	29004	69645	27831	0	56875	0
May-19	2	A4	13517	18792	82378	130455	51702	0	0	46121	0
May-19	2	A5	8	717	8631	2978	161756	142630	0	63597	52775
May-19	2	A6	0	0	0	48	18567	123137	11679	89379	272202
May-19	6	A1	569083	94526	14868	0	0	0	0	0	0
May-19	6	A2	153949	549048	74010	140	5669	0	0	1129	0
May-19	6	A3	50197	29523	163934	11138	86599	17695	0	38143	0
May-19	6	A4	13408	21770	72057	68162	69420	0	0	61353	0
May-19	6	A5	0	895	9486	2088	157604	162277	64	60401	31079
May-19	6	A6	0	0	0	0	15510	213131	14540	53554	217044
May-19	10	A1	543655	121407	10229	0	0	0	0	0	0
May-19	10	A2	118027	582462	82085	0	404	0	0	24	0
May-19	10	A3	52977	26251	147725	7561	101620	6376	9	34253	0
May-19	10	A4	6113	23348	99031	72894	24460	0	0	67060	0
May-19	10	A5	127	536	11979	633	206702	159107	307	46867	1526
May-19	10	A6	0	0	0	0	21647	243828	55139	53546	136623
April-18	2	A1	150905	825068	310745	32	233268	279	0	11514	0
April-18	2	A2	1801	373826	125832	75	245555	47958	0	10621	0
April-18	2	A3	0	1898	26678	743	149525	199702	8241	14673	25
April-18	2	A4	21	8159	61266	13775	107683	1305	0	14844	0
April-18	2	A5	0	0	3485	9	31573	156022	242513	10434	272
April-18	2	A6	0	0	0	0	669	160823	319922	1766	14069
April-18	6	A1	36032	56458	85530	51	37679	0	0	3119	0
April-18	6	A2	484	536413	142050	83	103211	9698	0	14109	0
April-18	6	A3	0	8057	76131	920	193830	88671	5023	29625	0
April-18	6	A4	13	5144	63650	26992	103678	314	0	7418	0

Cont.

Month	Depth	Source	A1	A2	A3	A4	A5	A6	A7	A8	A9
April-18	6	A5	0	0	4901	0	73089	230229	128155	6167	0
April-18	6	A6	0	0	0	0	1494	121917	300619	18766	39019
April-18	10	A1	239271	1051627	220658	305	14599	0	0	5193	0
April-18	10	A2	1649	591256	162445	133	34977	350	0	15389	0
April-18	10	A3	0	12535	143695	761	157582	31290	924	55156	0
April-18	10	A4	7	4465	55192	61440	74505	0	0	11637	0
April-18	10	A5	0	1	8404	3	151230	176087	90193	15819	0
April-18	10	A6	0	0	0	0	2514	60950	324057	47584	37971
March-18	2	A1	18451	67251	54583	2663	47348	0	0	28087	0
March-18	2	A2	1149	255227	298541	8030	144810	3625	0	88791	0
March-18	2	A3	0	1346	103643	9929	92412	73779	1854	99557	0
March-18	2	A4	0	3251	73416	24423	47157	0	0	58982	0
March-18	2	A5	0	0	8824	38	24804	244033	83079	85030	766
March-18	2	A6	0	0	0	0	445	158781	146721	78659	88128
March-18	6	A1	31097	73759	60932	1325	29711	0	0	21726	0
March-18	6	A2	5	408436	262520	4143	65078	1214	0	60431	0
March-18	6	A3	0	8239	112143	7974	118703	43759	4882	94507	0
March-18	6	A4	0	2676	84468	17877	41621	0	0	60635	0
March-18	6	A5	0	4	10929	51	121358	201209	74454	34616	0
March-18	6	A6	0	0	0	0	739	81700	167435	133356	125911
March-18	10	A1	386710	676002	375051	2311	46986	0	0	43561	0
March-18	10	A2	0	498438	218053	1123	54162	531	0	32251	0
March-18	10	A3	0	14561	194532	2706	102476	20459	4334	53421	0
March-18	10	A4	0	2000	84022	44905	32610	0	0	43761	0
March-18	10	A5	0	4	10929	51	121358	201209	74454	34616	0
March-18	10	A6	0	0	0	0	997	140224	190901	133545	44712
May-18	2	A1	222067	364540	846149	7	58199	0	0	40427	0
May-18	2	A2	1086	644385	77605	1016	43330	714	0	32713	0
May-18	2	A3	21	27	6530	2221	153072	50859	0	127958	0
May-18	2	A4	607	2821	40519	19283	26733	71	0	97163	0
May-18	2	A5	0	0	308	24	77459	245113	58444	30068	8444
May-18	2	A6	0	0	0	0	480	200570	304517	28	12710
May-18	6	A1	23667	108029	86377	0	105	0	0	60	0
May-18	6	A2	6631	612317	151419	279	22707	8	0	12283	0
May-18	6	A3	582	2477	106615	1896	209186	3384	0	61497	0
May-18	6	A4	5093	4722	66097	43221	39067	0	0	49092	0
May-18	6	A5	0	0	1313	38	147401	182331	60644	28429	10501
May-18	6	A6	0	0	0	0	3169	157854	321612	2371	30536
May-18	10	A1	46408	166420	6026	0	0	0	0	0	0
