Investigating Integrated Multi-Trophic Aquaculture at different spatial scales

A thesis submitted to the University of Stirling for the degree of Doctor of Philosophy

by

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DECLARATION

I declare that this thesis in an original piece of work conducted independently by myself and the work contained here has not been submitted for any other degree. All research material and sources of information have been duly acknowledged and cited.

Signature of Candidate

Anastasios Baltadakis

ABSTRACT

Integrated Multi-Trophic Aquaculture is being promoted as one of the solutions for the sustainable growth of Aquaculture in the coming years. Open water IMTA endeavours to efficiently use the wastes originating from fin-fish cage farming as a feed source for extractive organisms, alleviating potential environmental impacts. IMTA is a relatively vague term, characterised by a variety of system designs, species combinations and services provided, constituting it complex and multidisciplinary. Regardless the positive suggestions of proof of concept through conceptual desk-based modelling studies, lab-based feeding experiments and in-situ small scale experimental sites. IMTA in the West is not being promoted into formal legislation as a mean for the strategic sustainable growth of aquaculture. Since IMTA is a spatial issue, there is a considerable lack of understanding in terms of planning and setting up and IMTA site

In order to achieve this, the aims of this thesis was to create a holistic view on IMTAs main challenges, opportunities and limitation presented in each spatial scale, taking into account the effect of the variability of the natural environment along with the different requirements of the extractive organisms in terms of biology and rearing design.

The results of this PhD study suggest that not all regions nor all farm sites are suitable for IMTA and even when a farm is suitable, suitability varies among combination of species chosen. Considering a farm is suitable for IMTA, there are variations in terms of where the IMTA extractive species could be located within the farms waste footprint originating from its biological and stocking density limitations, influencing the bioremediation efficiency of the system. Following the modelling of bioremediation of IMTA, there needs to be a demonstrated *in-situ* trophic connection between the fed species and extractive species through the use of biological tracers which efficacy varying depending the type of extractive organism targeted. In the case of dissolved inorganic waste, a more suitable approach would be to assess IMTA nutrient budgets at regional waterbody scale (RIMTA). However, each sub-region within the water-body will vary in terms of species produced and biomass of the species produced divided into nutrient

source and sinks. Consideration of the hydrodynamic conditions of each sub-region could aid on planning activities within a water-body scale to mitigate potential pelagic impacts, offering an integrated ecological management approach.

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Chapter 1 Introduction

The global population continues to increase and there is a need to increase food production to meet growing demands (Tilman et al. 2011; Bodirsky et al. 2015). Aquatic food has a superior nutritional profile against conventional terrestrial meat, characterised by high quality of proteins, essential amino acids, omega-3 fatty acids, minerals and vitamins (Tacon and Metian 2013). In addition, aquaculture food production compared to land-based livestock products is considered to be a more sustainable form of production with less greenhouse gas emissions (Tsakiridis et al. 2020). Aquaculture is the fastest growing food production sector and it is considered the only way of increasing the supply of aquatic products due to limited capacity of capture fisheries to increase their landings (FAO 2018). However, although it is important to increase aquaculture production, there is also a need to ensure that any development is sustainable. This has been promoted through the Ecosystem Approach to Aquaculture (EAA) as a strategy to integrate any aquaculture activity with the surrounding ecosystem in a manner that promotes sustainable development, equity and resilience of social-ecological systems (Soto et al. 2008)

Over the last few decades, Integrated Multi-trophic Aquaculture (IMTA), has gained increased attention as way of increasing aquaculture production sustainably (Chopin et al. 2001; Neori et al. 2004; Ridler et al. 2007; Troell et al. 2009), adhering practically to the principles of the EAA at farm level (Barrington et al. 2009). IMTA is a system where species from lower trophic levels utilise the waste from species at higher trophic levels (Chopin et al. 2001). For example, waste from fed finfish species become inputs for lower trophic level species, either organic waste (e.g. deposit feeders, suspension feeders) or dissolved inorganic waste (e.g. seaweeds) (Troell et al. 2009; Barrington et al. 2009), as illustrated in Figure 1-1. The concept originates from polyculture systems that have been common practice in Asia for many years, in small rice ponds with shrimp or fish and fish with crabs (Troell et al. 2009), and in coastal open water systems as in Sanggou Bay, China, where abalone, kelp and finfish are cultured within the same system in close proximity (Fang et al. 2016). However, the difference with polyculture in the rest of the world is that IMTA attempts the deliberate, planned and in right proportion integration of different trophic level species under more intensive cultivation open water systems (Barrington et al. 2009).

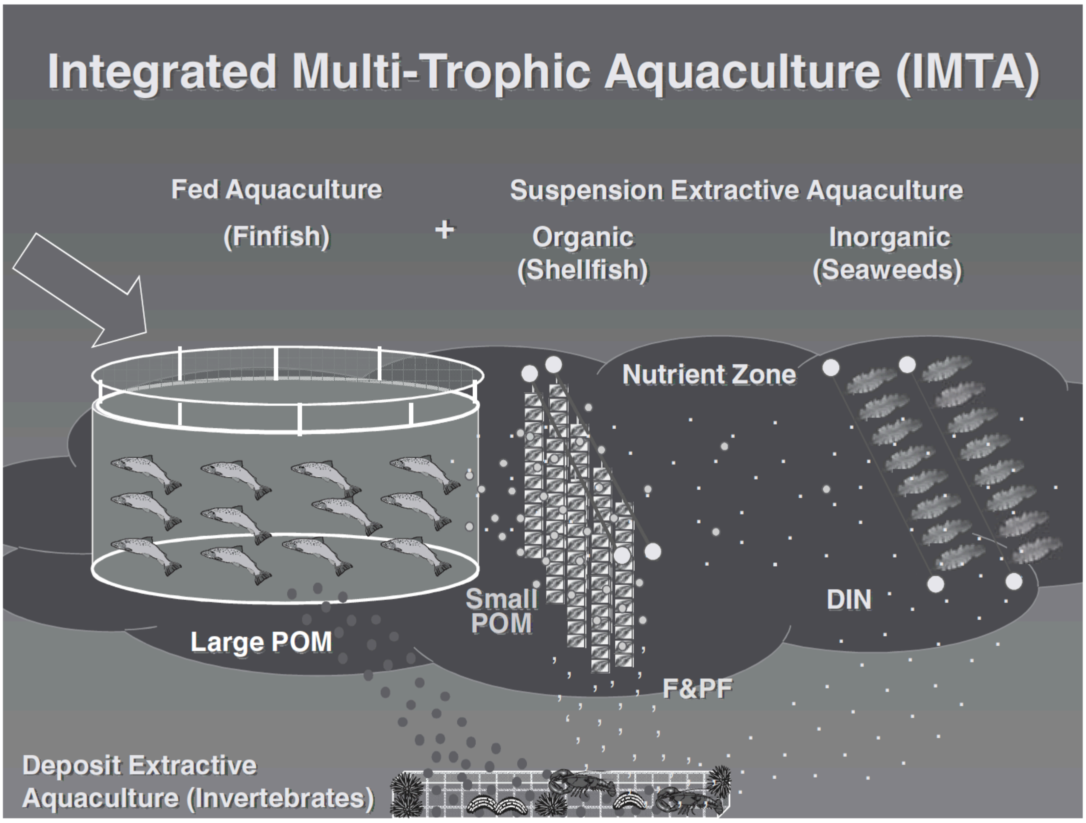

Figure 1-1 Conceptual diagram illustrates the sources and sinks of an IMTA system. Suspension organic extractive species (e.g. shellfish) feed on the small POM (particulate organic matter), inorganic suspension extractive organisms (e.g seaweeds) feed on dissolved nutrients, deposit extractive invertebrates consume particulate nutrients. Figure sourced from Chopin et al. 2006

There are different reasons and purposes for setting up an IMTA system. As the extractive organisms are using the waste from other species, they can act as bioremediators and reduce environmental impact (Chopin et al. 2012). The bioremediation potential is also a route to increase public perception and social acceptability of aquaculture (Barrington et al. 2008; Ellis and Tiller 2019). Growing multiple species within the same farm increases the diversity of production and this provides additional products that can be sold (Shi et al. 2013). In some areas, aquaculture development is limited by lack of available space so

IMTA is a way of increasing production at existing farm sites. However, although IMTA has received a lot of attention in recent years, there are still few examples of commercial systems (Alexander and Hughes 2017; Kleitou et al. 2018).

IMTA can take place in tanks (Shpigel et al. 2016), ponds (Kibria and Haque 2018), and coastal and open-water environments (Fossberg et al. 2018; Neofitou et al. 2019; Sanz-Lazaro and Sanchez-Jerez 2017; Wang et al. 2014). Coastal and open water systems are more complicated type of systems, characterised by their 'leaky' nature due to the rapid dilution of the nutrients to the surrounding environment (Filgueira et al. 2017). Whereas, on the contrary, closed systems usually offer the opportunity to control the nutrient flow, water circulation, temperature and other water quality variables or often mechanically remove some nutrients. In addition, land based IMTA systems can easily monitor the inflow and outflow of nutrients, providing a more robust assessment of the performance of the system (Reid et al. 2018). Coastal and open-water environments represent the dominant production method of fed finfish in the West, however, there is great concern regarding the environmental impacts, hindering development. Therefore, the premise of IMTA is primarily targeted on such systems. IMTA systems on open water coastal environments are more complicated and thus difficult to establish and test. The design is largely dependent on the hydrodynamics of the surrounding environment, with amount of waste and direction of the waste stream being variable based on local conditions (Filgueira et al. 2017; Reid et al. 2011), influencing the spatial extent of the influence (Fossberg et al. 2018). Limitations also occur from the capacity of each species to consume waste, its rearing design (Yu et al. 2011) and the available space needed.

In coastal and open-water environments, a range of species combinations have been explored in field trials and modelling studies. Bivalves, such as mussels and scallops are often considered in IMTA system designs (Gao et al. 2006; Handa et al. 2012; Lander et al. 2013; Sara et al. 2009). Studies of bivalves in open water systems have shown varying results concerning growth ranging from positive (Handa et al. 2012; Lander et al. 2013) to no effect on growth (Navarrete-Mier et al. 2010). In addition, trophic connectivity between the wastes of the fed finfish and bivalves has been tested through the use of biological tracers. Stable isotopes and fatty acids indicating that the contribution of aquaculture derived nutrients to bivalves nutrition is relatively minimal (Handa et al 2012; Irisarri et al. 2015; Sanz-Lazaro and Sanchez-Jerez 2017). Likewise, seaweed is commonly employed for IMTA systems and has been shown to have the capacity to utilize dissolved inorganic nutrients released from salmon open-water farms (Buschmann et al. 1996; Wang et al. 2014). Equally, biological tracers such as stable isotopes have been proved to be useful indicators for the detection of farm derived nitrogen in seaweeds (Carballeira et al. 2013; Fossberg et al. 2018). However, trophic connectivity does not equate to bioremediation capacity since there are scalability issues involved. As an example, it has been highlighted that in order to mitigate a considerable amount of dissolved inorganic nitrogen (DIN), there needs to be an unrealistic cultivation of *S.latissima* (Wang et al. 2014). The same practicality issue was raised by Reid et al. 2013 where number of rafts necessary for the sequestration of nutrients would exceed the typical site leases area of the farm site, by using a mass balance modelling approach. That brings in questions the applicability of open water IMTA systems to serve as a bio mitigation mechanism since production of secondary organisms has to be in large quantities.