May-18	10	A2	25509	623554	136896	273	11193	0	0	8901	0
May-18	10	A3	12621	7619	160949	7112	161420	2054	0	50341	0
May-18	10	A4	10376	4242	48350	74284	24802	0	0	45248	0
May-18	10	A5	0	27	8745	144	208530	149855	55384	18605	3716
May-18	10	A6	0	0	0	0	15804	136257	332383	5967	22526
April-17	2	A1	26099	192776	5	0	0	0	0	0	0
April-17	2	A2	17003	750908	37791	0	554	0	0	0	0
April-17	2	A3	10238	83529	195760	7	108882	4	0	4423	0
April-17	2	A4	9218	99242	87167	9372	2175	0	0	0	0
April-17	2	A5	6	288	45160	0	268030	123930	0	11525	0
April-17	2	A6	0	84	5497	0	27208	248529	215146	20002	79
April-17	6	A1	34449	182615	1751	0	0	0	0	0	0
April-17	6	A2	35063	670324	95421	0	4173	0	0	1030	0
April-17	6	A3	9793	64969	191962	89	116074	0	0	20156	0
April-17	6	A4	15239	80130	68299	34740	7603	0	0	1287	0
April-17	6	A5	0	684	31305	19	276269	111219	0	19872	0
April-17	6	A6	0	230	6783	359	9505	190240	275998	15137	8067
April-17	10	A1	61903	155532	1423	0	0	0	0	0	0
April-17	10	A2	35933	641770	119953	0	7112	0	0	1250	0
April-17	10	A3	13481	54078	214237	452	100625	0	0	20072	0
April-17	10	A4	19896	52495	53508	55241	20425	0	0	5669	0
April-17	10	A5	45	753	22638	70	257749	105018	131	57193	12
April-17	10	A6	0	0	2565	8826	3992	207049	276236	2850	13695
March-17	2	A1	25765	181902	11181	0	0	0	0	0	0
March-17	2	A2	38	660790	93659	0	43878	4787	0	3226	0
March-17	2	A3	0	3660	69821	108	246761	57066	192	25589	0
March-17	2	A4	0	7911	89876	8834	60733	3816	0	36171	0

Cont.

Month	Depth	Source	A1	A2	A3	A4	A5	A6	A7	A8	A9
March-17	2	A5	0	0	3485	0	89893	281751	73162	301	52
March-17	2	A6	0	0	0	0	1723	155644	360133	0	48
March-17	6	A1	11228	205227	2416	0	0	0	0	0	0
March-17	6	A2	17	659596	122031	39	22557	739	0	1317	0
March-17	6	A3	1	13873	156128	511	197225	18300	192	16943	0
March-17	6	A4	15	25563	89939	29200	40680	91	0	21822	0
March-17	6	A5	0	123	137776	15	1186041	1208611	600453	7312	61
March-17	6	A6	0	0	0	0	12132	128315	368564	1033	6850
March-17	10	A1	63760	118099	36727	0	43	0	0	3	0
March-17	10	A2	2983	647408	147291	7	7759	0	0	832	0
March-17	10	A3	356	20823	229174	333	133285	5388	0	13774	0
March-17	10	A4	308	22370	80646	57370	28543	0	0	18040	0
March-17	10	A5	0	444	22794	0	133383	151952	130499	8554	227
March-17	10	A6	0	0	0	0	8468	82515	393227	14611	9329
May-17	2	A1	130	117404	62670	0	38253	0	0	383	0
May-17	2	A2	89	473736	139136	0	176585	14231	0	2456	0
May-17	2	A3	26	3118	58388	1234	202558	116580	3325	17568	0
May-17	2	A4	344	4164	44405	11505	124274	0	0	22636	0
May-17	2	A5	0	0	1190	0	27417	195387	222211	153	0
May-17	2	A6	0	0	0	0	883	86780	388931	597	40454
May-17	6	A1	18019	147918	44161	0	8619	0	0	138	0
May-17	6	A2	1691	650350	117874	0	35530	24	0	491	0
May-17	6	A3	567	14092	122372	1205	194151	48494	960	21269	0
May-17	6	A4	1987	10104	71068	37830	71463	0	0	14851	0
May-17	6	A5	0	0	7835	0	83320	215740	133687	3967	0
May-17	6	A6	0	0	0	0	549	191434	305540	1477	17640
May-17	10	A1	63484	131442	19664	0	4043	0	0	220	0
May-17	10	A2	2282	625119	152492	0	25571	0	0	659	0
May-17	10	A3	377	31635	158808	4763	167255	15589	0	24647	0
May-17	10	A4	1037	20023	56802	68339	50720	0	0	10329	0
May-17	10	A5	0	0	8860	0	188294	193999	40527	10231	0
May-17	10	A6	0	0	0	0	23303	208454	242381	21243	11386

TABLE B.2: Mean and standard deviation calculation for the particles connectivity between source and destination area. The particle accumulations from Single day release setup, where the accumulation for each source regions have been averaged for March, April, and May at 2 m, 6 m, and 10 m depth for the years 2021 to 2017; 30-day release and 45-day release with beginning points on 1st March, 1st April, and 1st May 2021 (Mean ±Standard error).