For coastal and open-water fish farms, there are a number of different issues that aquaculture industry must consider if planning and IMTA site. Field trials and modelling studies provide important information to understand the role of different species within IMTA designs and impact of the IMTA system on the environment. Field trials are used to map performance of IMTA systems under more realistic scenarios, looking at diet access of the extractive organisms (Irisarri et al. 2015), potential growth in relation to the stream of waste (Jiang et al. 2012) as well as growth based on the type of rearing design selected (Yu et al.2012). Models are a powerful forecasting tool (Reid et al. 2018), that allow investigation of different production and environmental scenarios, species combinations and wider impacts on the ecosystem. For example, models have been constructed to calculate the culture area and density of seaweeds necessary to sequester a considerable weight of soluble inorganic nutrients derived from Atlantic salmon (*Salmo salar)* farms (Reid et al. 2013). In addition other models have used hypothetical aquaculture sites scenarios to calculate different components of the seston such as fish faeces, shellfish faeces, feed wastes and consequently measure the mitigation efficiency of an IMTA system comprised of mussels and fin-fish, with different spatial arrangements (Filgueira et al. 2017). Other models have been used to access bio-mitigation capacity of fish and sea cucumbers, by modelling the deposition rates of hypothetical farm sites of particulate organic matter in relation to absorbance rates of sea cucumbers (Chary et al. 2019; Cubillo et al. 2016; Zhang and Kitazawa 2016). These studies demonstrate that there is a great need to investigate how nutrients are transferred between species within IMTA systems.

The mechanism of uptake of nutrients from fish cage wastes will depend on the type of waste and also the species. Particulate and soluble wastes are released from fish in sea cages (Chen et al. 2003; Redmond et al. 2010; Wang et al. 2012). Fish farming activities release nutrients in the form of Carbon (C), Nitrogen (N) and Phosphorous (P). Dissolved inorganic N and P, DIN and DIP respectively are released through excretion. Inorganic C is released through respiration (Wang et al. 2012). Wastes in the particulate forms are released as particulate organic carbon, nitrogen and phosphorous (POC, PON and POP) through defecation and loss of feed. Dissolved organic C, N and P (DOC, DON and DOP) are released through the dissolution of the particulate organic fractions (Cromey et al. 2002; Wang et al. 2012). The different components of the nutrient wastes also have different distribution patterns. Dissolved inorganic nutrients such as DIN and DIP are readily available for phytoplankton and macroalgae or it might take several days before phytoplankton biomass responds (Olsen and Olsen 2008; Troell et al. 2009). Dissolved inorganic nutrients are travelling over greater distances, remaining for longer periods in the water column. Large particulate organic particles tent to sink rapidly within few tens of meters from the farm site, accumulating into the sediments on the seafloor, ultimately consumed by detritus eating animals (Brigolin et al. 2009; Cromey et al. 2002; Filgueira et al. 2017). Small suspended particulates can remain in suspension for longer periods and ultimately consumed filter-feeding zooplankton or mussels (Olsen and Olsen 2008). Therefore, each type of waste will require different spatial and ecological considerations as well as different targeted extractive species with the demonstrated physiological capacity to absorb the type of waste targeted. This will also greatly influence the spatial allocation of those extractive organisms in order to have an efficient connectivity. As an example, it has been suggested that for the case of particulate organic waste, a more reasonable approach would be the use of benthic detrivores such as sea cucumbers located in close vicinity to the farm site where majority of the wastes settles, requiring a farm scale approach (Filgueira et al. 2017). On the contrary for the case of dissolved waste, a more reasonable approach would be at watershed bay scale looking at nutrient budgets with the use of seaweeds and bivalves as extractive organisms.

Another component which influences the spatial management considerations of IMTA is also the mechanism of uptake of nutrients through the extractive organisms, being either direct or indirect. Studies have demonstrated the ability of IMTA extractive organisms to directly uptake waste particles (Bergvik et al. 2019; Handa et al. 2012). However, in the natural environment there is a wider dietary choice. Bivalves has been shown to prefer living organisms rather than non-living wastes, therefore, direct consumption of suspended solids has been suggested to be minimal (Sanz-Lazaro and Sanchez-Jerez 2017). Bivalves have demonstrated that removal of dissolved nutrients can be done indirectly by consuming phytoplankton and detritus (not dissolved nutrients in the water) stored in their tissues and shells (Reitsma et al. 2017; Rose et al. 2014; Rose et al. 2015). Therefore, direct and indirect uptake of nutrients influences the spatial management considerations of an IMTA system. For the case of bivalves, structures do not necessarily have to be close to the vicinity of the farm site. But within the same watershed scale system, following a nutrient budget approach. That brings another aspect of IMTA which has been surfaced under the term of Regional Integrated Multi-Trophic Aquaculture (RIMTA) (Sanz-Lazaro and Sanchez-Jerez 2020). Clearly, for both particulate and soluble wastes uptake of nutrients by extractive organisms in a coastal and open-water IMTA system will depend on location and other spatial factors. IMTA is inherently a spatial system and where the different species are placed will influence the transfer and consumption of nutrients. Consequently, there are considerations at different spatial scales that must be addressed when planning an IMTA system in coastal and open-water conditions.

From the outset, in the planning stage, it is essential to understand spatial issues associated with the environment that may affect the development and set-up of an IMTA system. Environmental conditions and biophysical parameters can vary considerably both spatially and temporally along a coastline (Chary et al. 2019b) and this will have great implications on the suitability of an IMTA system, both in terms of suitability of the physical structures but also suitability of the biological conditions of the extractive organism. Fish farms occupy a range of different settings and there may be differences in the suitability regarding the potential for installing an IMTA system. Spatial models can be used to assess the suitability of an area for aquaculture (Falconer et al. 2020), however most research has focused on monoculture and there is a need for more research into the spatio-temporal variability that may affect all components of an IMTA system.

If the physical conditions allow installation of the IMTA system, there is a need to understand how waste is dispersed into the environment as this will also affect the suitability of the site for IMTA. It is important to ensure that the extractive organisms have access to the waste, otherwise there is no trophic link, so the amount of feed available for extractive organisms (Chary et al. 2019; Cubillo et al. 2016; Ren et al. 2012; Watanabe et al. 2015). However, feed availability is just one consideration as conditions must also be suitable for the extractive organism to survive and grow. This is particularly important for benthic IMTA species as high levels of organic deposition underneath cages can alter the physicochemical profile of the sediments and cause anoxic conditions (Kalantzi and Karakassis 2006; Soto 2009). Thus, IMTA systems that plan to use benthic organisms to directly feed on the waste, there may be a spatial trade-off between locations that have sufficient feed, and areas that species can survive.

Even if an organism is placed in the waste stream, deposition of particulate matter might not equate to access to the feed source. This is important when exploring the potential for new species in IMTA. There is a need to understand how a species will uptake waste and ensure that the design of an IMTA accounts for this (Sanz-Lazaro and Sanchez-Jerez 2017). Trophic connectivity is easier to map under a controlled environment where the only source of feed is provided in the form of feed pellets and fin-fish waste. On the contrary in-situ studies are in nature more complicated since there are other sources of food available to the secondary organism. Concerning in-situ experimental studies, empirical observations are being used to measure physiological responses such as growth. Comparisons of growth between co-cultured organisms close in the vicinity with fed species in relation to reference sites has been performed for shellfish Lander et al. 2013; Sara et al. 2009) sea urchins (Cook and Kelly 2007) and sea cucumbers (Yokoyama, 2013, 2015). In order to assess whether there is a trophic relationship between the species in the open-water environment biological tracers are used. Fatty acids and stable isotopes originally where used as markers for assessing the fin-fish farming and the potential ecological impact they have on the wider trophic systems (Fernandez-Jover et al. 2009, 2011; White et al. 2017, 2019; Woodcock et al. 2018), but they also offer a way to examine the trophic relationship within an IMTA system (George and Parrish 2015; Irisarri et al. 2015; Redmond et al. 2010).

The overall aim of this thesis was to investigate the concept of IMTA at different spatial scales, through fieldwork and environmental modelling. This will allow to approach IMTA from different spatial angles, offering a holistic view over IMTAs limitations and future considerations when planning to establish13 a system. The aspect of IMTA on which this thesis is focused on is open cage fin-fish aquaculture using salmon as the fed species, whilst deposit feeders and suspended feeders were used as the secondary organisms. The specific objectives were:

1. To assess already commercial farm sites and regions for the suitability for IMTA through national scale site suitability assessment.
2. To assess the bio-mitigation capacity of an IMTA system and identify whether farm conditions affect the systems performance.
3. To investigate potential trophic relationships between salmon and a novel secondary trophic organism in a costal open-water IMTA system.
4. To investigate the concept of Regional Integrated Multi-Trophic Aquaculture (RIMTA) by looking at the interactions between not physically

connected aquaculture activities at water body scale and its consequent effect on the nutrient budgets.

The following chapters address these aims by investigating IMTA at the various spatial scales, with each chapter being a distinct study. Chapter 2 looks at IMTA at national scale using aquaculture site suitability methodologies for three different IMTA candidate species. This is a broad scale evaluation that can be used to identify potential areas for more detailed assessment. Chapter 3 focuses on the development of a farm scale spatial management approach to categorise farms based on their bioremediation potential, and suitability of locations for benthic extractive species immediately adjacent to fish cages. Ecosystem services provided by this type of IMTA will also be evaluated against the investment costs needed. Chapter 4 assesses the trophic relationship between salmon and lobsters at a small-scale experimental site by means of fatty acids and stable isotopes analysis. The effect on growth of lobsters under these conditions is appraised. Chapter 5 investigates the concept of RIMTA using water body scale mapping of the ecological interactions between Atlantic salmon and bivalve production.

Chapter 2 Investigating the spatial variability of suitable coastal ocean locations for IMTA at a national scale level

2.1 Introduction

Most mariculture takes place in sheltered, coastal environments that are close to the coast (Gentry et al. 2017). However, there are many activities competing for space and resources within coastal locations (e.g. tourism, fishing, military, renewable, ferry routes, conservation) (Lester et al., 2018; Sanchez-Jerez et al. 2016; Soto et al. 2009). Along the crowded coastlines, the sustainable development and expansion of aquaculture requires identification of locations that have suitable physical and biological conditions with no or minimal conflict with other resource users (Brugere et al. 2018; Sanchez-Jerez et al. 2016; Ross et al. 2013; Soto et al. 2009). However, site selection is complex, as multiple factors must be considered within any assessment (Falconer et al. 2016). Failure of mariculture planning in the past, and selection of unsuitable sites has led to a series of adverse environmental, social and economic impacts (FAO 2015; Suplicy et al. 2015).

Carrying capacity is an important concept for sustainable aquaculture development (Ross et al. 2013; Weitzman and Filgueira 2020). In broad terms, carrying capacity refers to the level of resource use that is sustainable in the long-term, and for aquaculture it has been divided into four categories; physical carrying capacity, production carrying capacity , ecological carrying capacity and social carrying capacity (Inglis et al. 2000; McKindsey et al. 2006; Ross et al., 2013). Physical carrying capacity is often considered first, since it evaluates the suitability of a site or an area with regards to the optimal conditions of the aquaculture setting, type of species cultured and the physical characteristics of the environment (Falconer et al. 2013; Ross et al. 2013). If a site cannot support aquaculture due to the physical characteristics, then that is a limiting factor and further assessment of other aspects of carrying capacity is redundant. Another reason for evaluating physical carrying capacity first is that it can be assessed at different spatial scales. Data collection and fieldwork can be expensive, especially if multiple sites are being considered, so a broad scale (e.g. national or regional level) assessment, of physical carrying capacity can be used to identify areas of interest, where more detailed assessment can take place (Falconer et al. 2013; Ross et al. 2013). It is important to note that physical carrying capacity does not address the level of production intensity, however it determines the total area which is potentially available for mariculture (Byron and Costa-Pierce 2013). Furthermore, the parameters considered will vary depending on the requirements of the species and system under investigation (Ross et al. 2013).

Physical environmental conditions tend to be characterised by a great deal of spatial and temporal heterogeneity which in turn creates significant implications for mariculture site selection (Falconer et al. 2013). For IMTA systems, this is a challenge as sites need to be able to support a number of species and different rearing systems. There are few examples of commercial-scale IMTA sites, but it as a start it is beneficial to investigate where IMTA can be established at existing finfish farms (Knowler et al. 2020). However, due to varying conditions between locations there is a need to identify farm sites that have meet the physical and biological requirements of the extractive species as well (Ross et al. 2013). It is also important to consider temporal variability of the locations as this may affect suitability of locations if species are to be grown all year round (Alexander and Hughes 2017).

Identification of farms that may be suitable for IMTA would also be an advantage for marine spatial planning. IMTA is considered as having potential for reducing conflicts in use of the sea areas, whilst increasing food production vertically, providing another source of income through the extractive organisms (Cavallo et al. 2020; Christie et al. 2013; Ellis and Tiller 2019; Wood et al. 2017). However, as highlighted by Barrington et al. (2009), both the location and species are important parts of the site selection process, and the different IMTA species will have their own requirements and services that they need and will provide. Therefore, when assessing the suitability of farm sites for IMTA it may be beneficial to evaluate several different combinations of species services provided.

Geographic Information Systems (GIS) are commonly used as a tool for the designation of suitable locations for aquaculture (Falconer et al. 2020). Examples of previous studies include assessment of shrimp pond aquaculture in Vietnam (Giap et al. 2006), shellfish in Japan (Radiarta et al. 2008), tilapia cages in Ghana (Asmah et al. 2021), shellfish in New Zealand (Longdill et al. 2008), and crabs and shrimp ponds in Bangladesh (Salam et al. 2003). It is expected that IMTA in coastal and open-water systems will be established at existing aquaculture sites. However, even within the same country, these can occupy a range of different conditions. A study by Gimpel et al. (2015) extended this to identify suitable locations for co-location of different aquaculture species and offshore windfarms in the German Exclusive Economic Zone (EEZ).

The aim of this chapter was to investigate IMTA at national scale level by assessing already commercial farm sites and regions for their suitability of three different IMTA candidate species. By using GIS based aquaculture site suitability methodologies, the level of spatio-temporal variability across and among the three extractive organisms suitability scores would be evaluated. This would considerably contribute and assist planning and management of IMTA at national scale level. In this study Scotland was used as a case study due to the large number of coastal fish farms that occupy a wide range of physical conditions.

2.2 Methodology

2.2.1 Study area

The coastline of Scotland covers over 9910 km of mainland and 8092 km island coastline. The west coast of Scotland is characterized by islands, high seas cliffs and fjordic inlets, typified by both exposed and sheltered conditions, whereas the east coast is more homogenous and is dominated by low-lying sedimentary shores (Baxter et al. 2011). Aquaculture plays an important role in the Scottish economy, providing a source of income and jobs. In 2018, production of Atlantic salmon (*Salmo salar*), reached 156,025 tonnes of whole fish weight, with an estimated farm gate value of £878 million (Munro 2018). More than 200 active commercial grow out salmon farms are located along the West coast, Highlands and Islands (Aquaculture Scotland 2020) (Figure 2-1). Along the East coast, there is a moratorium on salmon aquaculture development to protect the wild salmon rivers (Marine Scotland Information. 2020).

Marine planning in Scotland comes under the EU Marine Strategy Framework Directive and fulfils the requirements of the Maritime Spatial Planning EU Directive (2014/89/EU) (European commission 2014). In turn these two directives are integrated within the devolved administrations of the UK by the UK Marine Policy Statement and the UK Marine and Coastal Access Act (2009). The Marine Scotland Act (2010) gave the Scottish Government powers to plan and manage its territorial waters, except activities such as defence. The Act initiated a process for the development of a National Marine Plan (NMP). Scotland's National Marine Plan was finalized and adopted in March 2015 and provides the framework for managing all the activities and developments within Scotland's territorial waters. The NMP shall be implemented following a regional approach through designating eleven Scottish Marine Regions (SMR), with each region having its own designated members, with the necessary skills and expertise of marine planning. These groups are responsible for developing Regional Marine Plans (RMP). RMPs are developed by Marine Planning Partnerships (MPPs) and part of their role is to take initiatives and recommend planning at regional scale. An example of such regional planning produced under Marine Scotland Act (2010) was completed in March 2016 was the Pentland Firth and Orkney Waters (PFOW) and Shetland. This is the third in place Scotland's Marine Spatial Plan preceded by the Shetland Marine Spatial Plan in 2015 and the Clyde Marine Spatial Plan in 2010 (Smith and Jentoft 2017). Thus far aquaculture has been incorporated in Marine Spatial Plans as the legal means for the development of the sector both nationally and regionally. IMTA however, is not being included yet to any marine spatial plans, with no legal binding framework in place.

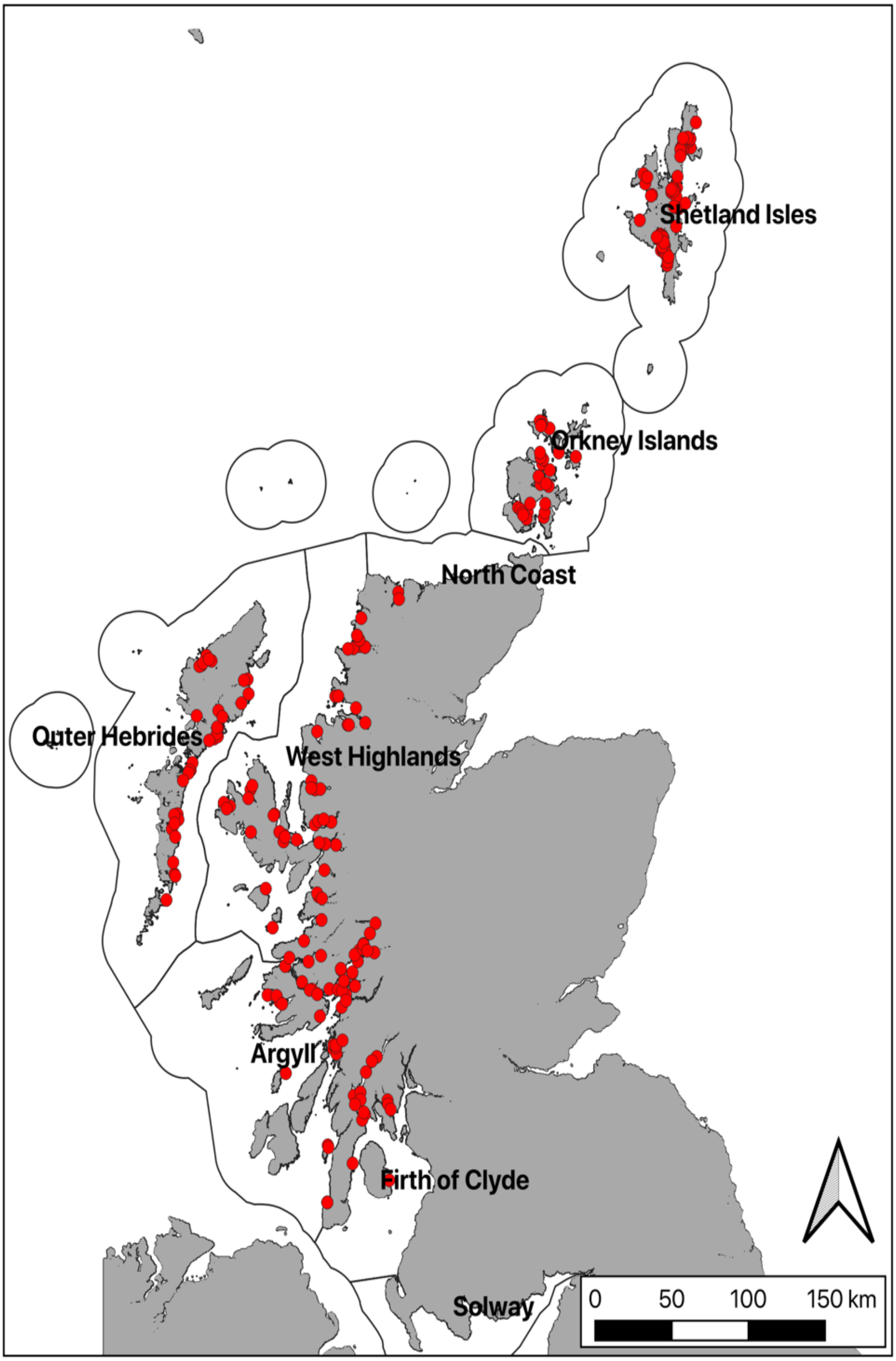

Figure 2-1 Illustration of the eight Scottish Marine regions with active Salmon farms (red dots). Data was sourced from http://aquaculture.scotland.gov.uk/map/map.aspx

2.2.2 IMTA species used within the model

Choosing an IMTA secondary organism for this chapter was based on native organisms along the Scottish waters, with each species selected for different reasons. The species were: European lobster (*Homarus gammarus*), chosen for the conservation services provided through restocking wild populations (Daniels et al. 2015). Sea cucumber (*Holothuria forskali*), and Sea urchin (*Paracentrotus lividus)*, for their proven record of waste consumption and economic significance*.* At present, there are no commercial scale IMTA systems that have been developed at Scottish salmon farms for these species. However, they are species which have been investigated within Europe for their potential to be included in IMTA systems (Baltadakis et al. 2020; Cook and Kelly 2007; Hannah et al. 2013)

2.2.2.1 Lobster rearing design

The European lobster is a decapod crustacean, ranging geographically from the North East Atlantic down to Morocco and the Mediterranean Sea. Lobster species constitute a valuable commercial fishery market, and the natural stocks are of great importance (FAO 2020). However, overexploitation led to stock depletion across Scandinavia and the Mediterranean around the 1930ss until 1970s and the recovery has since been slow (Ellis at al. 2015). This has led to the growing of lobster larvae in land-based hatcheries, for re-stocking of natural wild populations (Addison et al. 1994; Daniels et al. 2015). To increase survivability of post release larvae, lobsters can be grown in the marine environment for a short period of time, providing natural stimulus and acclimatisation (Drengstig and Bergheim 2013; Halswell et al. 2016).