Simulation	Year	Source/ Target area	A1	A2	A3	A4	A5	A6	A7	A8	A9
30-day	2021	A1	81.7 ±3.2	16.6 ±2.7	1.6 ±0.5	0	0.1 ±0.1	0	0	0	0
30-day	2021	A2	23.1 ±2.9	62.7 ±3.9	12.0 ±1.1	0.1 ±0.1	1.4 ±0.3	0	0	0.6 ±0.2	0
30-day	2021	A3	31.0 ±5.0	8.8 ±0.7	21.1 ±3.0	2.1 ±0.5	16.8 ±2.8	3.5 ±0.6	0.3 ±0.2	16.2 ±5.9	0.3 ±0.2
30-day	2021	A4	17.3 ±3.3	5.1 ±1.3	6.0 ±1.0	26.5 ±4.6	3.3 ±1.1	5.3 ±2.5	1.7 ±0.9	34.8 ±6.3	0
30-day	2021	A5	0.3 ±0.1	0.3 ±0.1	2.5 ±0.6	0.2 ±0.0	22.7 ±2.4	30.7 ±2.1	20.9 ±6.3	11.3 ±3.2	11.2 ±4.4
30-day	2021	A6	0	0	0	0	1.6 ±0.2	16.1 ±1.5	43.8 ±10.5	4.2 ±1.1	34.4 ±9.6
30-day	2020	A1	72.3 ±5.8	18.0 ±3.2	5.9 ±2.8	0.3 ±0.1	1.7 ±0.5	0	0	1.8 ±0.6	0
30-day	2020	A2	16.7 ±2.9	61.2 ±2.2	14.9 ±1.4	0.3 ±0.2	2.8 ±0.7	0.5 ±0.2	0.5 ±0.2	3.1 ±1.2	0
30-day	2020	A3	10.8 ±3.2	4.8 ±1.7	20.3 ±4.7	1.8 ±0.7	18.4 ±2.5	16.3 ±4.2	12.9 ±6.6	13.4 ±3.0	1.3 ±0.8
30-day	2020	A4	5.1 ±1.1	4.2 ±1.6	7.7 ±1.7	23.5 ±5.9	12.8 ±3.4	10.7 ±2.9	4.6 ±2.4	29.4 ±3.7	1.9 ±1.3
30-day	2020	A5	0	0.2 ±0.1	0.9 ±0.3	0	18.5 ±2.7	33.5 ±6.4	42.6 ±8.8	1.7 ±0.2	2.6 ±1.3
30-day	2020	A6	0	0	0	0	1.0 ±0.3	22.1 ±2.8	62.9 ±6.0	0.6 ±0.2	13.4 ±3.5
30-day	2019	A1	63.0 ±7.9	22.8 ±6.9	7.4 ±3.3	0.1 ±0.1	4.7 ±2.7	0.6 ±0.6	0	1.4 ±0.8	0
30-day	2019	A2	15.2 ±4.6	68.9 ±4.1	8.5 ±1.5	0.3 ±0.1	3.8 ±1.9	1.5 ±0.9	0.3 ±0.3	1.4 ±0.7	0
30-day	2019	A3	19.3 ±7.2	5.6 ±2.5	23.3 ±4.5	2.2 ±0.8	25.1 ±3.4	14.7 ±6.4	3.4 ±2.3	6.3 ±1.4	0.1 ±0.1
30-day	2019	A4	14.7 ±7.0	5.7 ±2.8	13.7 ±3.7	20.0 ±5.0	17.5 ±3.2	10.7 ±6.3	0.4 ±0.2	17.3 ±3.2	0
30-day	2019	A5	0.2 ±0.1	0.1 ±0.0	3.6 ±1.0	0.2 ±0.1	30.2 ±3.8	27.8 ±3.4	29.3 ±8.3	5.3 ±2.0	3.3 ±1.5
30-day	2019	A6	0	0	0	0	1.8 ±0.5	22.1 ±4.8	50.6 ±12.2	5.2 ±2.1	20.3 ±6.0
30-day	2018	A1	15.2 ±1.8	45.1 ±6.2	27.6 ±5.1	0.2 ±0.1	8.4 ±2.8	0	0	3.4 ±1.6	0
30-day	2018	A2	0.5 ±0.3	62.6 ±5.5	21.8 ±2.9	0.2 ±0.1	10.0 ±3.1	0.9 ±0.6	0	3.8 ±1.1	0
30-day	2018	A3	0.4 ±0.3	1.6 ±0.4	26.3 ±5.1	1.0 ±0.3	38.3 ±3.4	14.6 ±5.1	0.7 ±0.2	17.2 ±3.5	0
30-day	2018	A4	0.9 ±0.6	2.0 ±0.3	31.2 ±2.3	17.6 ±3.4	26.9 ±5.1	0.1 ±0.1	0	21.4 ±5.0	0
30-day	2018	A5	0	0	1.3 ±0.3	0	23.8 ±4.8	45.2 ±3.0	22.5 ±4.7	6.5 ±1.9	0.7 ±0.3
30-day	2018	A6	0	0	0	0	0.6 ±0.3	27.1 ±2.7	53.6 ±5.1	9.4 ±3.7	9.3 ±2.5
30-day	2017	A1	15.5 ±3.6	72.7 ±5.0	9.1 ±3.5	0	2.6 ±1.9	0	0	0	0
30-day	2017	A2	1.3 ±0.6	79.6 ±3.0	14.1 ±1.5	0	4.5 ±2.3	0.3 ±0.2	0	0.2 ±0.0	0
30-day	2017	A3	1.0 ±0.5	8.0 ±2.4	38.5 ±5.0	0.2 ±0.1	40.4 ±4.2	7.2 ±3.2	0.1 ±0.1	4.5 ±0.5	0
30-day	2017	A4	2.6 ±1.2	17.3 ±5.5	34.4 ±2.7	16.7 ±3.6	21.8 ±6.1	0.2 ±0.2	0	7.0 ±1.9	0
30-day	2017	A5	0	1.4 ±1.4	8.4 ±4.5	1.6 ±1.6	35.3 ±7.0	34.4 ±6.0	14.9 ±6.0	4.0 ±1.5	0
30-day	2017	A6	0	0	0.3 ±0.2	0.2 ±0.2	1.9 ±0.6	32.4 ±3.7	61.1 ±4.2	1.7 ±0.6	2.3 ±0.8
45-day	2021	A1	83.3 ±3.7	13.2 ±2.9	2.7 ±0.9	0	0.4 ±0.1	0	0	0.4 ±0.2	0
45-day	2021	A2	34.9 ±5.2	47.6 ±4.2	10.7 ±2.0	0.1 ±0.0	3.1 ±0.8	0.5 ±0.3	0	3.0 ±1.1	0
45-day	2021	A3	40.7 ±6.6	5.9 ±0.8	11.4 ±2.2	1.5 ±0.6	10.0 ±1.2	13.0 ±4.7	2.6 ±1.3	11.8 ±1.5	3.2 ±1.0
45-day	2021	A4	23.6 ±4.7	2.1 ±0.4	3.4 ±0.6	19.3 ±4.3	1.6 ±0.3	11.8 ±4.3	3.3 ±1.4	30.1 ±2.9	4.8 ±1.0
45-day	2021	A5	1.0 ±0.3	0.3 ±0.1	1.2 ±0.3	0.1 ±0.0	14.9 ±2.6	20.1 ±2.0	32.3 ±6.3	8.0 ±1.6	22.1 ±6.7
45-day	2021	A6	0	0	0	0	1.1 ±0.5	18.8 ±2.5	65.0 ±6.4	4.2 ±1.5	10.9 ±3.5
45-day	2020	A1	79.5 ±5.0	14.4 ±3.8	2.6 ±1.5	0.4 ±0.2	1.7 ±0.7	0	0	1.5 ±0.6	0
45-day	2020	A2	20.6 ±2.3	56.1 ±3.6	14.1 ±2.7	0.5 ±0.2	3.3 ±0.9	1.5 ±0.7	1.0 ±0.6	2.6 ±1.0	0.2 ±0.2
45-day	2020	A3	14.9 ±4.4	6.5 ±2.0	12.4 ±3.4	2.3 ±0.7	10.8 ±1.9	15.7 ±1.9	18.4 ±9.1	12.3 ±2.6	6.8 ±4.3
45-day	2020	A4	10.0 ±2.4	2.5 ±1.0	5.6 ±1.6	19.8 ±5.0	7.1 ±1.3	13.9 ±3.3	8.4 ±4.7	25.9 ±5.1	6.7 ±3.2
45-day	2020	A5	0.2 ±0.1	0.1 ±0.0	0.5 ±0.2	0.1 ±0.0	13.5 ±2.1	26.5 ±5.0	46.8 ±9.6	4.7 ±1.3	7.6 ±4.1
45-day	2020	A6	0 ±0.1	0	0 ±0.2	0	1.2 ±2.1	14.8 ±5.0	58.9 ±9.6	2.3 ±1.3	22.8 ±4.1
45-day	2019	A1	51.3 ±0.0	29.5 ±0.0	8.2 ±0.0	0.4 ±0.0	5.4 ±0.3	0.5 ±1.7	0 ±7.5	4.3 ±0.9	0.4 ±6.2