The system design for the lobster culture was selected based on the Sea Based Container Culture (SBCC) system (Daniels et al. 2015). In this design, the SBCC was suspended from a buoy on moorings attached to a concrete block and deployed on the seabed. The length of the mooring rope was adjusted so the SBCC structures were at the preferred depth. Riser lines and buoys at both ends of the system signal location (see Figure 2.2).

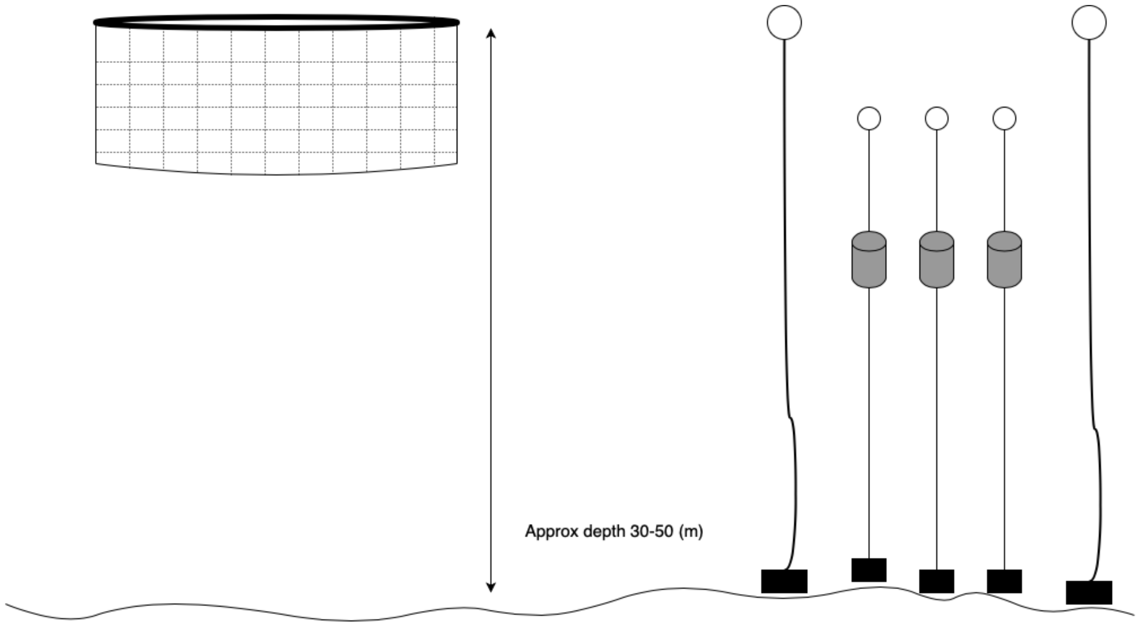

Figure 2-2 Conceptual design for the IMTA lobster set-up. Lobsters reared within the SBCC structures adjacent to the salmon cage. The SBCC (grey cylinder) is suspended from a buoy (white circle) and moored to a concrete block on the seabed (black rectangle).

2.2.2.2 Sea cucumbers rearing design

The sea cucumber *Holothuria forskali* can be found in the North East Atlantic and in the Mediterranean Sea. They live in benthic sediments between depths of 1-50 m (Hayward and Ryland 2017) and are deposit feeders which ingests sediment rich in organic matter (Zamora et al. 2016). Sea cucumbers are a popular sea food delicacy in Asia with an important market and high economic value (Purcell et al. 2011). Increasing global demand has overstretched wild stock landings leading to the collapse of natural wild populations (Anderson et al. 2010; Purcell et al. 2011). Efforts are being made to cultivate sea cucumbers in land and sea-based environments. The combination of high market value and their feeding behaviour has made sea cucumbers potential candidates for IMTA. Sea cucumbers have been shown to consume particulate organic waste derived from other fed organisms across different parts of the world (Slater and Carton 2009; Yokoyama 2013).

Grow-out systems for sea cucumbers used with fish cages range from suspended pearl nets (Yokoyama et al. 2013) to pallets, mesh containers or metal crates directly deployed to the seabed (Neofitou et al. 2019). Here, a seabed deployed system was used to assess suitability of locations. The metal crates arranged on the seabed allows sea-cucumbers to feed on and bio-accumulate enriched organic sediment accumulating near to fish cages (Figure 2.3). The design used metal crates moored on a single bottom line between two concrete blocks. A buoy at the end of the line is used to indicate location.

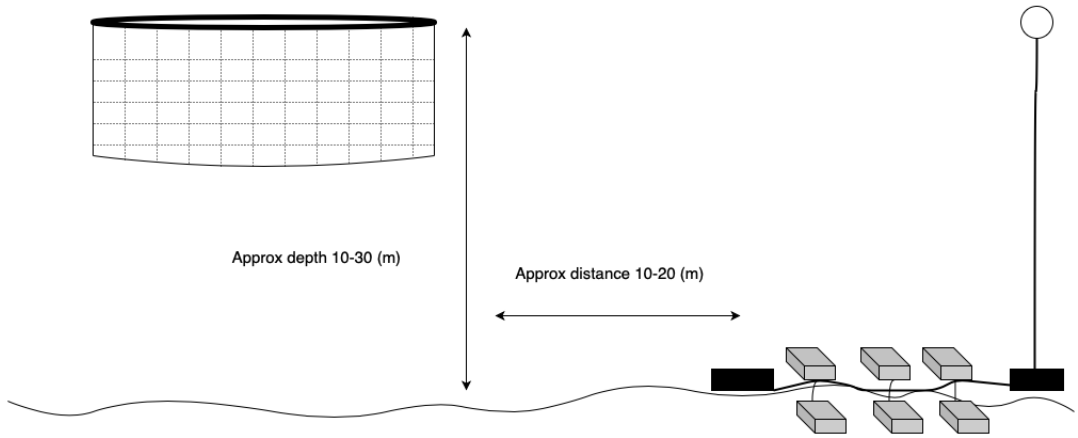

Figure 2-3 Conceptual design for the IMTA sea cucumber set up. Metal crates (light grey) are attached on a single bottom line between two concrete blocks (black) with crates perpendicular to each other along its length. A surface buoy (white) is attached at the beginning of the bottom line to show location.

2.2.2.3 Sea urchin rearing design

The purple sea urchin *Paracentrotus lividus* is distributed across the Mediterranean and North-eastern Atlantic. Distribution is common on the subtidal zones with occurrence depth ranging from 10-20 m (Lawrence 2013). In their natural habitat, when there is no limited food source, they prefer herbivorous diets, however when there is a limited food supply they can exploit any type of food source available, including sponges, hydrozoans and particulate organic matter (POM) (Boudouresque and Verlaque 2013). Sea urchins are a significant food trend globally reaching a value of 150 euros/kg (Stefansson et al. 2017). The role of sea urchins in most of its geographical range is considered a delicacy leading to commercial exploitation and high market value (Rakaj et al. 2018). High market value however has led to overexploitation with cases of illegal fishing and a collapse of wild stocks (Giglioli et al. 2021). Efforts are being made to culture sea urchins in land-based facilities for commercial harvest and restocking purposes. Nevertheless, there are still challenges hindering the aquaculture development of sea urchins (Cirino et al. 2017; Shpigel et al. 2005; Vizzini et al. 2019).

Due to their high market value and feeding habits, sea urchins have been investigated as a candidate species for IMTA (Cook and Kelly 2009; Shpigel et al. 2018). Lab based studies have shown that sea urchins can accumulate particulate organic matter originating from fed species (Grosso et al. 2021; Lamprianidou et al. 2015; Shpigel et al. 2018). Whilst open water experimental scale studies in Scotland have shown that sea urchins could grow significantly when reared close to sea cages and consume part of the salmon waste, they have been of poor market quality (Cook and Kelly 2007).

Based on previous IMTA open water studies, the stocking design of sea urchins used was suspended pearl nets around the sea cages (Figure 2-4) (Cook and Kelly 2009). In the design, moorings are attached on a concrete block deployed to the benthos whilst pearl nets are attached to the concrete block through an adjustable rope for preferred depth.

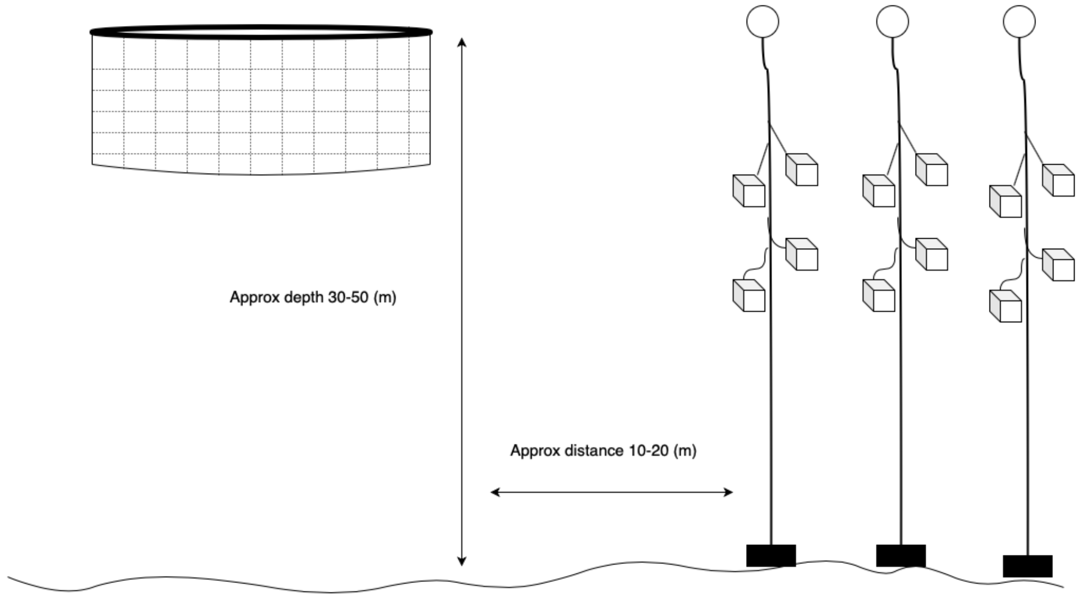

Figure 2-4 Conceptual design for the IMTA sea urchins set-up. Pearl nets (light grey) hosting sea urchins are suspended at the preferred depth on a mooring attached to a concrete block (black) on the seabed. A surface buoy (white) is attached at the top of the mooring line to signal location.