45-day	2019	A2	8.4 ±9.4	68.0 ±8.9	12.0 ±2.5	0.3 ±0.3	4.9 ±2.1	1.5 ±0.5	0.1 ±0.0	4.2 ±2.5	0.6 ±0.4
45-day	2019	A3	5.8 ±4.0	17.8 ±3.9	21.9 ±2.3	1.1 ±0.1	17.9 ±1.3	18.4 ±0.9	1.8 ±0.1	11.8 ±1.6	3.6 ±0.5
45-day	2019	A4	6.3 ±2.1	10.5 ±7.3	22.3 ±8.3	7.5 ±0.5	18.7 ±3.6	11.7 ±5.3	0.1 ±0.9	16.0 ±2.1	6.8 ±2.6
45-day	2019	A5	0.1 ±2.1	0.3 ±5.0	1.3 ±7.7	0.1 ±3.1	21.7 ±3.8	35.8 ±3.8	28.8 ±0.1	5.6 ±2.9	6.3 ±4.7
45-day	2019	A6	0	0 ±0.1	0 ±0.3	0	1.3 ±4.3	19.2 ±4.1	46.6 ±6.7	3.7 ±1.2	29.1 ±2.3
45-day	2018	A1	12.7 ±0.0	28.0 ±0.0	33.0 ±0.0	0.7 ±0.0	15.3 ±0.5	0.9 ±3.5	0 ±11.3	9.4 ±1.2	0 ±7.6
45-day	2018	A2	0.6 ±3.8	53.1 ±5.0	19.4 ±7.3	0.8 ±0.4	13.0 ±5.6	3.1 ±0.8	0	9.9 ±5.2	0
45-day	2018	A3	0.1 ±0.4	1.8 ±7.2	19.4 ±2.7	1.2 ±0.5	27.3 ±3.7	25.5 ±1.6	2.5 ±0.0	21.8 ±5.3	0.4 ±0.0
45-day	2018	A4	0.9 ±0.5	2.1 ±0.8	25.8 ±3.8	11.3 ±2.3	28.3 ±5.6	6.7 ±2.7	0	24.9 ±5.3	0
45-day	2018	A5	0	0	1.3 ±0.5	0	16.6 ±3.2	37.8 ±4.3	30.5 ±8.8	10.0 ±3.7	3.8 ±2.0
45-day	2018	A6	0	0	0	0	0.3 ±0.2	19.2 ±3.4	54.5 ±10.3	12.4 ±4.5	13.6 ±4.0
45-day	2017	A1	16.1 ±4.5	46.2 ±7.3	29.5 ±7.9	1.1 ±1.1	6.0 ±4.3	0	0	1.0 ±0.6	0
45-day	2017	A2	1.6 ±0.4	57.0 ±7.0	28.4 ±4.4	0.5 ±0.5	10.6 ±6.6	0.3 ±0.2	0	1.6 ±0.7	0
45-day	2017	A3	1.2 ±0.3	5.7 ±1.6	31.7 ±4.7	1.3 ±0.8	39.4 ±5.8	10.3 ±3.2	0.6 ±0.2	9.8 ±4.7	0
45-day	2017	A4	4.1 ±1.0	11.2 ±2.2	34.8 ±4.0	8.9 ±3.1	30.6 ±7.9	0.9 ±0.6	0	9.6 ±3.2	0
45-day	2017	A5	0	0.1 ±0.0	2.7 ±0.6	0.1 ±0.1	22.4 ±4.5	40.4 ±5.4	28.2 ±8.3	5.9 ±2.8	0.2 ±0.1
45-day	2017	A6	0	0	0.2 ±0.1	0	0.6 ±0.2	22.9 ±3.8	65.8 ±6.5	6.1 ±2.5	4.4 ±1.4

TABLE B.3: Mean and standard deviation calculation for the particles connectivity between source and destination area. The data obtained is from the "Single day" release set up. The table shows data averaged for March, April, and May at each different depth (2 m, 6 m, and 10 m) for year 2021 (Mean ±Standard error).