2.2.3 Site suitability model development

A GIS-based site suitability framework was developed and used to assess the suitability for the three different IMTA candidate species. The overall framework and model structure was the same but the specifications of the individual layers and models varied by species depending on their tolerances. The model was developed using IDRISI TerrSet GIS software (Clarks Labs, Massachusetts, USA) (Eastman 2012) and QIGIS.10.3 Las Palmas (Quantum GIS Development team, 2020). The spatial extent is outlined in Table 2-1.

Table 2-1 Spatial extent of model domain

Properties	Value
Number of columns	1758
Number of rows	2561
Reference system	UTM-29n
Minimum X coordinate	559800
Maximum X coordinate	1087200
Minimum Y coordinate	6056100
Maximum Y coordinate	6824400
Y Resolution	300 metres
X Resolution	300 metres

A conceptual model diagram, shown in Figure 2-5, illustrates the model framework. There were five different stages within the model: 1) Data identification and pre-processing, 2) Reclassification of data layers 3) Multi-Criteria Evaluation (MCE), 4) Preliminary model output showing seasonal changes, and 5) Final model output.

The following subsections outline the steps taken in each stage of model development.

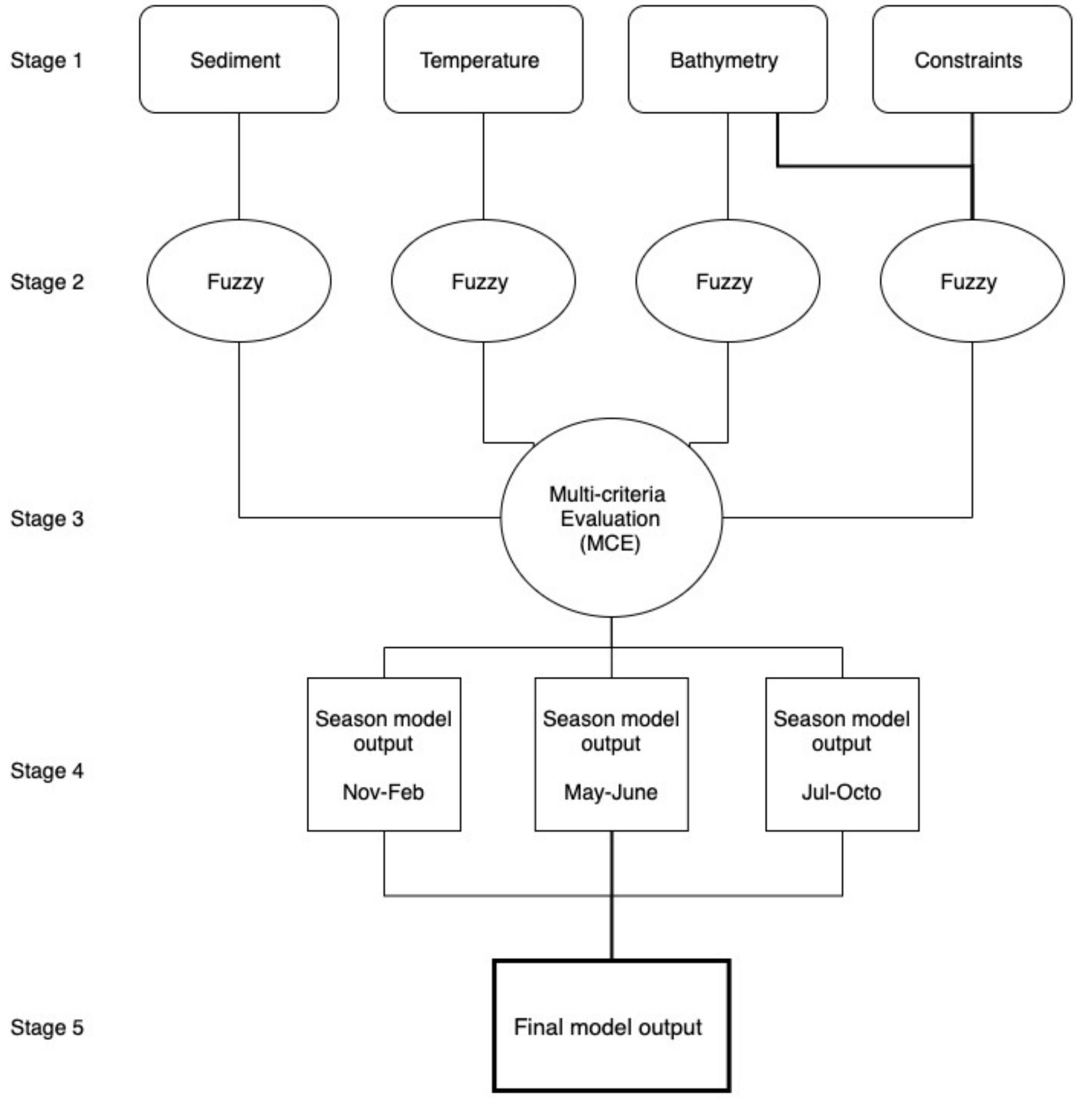

Figure 2-5 Conceptual diagram of the site suitability model. Rectangles represent data layers and ellipses represent processes. The final model output is presented as a bold rectangle.

2.2.3.1 **Stage 1: Data identification and pre-processing**

The first stage of the model included the identification, selection and sourcing of input data layers. The data layers represent factors and constraints. Factors are the important parameters which are relevant to the model framework. Selection of factors was determined by the author and a group of international experts in the field of aquaculture site suitability and sustainable aquaculture. Furthermore, selection and inclusion of factors depends on data availability and quality, while constraints are used to identify areas where aquaculture development is not permitted or not possible due to conflict with activities such as shipping lanes, port locations and areas of ecological significance.

During pre-processing, data were imported, inspected, cleaned, and then reprojected and cropped to ensure each layer had the same dimensions as outlined in Table 2.1. Some datasets were imported and inspected but were found to be of insufficient resolution or not appropriate to be used within the model. The data layers used in the model are outlined in Table 2-2; the following subsections provide further information on each.

Table 2-2 Data layers used within the model

Data layer	Unit	Resolution	File type	Source
Bathymetry	(m)	30 (m)	raster	GEBCO
Temperature	(°C)	1.5 (km)	netCDF	Marine Scotland
Sediment type	Type of sediment	100(m)	raster	JNCC
Constraints	n/a	n/a	vector	Marine Scotland

Bathymetry

Bathymetry is an important factor for the deployment of suspended and seabed structures. Suspended structures are limited by depth when it comes to the deployment of the concrete blocks holding the rearing design in place. Organisms suspended in boxes can then be easily adjusted with ropes at a preferred depth. Therefore, determining suitable locations for suspended culture is not constrained by the biological tolerances of the cultured species but from the requirements of the physical structure of the system. Seabed structures require different considerations when determining optimal depths, as benthic IMTA systems requires an suitable depth and distance to ensure the spread of particulate waste for trophic connectivity between the fin-fish cage system and the extractive organism. Furthermore, seabed structures require regular maintenance, such as scuba diving for antifouling, and harvesting. Seabed structures should also be within the optimal depth range of the cultured species.

Bathymetry data were sourced from the General Bathymetric Chart of the Oceans (GEBCO - The General Bathymetric Chart of the Oceans, 2020). By using the easily accessible online map application provided by GEBCO, areas of interest were selected, and the subsequent gridded data sets were available for download in the form of a netCDF file at a resolution of 30m. The original data set was inserted into IDRISI TerrSet using the "netCDF" function and reprojected using the "project" function at a resolution of 300 m.

Temperature

In any coastal mariculture setting temperature is considered the most influential factor for the growth of the cultivated species, while studies have related the temperature of the water to the thermal tolerance of the species (Gentry et al. 2017; Longdill et al. 2008; Porporato et al. 2020; Radiarta et al. 2008) For the development of an IMTA system, there needs to be careful consideration of the optimal temperature ranges of the cultured species. Even though, it has been suggested that extractive organisms tend to be adaptable towards a wide range of temperatures (Troell et al. 2009). Variation of temperature and particularly due to seasonal changes have been shown to be crucial for the production cycle of the extractive organisms, affecting growth and bio-mitigation capacity (Handa et al. 2013).

The temperature layer was downloaded from a dataset made available by Marine Scotland Science (De Dominicis et al. 2019). The temperature dataset is an output of the Scottish Shelf Model (SSM), that represents the climatological average boundary forcing's from 1990-2014 around the Scottish continental shelf, depicting physical variables. The main output of the model is one year of climatological conditions divided into three periods (November-February, March - June, July - October).

The original SSM dataset has a resolution of 1.5km in netCDF format. In order to reflect the same georeferencing and resolution conditions of the model, the data set was resampled through the “resample” function in TerrSet using background information of the bathymetry layer mentioned above. To address empty data cells due to the difference in resolution, the “Fillnodata” function was performed in QGIS.10.3 Las Palmas [Quantum GIS development team, 2020] to assign values to empty cells with those from neighbours. Then, a visual inspection over each temperature layer was performed to ensure they were fit to be used in the model. The final output of the temperature layer was reflecting the model’s whole spatial extent at 300 m resolution for the three periods.

Sediment type

Sediment type is an important consideration for the establishment of the moorings for cage and suspended culture installations (Cardia and Lovatelli 2015). Locations with rocky substrate can be problematic in terms of installations difficulties as well as expensive (Beveridge 2004). In addition, sediment type suitability is dependent on the structure and the type of organism. Suspended culture relies on the deployment of concrete blocks and therefore the suitability ranges are only dependent on the requirements of the moorings for cage farming. On the contrary, if the purpose of the structure is for culture of seabed structures hosting benthic organisms, sediment type is crucial since it should allow them to feed on fine participles (Neofitou et al. 2019).

The Sediment type layer was sourced from the UKSeaMap 2018v2 (JNCC, 2019), a broad scale physical habitat map for UK waters developed by the Joint Nature Conservation Committee (JNCC). The dataset contained 100m resolution broad scale seabed habitat map in the form of a GeoTIFF file. The categorization of sediment types was based on European Nature Information System habitat classifications (EUNIS), MSFD Benthic broad habitat types and the marine habitat classification for Britain and Ireland (European Commission, 2008). The file was digitized and categorized under a 10-category sediment type profile based on the European Nature System habitat classification at a resolution of 300m in the form of a raster file.

Constraints

Constraints are activities or designated areas of environmental interest that prevent any mariculture activity (Falconer et al. 2018). Scottish waters, like many coastal areas throughout the world, are crowded with many different users and activities taking place (Smith and Jentoft 2017). In Scottish territorial seas activities range from ferry routes, dredging areas, underwater cables, fishing grounds, pipelines. The Crown Estate is responsible for leasing areas for the renewable sector such as tidal and wind turbines (Neill et al. 2017). Lastly, areas of conservation were designated as constraints, and this included the UK Special Areas of Conservation (SAC), UK Special Protection Areas (SPA), and Marine Protected Areas (MPA). Data on relevant activities were sourced from Marine Scotland Website (Marine Scotland Information 2020) (Table 2.3).

Table 2-3 List of activities and reasons for selecting for the development of the constraints layer

Activities	Reason for selecting
Underwater cables	Constraint for development
Dredging	Constraint for development
Energy infrastructure designated areas	Constraint for development
Energy leases	Constraint for development
Ferry routes	Constraint for development
Fishing grounds	Constraint for development
Pipelines	Constraint for development
Tidal leases	Constraints for development
UK Special Areas of Conservation (SAC)	Protected areas
UK Special Protections Areas (SPA)	Protected areas
UK Rasmar	Protected areas
Wave leases	Constraint for development

2.2.3.2 Stage 2: Reclassification of data layers

Each data layer was reclassified to a common classification scheme by using the reclassification process. Each factor has different units; therefore reclassification attempts to translate all the different values of every parameters into a common value system, allowing their integration within a model. There are several different methods that can be used for reclassification, each with their own advantages and disadvantages. The simplest form of reclassification is the Boolean method where values are either given a score of 0 (e.g. not suitable) or 1 (e.g. suitable). This method is commonly used to develop constraint layers as the option is binary and therefore distinct. However, Boolean reclassification is very simplistic as different parameters will have a range of different levels of suitability (e.g. temperature). In order to account for this, user defined categories can be applied to assign a suitability score on a range of values. Furthermore, the user can also assign values to classes ranging from 0 to 1. The advantage with this approach is that someone can classify categories and choose the scoring scheme such as different types of sediments. However, this approach still entails hard boundaries.

Another approach is to use fuzzy reclassification and assign gradual suitability scores between suitable and not suitable (Zadeh 1999; Eastman 2012). The suitability scores range between 0 and 1 having a continuous increase from non-membership to complete membership. There are four types of fuzzy membership: Sigmoidal, J-shaped, Linear and User-defined (Figure 2-6). The IDRISI GIS software has a built in Fuzzy module that facilitates fuzzy reclassification. The reclassification is based on the position of 4 inflection points, a, b, c, and d, as indicated on Figure 2.6. The function takes different forms, Figure 2-6A shows a monotonically increasing function where “a” rises from zero to 1 without dropping. The second curve in Figure 2-6B is a monotonically decreasing function which begins from 1 and falls and stays at 0, with a, b and c having identical values whereas d is the point where it reaches 0. Finally, the last two functions Figures 2-6 C-D show a symmetric function as the score rises and falls again. In the case of Figure 2-6C the function rises and immediately falls with b and c having the same value. Finally, Figure 2-6D shows a function where the score rises and stays at 1 for a while. The positive aspect of a fuzzy reclassification is that it does not incorporate hard boundaries and therefore is useful for disseminating information on stakeholders, legislators aiding decision support.

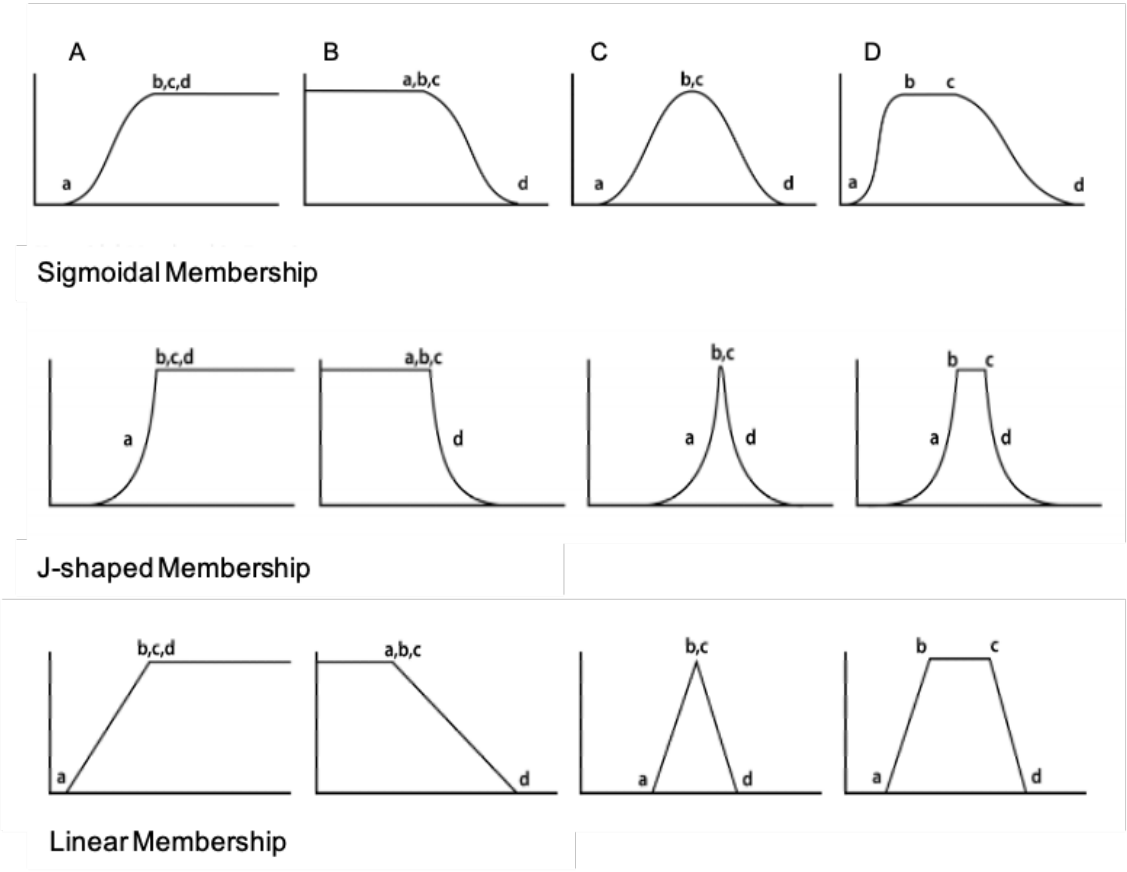

Figure 2-6 Fuzzy membership linear functions. Sourced from IDRISI TerrSet Handbook

Bathymetry

The bathymetric dataset was reclassified for the three different species based on the systems that would be used for culture (Table 2.4). The lobster design was suspended container SBCC systems moored on concrete blocks deposited to the benthos. Suspension of the structures would allow flexibility having an adjustable depth, with bathymetry limiting only the deployment of the concrete blocks rather the biological characteristics of the lobsters. Therefore, reclassified depth was based upon the concrete blocks, irrelevant of the species biological tolerances. From the meeting with the groups of experts it was decided that suspended cultures such as the lobsters should share the same requirements as of fin-fish cages in Scotland retrieved from Falconer et al. 2013. Therefore, a sigmoidal fuzzy membership function was applied with most suitable depth covering the range of 25-35 m.

Sea cucumbers metal crates are directly deployed onto the seabed. Therefore, depth needed to be considerably shallower, for regular inspection and maintenance activities such as anti- fouling maintenance purposes by divers (Neofitou et. al. 2019). Following the discussion with the group of experts it was decided that the bathymetry layer should be reclassified using a fuzzy sigmoidal symmetric membership with high suitability score given in areas with a depth range between 10-30 m.

Finally, sea urchins would be suspended in mid water in oyster baskets, and would not be limited by bathymetry, sharing the same characteristic with the lobsters species design, therefore same reclassification was performed using a sigmoidal symmetric function with the most suitable depth covering the range of 25-35 m. (Table 2-4).

Table 2-4 Reclassification of bathymetry (m) in terms of suitability for each IMTA scenario.

Candidate species	Boolean		Fuzzy						Source adapted from
	Non constraint	Constraint	Membership function		Control points				
			Type	Shape	a	b	c	d	
Lobsters	15-50	<15 and 50>	Sigmoidal	Symmetric	15	25	35	50	Falconer et al. 2013
Sea cucumbers	10-40	<10 and 40>	Sigmoidal	Symmetric	0	10	30	40	Neofitou et al. 2019
Sea urchin	15—50	<15 and 50>	Sigmoidal	Symmetric	15	25	35	50	Falconer et al. 2013

The reclassified bathymetry layer for each species is shown in Figure 2.7. From the maps it can be seen that suspended structures of the lobster and the sea urchins have the same suitability scores for bathymetry, whereas the sea cucumber has less areas suitable for the deployment of benthic crates