Year	Source/ Target area	Depth	A1	A2	A3	A4	A5	A6	A7	A8	A9
2021	A1	2	84.6 ±6.1	13.3 ±4.8	1.8 ±1.1	0	0.3 ±0.3	0	0	0	0
2021	A1	6	83.1 ±5.4	15.6 ±4.7	1.3 ±0.7	0	0	0	0	0	0
2021	A1	10	77.3 ±6.4	20.8 ±5.5	1.8 ±0.9	0	0	0	0	0	0
2021	A2	2	30.4 ±5.6	54.1 ±8.9	12.3 ±3.1	0.2 ±0.1	2.3 ±0.8	0	0	0.7 ±0.6	0
2021	A2	6	21.5 ±4.3	64.5 ±5.9	12.3 ±2.3	0.1 ±0.1	1.0 ±0.1	0	0	0.7 ±0.6	0
2021	A2	10	17.5 ±2.4	69.5 ±2.0	11.4 ±0.2	0.1 ±0.0	1.0 ±0.3	0	0	0.5 ±0.3	0
2021	A3	2	32.9 ±12.9	7.4 ±1.0	20.0 ±9.6	1.5 ±0.9	15.4 ±5.5	3.1 ±0.5	0.4 ±0.3	18.6 ±14.8	0.7 ±0.6
2021	A3	6	30.2 ±9.3	11.1 ±0.6	18.9 ±1.4	1.4 ±0.4	16.7 ±5.3	4.6 ±1.6	0.1 ±0.1	16.9 ±12.2	0.1 ±0.1
2021	A3	10	29.9 ±6.6	7.9 ±0.9	24.3 ±2.7	3.4 ±0.9	18.3 ±5.9	2.7 ±0.9	0.3 ±0.3	13.0 ±6.2	0
2021	A4	2	16.1 ±4.5	6.3 ±3.5	7.3 ±2.6	13.9 ±4.7	4.1 ±1.8	9.4 ±7.0	2.0 ±2.0	40.8 ±13.4	0.1 ±0.1
2021	A4	6	16.1 ±4.5	6.3 ±3.5	7.3 ±2.6	13.9 ±4.7	4.1 ±1.8	9.4 ±7.0	2.0 ±2.0	40.8 ±13.4	0.1 ±0.1
2021	A4	10	18.1 ±7.2	3.4 ±0.6	6.5 ±1.4	41.6 ±4.3	1.5 ±1.0	2.8 ±2.6	1.6 ±1.6	24.4 ±8.8	0
2021	A5	2	0.3 ±0.2	0.3 ±0.1	3.5 ±1.7	0.2 ±0.1	16.1 ±3.1	28.3 ±5.8	19.9 ±11.1	13.9 ±8.0	17.5 ±12.2
2021	A5	6	0.3 ±0.1	0.4 ±0.2	1.8 ±0.5	0.2 ±0.1	22.5 ±3.4	31.2 ±2.5	22.5 ±13.6	9.9 ±5.3	11.1 ±6.4
2021	A5	10	0.3 ±0.2	0.2 ±0.1	2.3 ±0.3	0.2 ±0.1	29.3 ±2.5	32.5 ±3.3	20.1 ±13.2	10.1 ±5.1	5.0 ±2.7
2021	A6	2	0	0	0	0	1.7 ±0.5	16.1 ±2.7	40.6 ±20.7	3.0 ±1.5	38.5 ±22.7
2021	A6	6	0	0	0	0	1.2 ±0.1	17.0 ±3.3	43.0 ±21.8	3.8 ±1.9	35.0 ±19.1
2021	A6	10	0	0	0	0	1.7 ±0.2	15.2 ±3.0	47.8 ±19.9	5.7 ±2.8	29.7 ±14.6

TABLE B.4: Mean and standard deviation calculation for the particle connectivity between the source and destination area for March, April, and May 2021, at 2 m, 6 m and 10 m. The particle accumulations from the Single day release setup, where the accumulation for each source regions have been averaged for one month release (March, April, and May) and 45-day release with beginning points on 1st March, 1st April, and 1st May.

Simulation	Month	Depth	Source/Target	A1	A2	A3	A4	A5	A6	A7	A8	A9
30-day	Mar-21	2	A1	93.0	6.4	0.2	0	0	0	0	0	0
30-day	Mar-21	2	A2	27.0	64.0	7.3	0	1.5	0	0	0	0
30-day	Mar-21	2	A3	24.0	6.1	39.0	0	26.0	2.1	1.1	1.6	0
30-day	Mar-21	2	A4	22.0	3.2	4.1	4.9	7.5	23.2	5.9	29.0	0.1
30-day	Mar-21	2	A5	0.2	0.3	6.8	0	22.0	31.9	38.2	0.4	0
30-day	Mar-21	2	A6	0	0	0	0	2.6	19.3	74.5	0	3.6
30-day	Mar-21	6	A1	92.0	7.5	0.1	0	0	0	0	0	0
30-day	Mar-21	6	A2	23.0	66.0	11.0	0	0.8	0.1	0	0.1	0
30-day	Mar-21	6	A3	31.0	11.0	21.0	0.6	27.0	7.0	0.4	2.3	0
30-day	Mar-21	6	A4	25.0	3.9	2.6	17.0	10.0	9.1	4.3	28.0	0.1
30-day	Mar-21	6	A5	0.4	0.4	2.3	0	20.0	29.2	47.2	0.4	0.1
30-day	Mar-21	6	A6	0	0	0	0	1.2	11.9	83.5	0	3.4