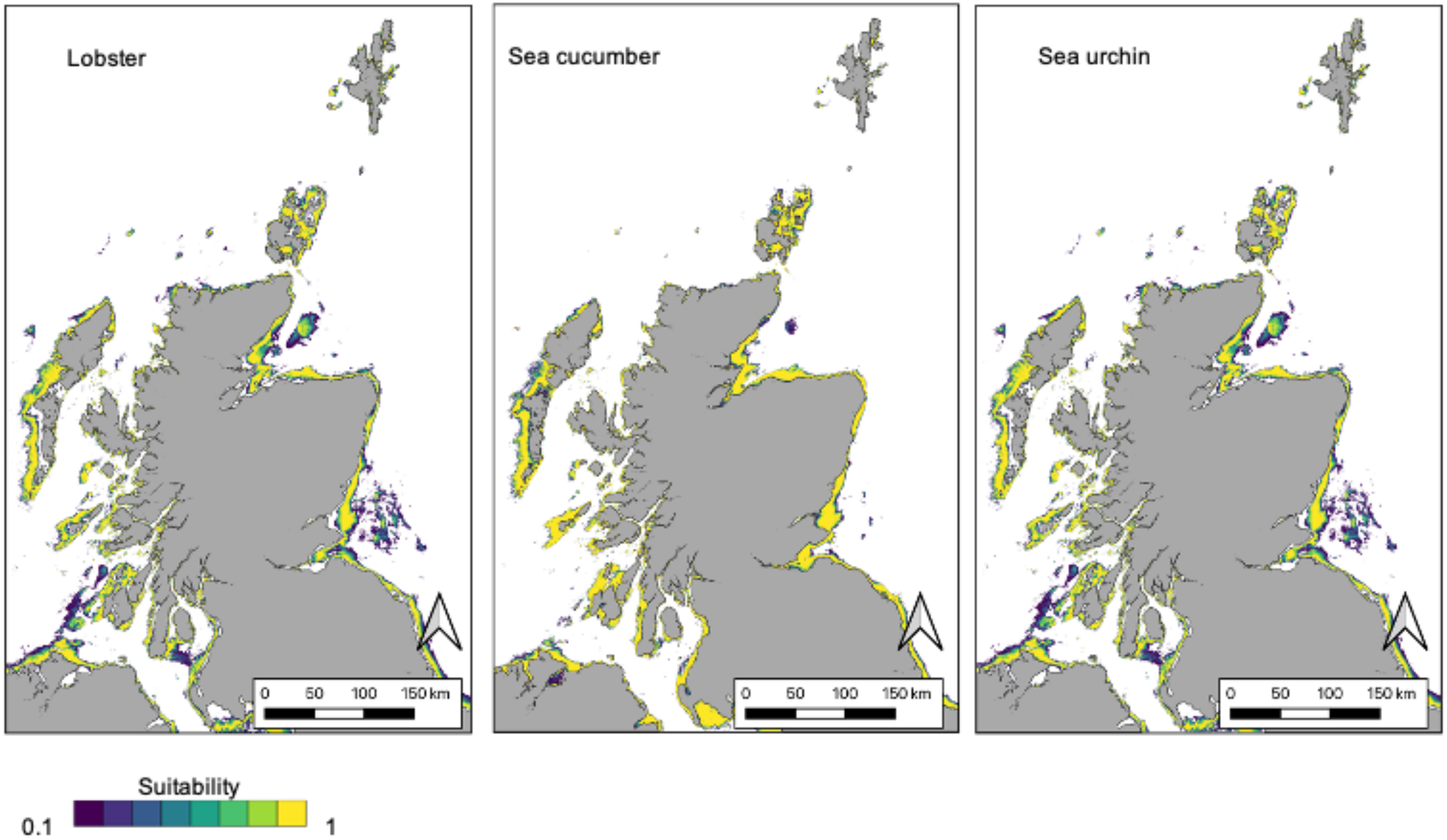

Figure 2-7 Map illustrates fuzzy classification species models for bathymetry. Suitability is assessed on a continuous scale: 0.1 low suitability, 1 high suitability. White background colour indicates areas with zero suitability score.

Temperature

The temperature layer was reclassified for each of the three species based on information regarding their thermal tolerance drawn from the literature. Lobsters tend to tolerate a wide range of temperatures, and literature (Wilson 2008; Albalat et al. 2019) suggested a range of 0-30 °C and with most suitable range being between 15-25 °C. Sea cucumbers such as *Holothuria forskali* are also known for their tolerance over a broad temperature range, maintaining a consistent metabolic performance, characterised as a thermal generalist (Kühnhold et al. 2019). The membership function chosen was a sigmoidal symmetric for a range of 8-19 °C with most suitable range being between 13-16 °C (OBIS 2020). Finally, the temperature range for the sea urchins was assigned values between 8-27 °C while ranges between 15-20 °C were more favoured (Spirlet et al. 2000) (Table 2.5). As shown in Figure 2-8 all species temperature outputs indicated a level of seasonality with scores increasing during the summer season. Lobster had less variability in terms of suitability scores with the rest two species experiencing a more rapid increased during the summer (July- October). Across all species outputs spatially variability was also observed with higher suitability scores prevailing towards the south of Scotland across all three seasons.

Table 2-5 Reclassification of Temperature (°C) for each species

Candidate species	Boolean		Fuzzy						Source adapted from
	Suitable	Not suitable	Membership function		Control points				
			Type	Shape	a	b	c	d	
Lobsters	0-30	<0 >30	Sigmoidal	Symmetric	0	15	25	30	Wilson. 2008
Sea cucumbers	10-19	<10 and 19>	Sigmoidal	Symmetric	8	13	16	19	OBIS.2020
Sea urchin	8-27	<8-27>	Sigmoidal	Symmetric	8	15	20	27	Spirlet et al. 2000

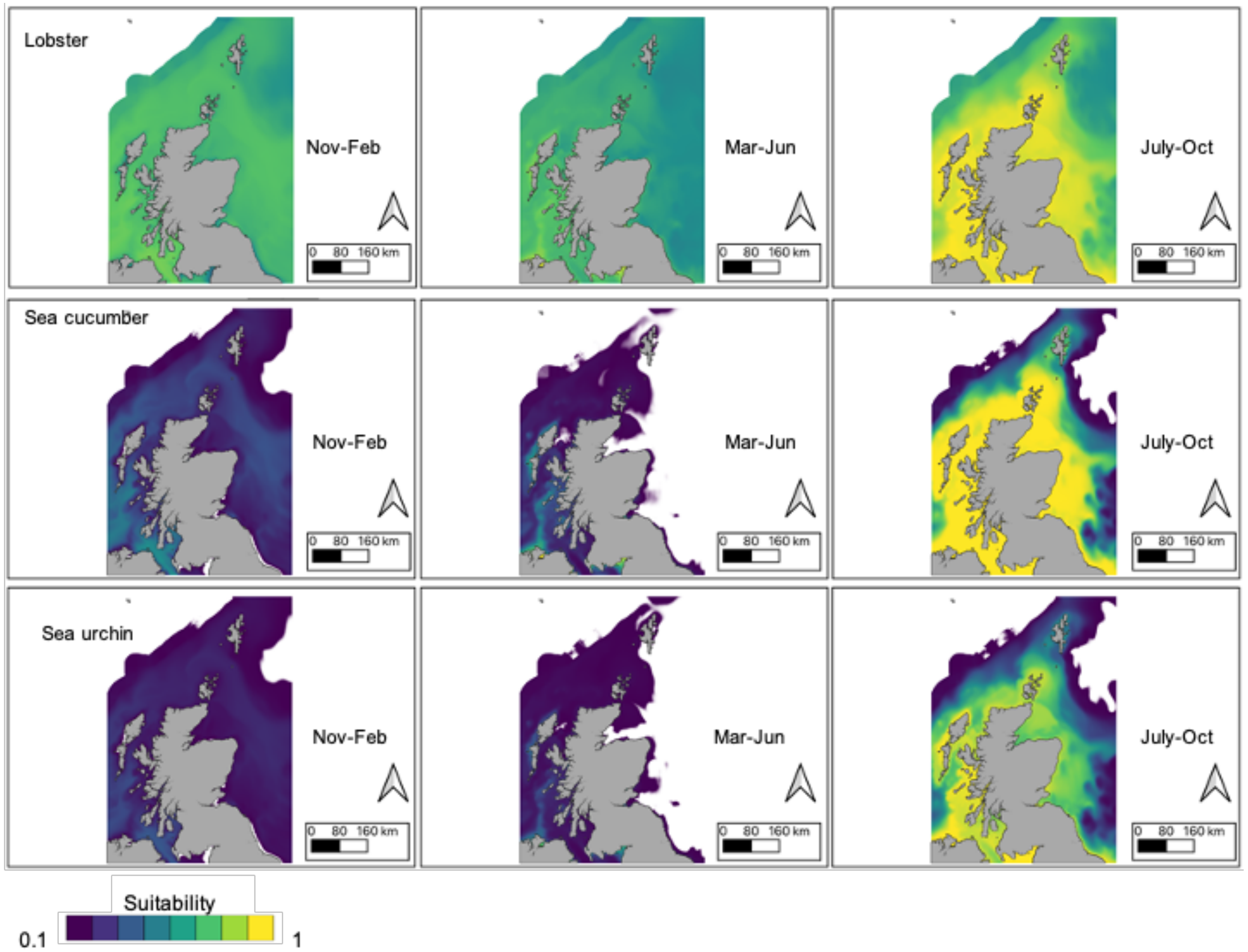

Figure 2-8 Map illustrates fuzzy classification species models for temperature for three seasons (November-February, March- June, July-October). Suitability is assessed on a continuous scale: 0.1 low suitability, 1 high suitability. White background colour indicates areas with zero suitability score

Sediment type layer

Unlike the bathymetric and temperature layers, which were reclassified on continuous scales, the sediment data layer had a discrete scale. Therefore, scores could not be assigned using fuzzy set memberships, but had to be assigned on the same scale. Based on the considerations of an expert focus group each sediment types were assigned as specific suitability score ranging from 0 (not suitable) to 1 (highly suitable).

Lobsters and sea urchins would have the same suitability score favouring sediments such as sand to muddy sand and coarse substrate, as these are most suitable for the deployment of the concrete blocks (Cardia and Lovatelli 2015). Benthic sea cucumbers, on the other hand, favour sand to muddy sand sediments. This is particularly important since part of their feeding mechanism is to digest fine sediment particles (Hou et al. 2017). Therefore, higher suitability scores for the suspended design were given to coarse substrate and sand to muddy sand, whereas for the deposit system higher suitability scores were given on sand and sand to muddy sand (Table 2-6)

Table 2-6 Reclassification of Sediment type in terms of suitability for each species design

	Lobsters	Sea cucumbers	Sea urchins
Coarse substrate	0.75	0.1	0.75
Fine mud	0.1	0.25	0.1
Mixed sediment	0.25	0.25	0.25
Mud to muddy sand	0.25	0	0.25
Rock or other hard substrate	0.25	0	0.25
Sand	0.5	1	0.5
Sand to muddy sand	1	0.75	1

Based on the reclassified map of the sediment for each species shown in Figure 2-9, sea cucumber had higher suitability scores on the East coast of Scotland, whereas on the South East of Scotland there were no suitable areas close to the shore within the littoral zone. Lobsters and Sea urchins had suitable areas within the whole of Scotland with suitability scores on the South-East being low. This map demonstrates that for suspended designs suitable areas were greater compared to the benthic designs.