30-day	Mar-21	10	A1	90.0	10.0	0	0	0	0	0	0	0
30-day	Mar-21	10	A2	17.0	69.0	12.0	0	1.4	0	0	0.3	0
30-day	Mar-21	10	A3	28.0	9.6	19.0	3.2	30.0	4.0	1.0	5.0	0
30-day	Mar-21	10	A4	23.0	4.5	9.2	33.0	3.6	8.0	4.9	14.0	0
30-day	Mar-21	10	A5	0.2	0.2	2.8	0.1	25.0	25.8	44.9	1.1	0
30-day	Mar-21	10	A6	0	0	0	0	2.0	10.3	83.5	0.1	4.2
30-day	Apr-21	2	A1	73.0	22.0	3.8	0	0.9	0	0	0	0
30-day	Apr-21	2	A2	41.0	36.0	18.0	0.2	3.9	0	0	0.3	0
30-day	Apr-21	2	A3	58.0	9.3	13.0	1.5	7.6	3.6	0.1	6.0	0.2
30-day	Apr-21	2	A4	19.0	13.0	12.0	21.0	3.3	5.1	0	26.0	0.2
30-day	Apr-21	2	A5	0.6	0.5	2.9	0.2	13.0	36.1	21.6	13.0	12.0
30-day	Apr-21	2	A6	0	0	0	0	1.8	18.2	44.4	4.8	31.0
30-day	Apr-21	6	A1	74.0	24.0	2.5	0	0	0	0	0	0
30-day	Apr-21	6	A2	28.0	54.0	17.0	0.2	1.0	0	0	0.2	0
30-day	Apr-21	6	A3	46.0	12.0	19.0	1.7	8.7	5.2	0	7.2	0
30-day	Apr-21	6	A4	25.0	10.0	6.1	27.0	1.4	2.3	0	28.0	0
30-day	Apr-21	6	A5	0.4	0.7	2.2	0.4	18.0	36.2	20.4	10.0	11.0
30-day	Apr-21	6	A6	0	0	0	0	1.5	23.3	36.5	6.2	33.0
30-day	Apr-21	10	A1	74.0	23.0	2.5	0	0	0	0	0	0
30-day	Apr-21	10	A2	22.0	66.0	12.0	0.1	0.4	0	0	0.1	0
30-day	Apr-21	10	A3	42.0	7.3	26.0	2.1	11.0	3.2	0	8.9	0
30-day	Apr-21	10	A4	27.0	3.5	4.8	46.0	0.5	0.3	0	18.0	0
30-day	Apr-21	10	A5	0.8	0.2	2.3	0.3	29.0	35.9	15.3	10.0	5.6
30-day	Apr-21	10	A6	0	0	0	0	1.8	14.5	45.0	8.5	30.0
30-day	May-21	2	A1	88.0	11.0	1.3	0	0	0	0	0	0
30-day	May-21	2	A2	23.0	62.0	12.0	0.5	1.4	0	0	1.8	0
30-day	May-21	2	A3	16.0	6.7	7.7	3.1	12.0	3.7	0	48.0	1.9
30-day	May-21	2	A4	7.3	2.4	5.3	16.0	1.5	0	0	68.0	0
30-day	May-21	2	A5	0	0.1	0.8	0.3	13.0	17.0	0	28.0	41.0
30-day	May-21	2	A6	0	0	0	0	0.8	10.8	3.0	4.2	81.0
30-day	May-21	6	A1	83.0	15.0	1.3	0	0	0	0	0	0
30-day	May-21	6	A2	14.0	74.0	9.4	0.1	1.1	0	0	1.8	0
30-day	May-21	6	A3	14.0	10.0	16.0	1.8	15.0	1.6	0	41.0	0.3
30-day	May-21	6	A4	2.5	2.7	3.7	28.0	1.0	0	0	62.0	0
30-day	May-21	6	A5	0	0.1	1.0	0.2	29.0	28.3	0	19.0	22.0
30-day	May-21	6	A6	0	0	0	0	1.1	15.7	8.9	5.1	69.0

Cont.

Simulation	Month	Depth	Source/Target	A1	A2	A3	A4	A5	A6	A7	A8	A9
30-day	May-21	10	A1	68.0	29.0	3.1	0	0.1	0	0	0	0
30-day	May-21	10	A2	13.0	73.0	11.0	0.1	1.3	0	0	1.2	0
30-day	May-21	10	A3	19.0	6.7	28.0	5.1	14.0	1.1	0	25.0	0
30-day	May-21	10	A4	3.9	2.2	5.7	46.0	0.4	0	0	42.0	0
30-day	May-21	10	A5	0	0.1	1.8	0.2	34.0	35.7	0.2	19.0	9.4
30-day	May-21	10	A6	0	0	0	0	1.3	20.7	14.8	8.4	55.0
45-day	Mar-21	2	A1	99.0	1.3	0.1	0	0	0	0	0	0
45-day	Mar-21	2	A2	61.0	35.0	1.6	0	0.7	0.3	0	1.1	0
45-day	Mar-21	2	A3	53.0	4.2	9.3	0.6	6.9	6.7	1.6	18.0	0.4
45-day	Mar-21	2	A4	30.0	0.9	1.2	4.5	0.8	6.8	6.9	42.0	7.3
45-day	Mar-21	2	A5	2.8	0.8	2.2	0.3	12.0	21.4	45.3	10.0	4.6
45-day	Mar-21	2	A6	0	0	0	0	0.3	16.9	76.9	1.0	4.8
45-day	Mar-21	6	A1	94.0	5.9	0.2	0	0	0	0	0	0
45-day	Mar-21	6	A2	42.0	51.0	5.0	0	0.6	0.2	0	0.4	0
45-day	Mar-21	6	A3	52.0	4.5	8.7	1.8	7.3	9.5	2.4	12.0	1.3
45-day	Mar-21	6	A4	36.0	1.1	1.0	16.0	0.8	15.5	4.3	22.0	3.7
45-day	Mar-21	6	A5	1.5	0.3	1.8	0.1	7.7	21.3	55.1	7.9	4.2
45-day	Mar-21	6	A6	0	0	0	0	0.3	10.1	85.1	0.4	4.1
45-day	Mar-21	10	A1	91.0	8.7	0.1	0	0	0	0	0	0
45-day	Mar-21	10	A2	36.0	52.0	11.0	0.1	0.8	0.2	0	0.4	0
45-day	Mar-21	10	A3	41.0	6.8	14.0	5.1	15.0	5.7	2.5	9.6	0.8
45-day	Mar-21	10	A4	33.0	3.1	5.9	28.0	0.8	8.1	5.5	12.0	3.7
45-day	Mar-21	10	A5	0.9	0.3	2.3	0.2	16.0	16.7	54.2	6.1	3.1
45-day	Mar-21	10	A6	0	0	0	0	1.8	7.8	84.7	0.4	5.4
45-day	Apr-21	2	A1	93.0	3.5	0.9	0	0.8	0	0	2.2	0
45-day	Apr-21	2	A2	54.0	25.0	5.7	0	4.0	0	0	11.0	0
45-day	Apr-21	2	A3	68.0	2.4	3.1	0.1	5.3	1.9	0	14.0	5.0
45-day	Apr-21	2	A4	33.0	3.1	4.1	12.0	3.2	0.2	0	37.0	7.1
45-day	Apr-21	2	A5	1.4	0.4	0.5	0	5.8	19.4	9.0	12.0	51.0
45-day	Apr-21	2	A6	0	0	0	0	1.7	12.5	38.0	6.6	41.0
45-day	Apr-21	6	A1	81.0	14.0	2.9	0	0.9	0	0	0.8	0
45-day	Apr-21	6	A2	36.0	41.0	11.0	0	6.2	0	0	6.9	0
45-day	Apr-21	6	A3	56.0	4.9	5.4	0.3	9.4	4.8	0.1	14.0	5.5
45-day	Apr-21	6	A4	36.0	1.6	2.1	18.0	2.8	0.5	0	31.0	7.7
45-day	Apr-21	6	A5	1.1	0.2	0.6	0	11.0	18.7	10.6	8.8	48.0
45-day	Apr-21	6	A6	0	0	0	0	1.6	17.7	25.1	3.6	52.0
45-day	Apr-21	10	A1	80.0	17.0	2.5	0	0.5	0	0	0.3	0
45-day	Apr-21	10	A2	28.0	58.0	10.0	0.2	2.1	0	0	2.1	0
45-day	Apr-21	10	A3	48.0	9.9	14.0	1.5	8.8	2.6	0.3	12.0	3.2
45-day	Apr-21	10	A4	31.0	2.5	3.6	24.0	0.9	0.7	0	34.0	3.0
45-day	Apr-21	10	A5	1.1	0.3	1.2	0.2	22.0	19.5	11.6	13.0	30.0
45-day	Apr-21	10	A6	0	0	0	0	1.7	12.5	38.0	6.6	41.0
45-day	May-21	2	A1	74.0	20.0	5.1	0	0.4	0	0	0.1	0
45-day	May-21	2	A2	24.0	44.0	21.0	0	7.1	2.3	0	2.1	0
45-day	May-21	2	A3	12.0	7.1	7.4	0	8.8	39.0	12.4	3.7	9.5
45-day	May-21	2	A4	5.1	4.0	5.4	6.3	1.6	26.0	11.4	32.0	8.7
45-day	May-21	2	A5	0	0.1	0.4	0	7.3	11.9	47.5	0.4	33.0
45-day	May-21	2	A6	0	0	0	0	0.9	13.0	75.5	0.1	11.0
45-day	May-21	6	A1	72.0	24.0	3.8	0	0.3	0	0	0.1	0
45-day	May-21	6	A2	16.0	62.0	17.0	0	3.1	1.1	0	1.1	0
45-day	May-21	6	A3	15.0	7.8	16.0	0.4	14.0	35.3	3.3	6.8	2.6
45-day	May-21	6	A4	3.6	1.6	3.6	17.0	2.5	37.8	1.1	30.0	2.2
45-day	May-21	6	A5	0.1	0.1	0.6	0	25.0	17.9	31.1	1.0	24.0
45-day	May-21	6	A6	0	0	0	0	2.5	24.1	55.7	0.3	17.0
45-day	May-21	10	A1	66.0	24.0	8.5	0	0.6	0	0	0.3	0
45-day	May-21	10	A2	17.0	61.0	15.0	0.1	3.6	0.2	0	2.7	0
45-day	May-21	10	A3	22.0	5.5	25.0	3.9	15.0	11.5	0.6	16.0	0.1
45-day	May-21	10	A4	6.2	0.7	3.7	47.0	1.3	10.9	0.1	30.0	0
45-day	May-21	10	A5	0.1	0.1	1.6	0.1	26.0	33.9	26.7	11.0	0
45-day	May-21	10	A6	0	0	0	0	0.3	19.0	51.0	1.4	28.0

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