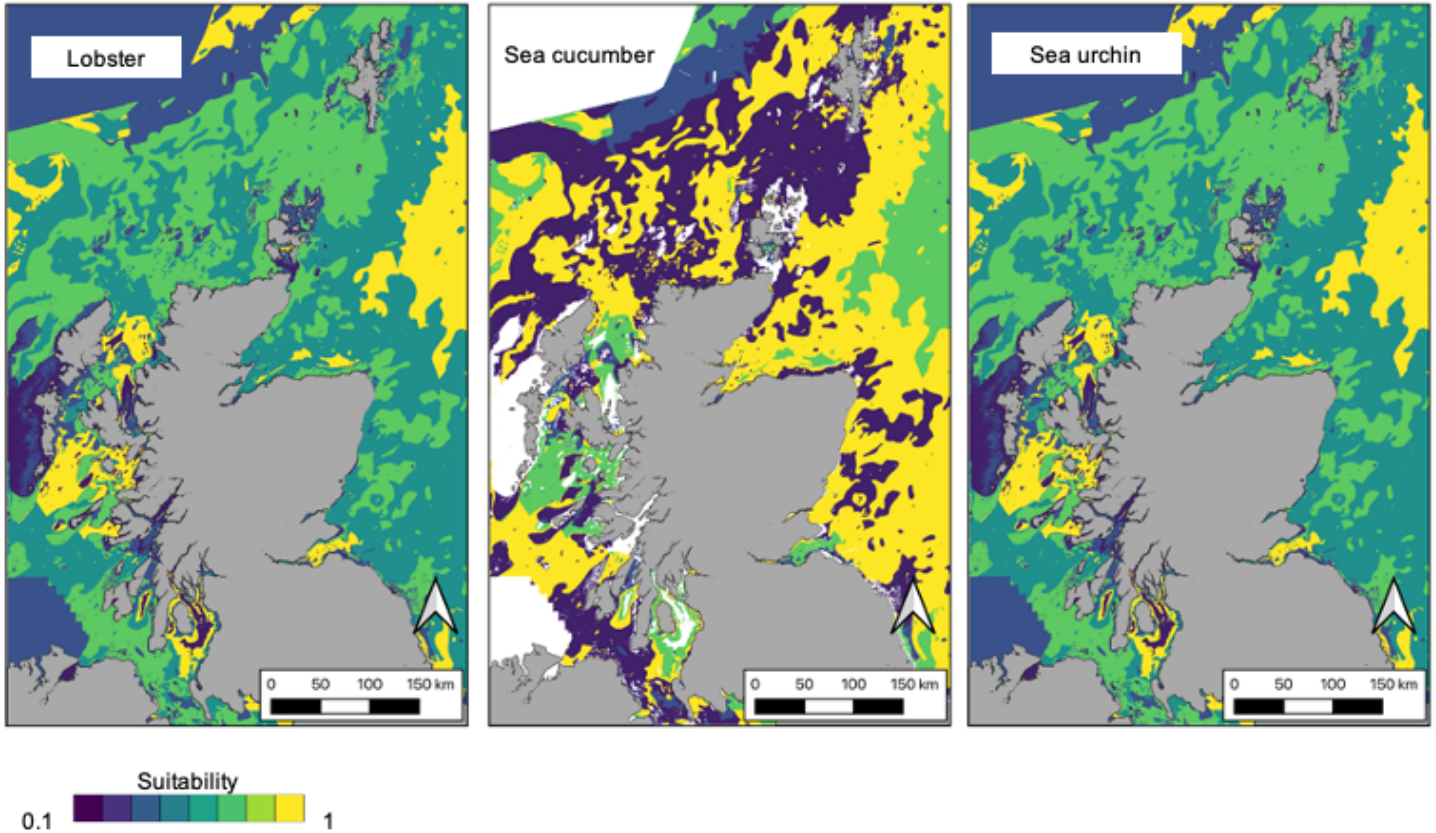

Figure 2-9 Map illustrates fuzzy classification species models for sediments. Suitability is assessed on a continuous scale: 0.1 low suitability, 1 high suitability. White background colour indicates areas with zero suitability score.

Constraint layer

The constraint layer was reclassified using a Boolean approach. Features that were considered a constraint were given a score of 0. Areas that were not considered a constraint were given a score of 1. Majority of the constraints where located on the East coast of Scotland and the Southeast (Figure 2-10). Constraints included were particularly, areas of conservation with priority marine features, leases for the energy sector, fishing and ferry routes.

Figure 2-10 Map illustrates the constraints layer, where development of any aquaculture activity was prohibited.

2.2.3.3 Stage 3: Multi-Criteria Evaluation (MCE)

The layers were combined using a Multi-Criteria evaluation (MCE) (Carver 1991, Eastman 2012). MCE is a common method to combine multiple factors in a structured manner (Ross et al. 2011). Factors are ranked against each other through a pair-wise comparison method, to meet a specific objective. The MCE is using a weighted overlay with weights assigned based on the opinion of legislators, stakeholders, scientists, being proportional to importance (Nath et al. 2000). This is advantageous since it allows the simultaneous assessment of spatial variability between the factors relevant to the aquaculture site. It categorises variables based on a level of importance, so multiple input layers are combined to produce the final output that is easier for decision makers to understand than evaluating the individual input layers.

Weighting of the factors included in the model development was followed after a group meeting with international experts on the field of sustainable aquaculture and marine spatial planning for aquaculture. The weights were applied following discussion on the factors incorporated in the model and it was decided that all layers should be weighted equally.

2.2.3.4 Stage 4: Preliminary model outputs

The fourth stage of the model was a preliminary output, depicting species site suitability results across the three different seasons. This would allow the model to indicate potential areas suitable for each species both spatially and temporally. Temporal component was driven by the temperature layer, whereas spatial variability was driven by all the factors and constraints. For the production of an organism the level of temporal variability is important to maintain a homogenous growing cycle.

2.2.3.5 Stage 5: Final model outputs.

For the fifth and final stage of the model, areas where suitability scores of each species model was over and equal 0.6 were across all three seasons were averaged to a single value, giving rise to the final model output, areas even with a suitability score less than 0.6 on a single seasons were considered not suitable and were not incorporated into the analysis. Thereafter, final model output for each species was overlayed on the existing salmon farms vector points. This would allow to assess which farm is more suitable for IMTA and explore the level of variability between species and farm sites.

2.3 Results

2.3.1 Lobster seasonal site suitability models

Maps showing seasonal suitability from Stage 4 of the modelling process, for lobsters are given in Figure 2-11. The results show, that in all seasons, there are areas of moderate to high suitability for lobsters around the Scottish coast and islands. The areas occupying the South West of Scotland had a considerable higher score, with suitability scores remaining constant across all three seasons.

The output for July – October had higher suitability scores in many areas than the other seasons, with 17,298 km^2 having a score greater than or equal to 0.6 compared to 11,865km^2 and 11,263km^2 for November-February and March-June respectively.

2.3.2 Sea cucumber seasonal site suitability models

Depicted map showing the seasonal variation from Stage 4 of the sea cucumbers model is given in Figure 2-12. The model output suggests that suitability varies across the three seasons and also depending the areas. The East coast of Scotland had higher suitability scores but were not included in the assessment due to the moratorium on salmon farming and therefore no potential to develop an IMTA. The South-West of Scotland had more areas with higher suitability scores compared to the North.

Results demonstrated a substantial level of temporal variability with the summer season having noticeable higher suitability scores. Mean suitability score for the November-February season was 0.47 (sd± 0.17), rising to a mean value of 0.5 (sd ± 0.19) for the March-June season. Mean suitability scores for the summer season increased considerably to 0.77 (sd ± 0.18). The same pattern was observed when looking at areas with a suitability score of >=0.6. First two seasons (Nov-Feb) and (March- June) occupied 2924820 km^2 and 3839130 km^2 respectively. With summer season having almost a double increase covering 8588520 km^2 .

2.3.3 Sea urchin seasonal site suitability models

The map showing the sea urchin seasonal suitability of the Stage 4 modelling process is given in Figure 2-13. The map suggests that areas with high suitability scores were located on the East Coast and South-West of Scotland, opposed to the North-West were scores were considerably lower. The impact of seasonal variation is present on this seasonal model output with differences in suitability scores occupying through the seasons.

The period of November- February and March -June had similar mean suitability scores of 0.4 (sd ± 0.14). During the summer period (July- Oct) mean suitability score increased to 0.6 (sd ±0.15). The same pattern was observed also in areas where suitability scores were >=0.6. First two seasons (Nov-Feb and March-June) high suitability score areas occupied almost the same areas with an average of 2159415 km^2 (sd ± 172145), whereas during the summer season area cover increased considerably to 14431860 km^2.

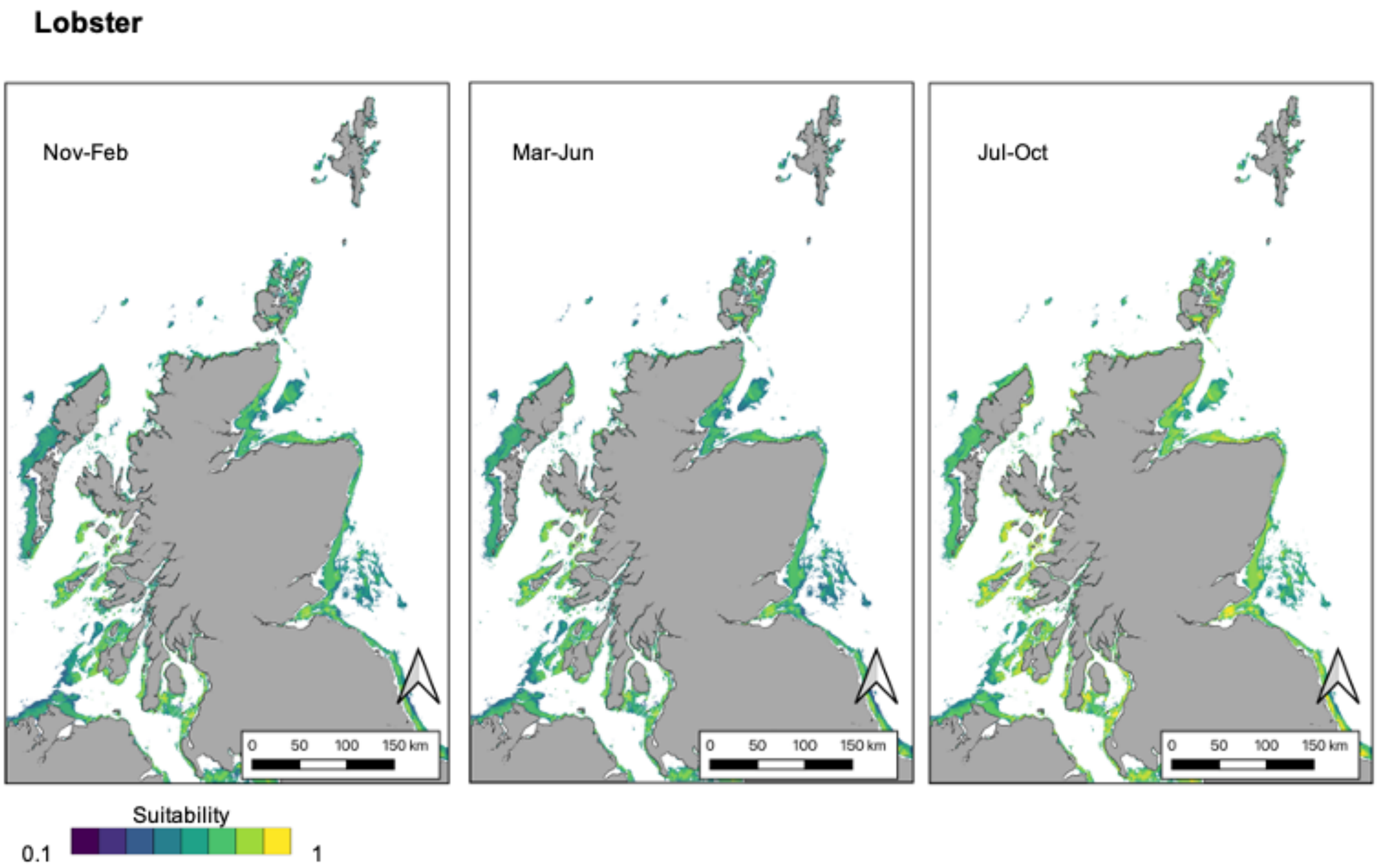

Figure 2-11Map illustrates the seasonal suitability results of the Lobster species model over the three seasons. Suitability is assessed on a continuous scale: 0.1 low suitability, 1 high suitability. White background indicates areas with a suitability score of 0

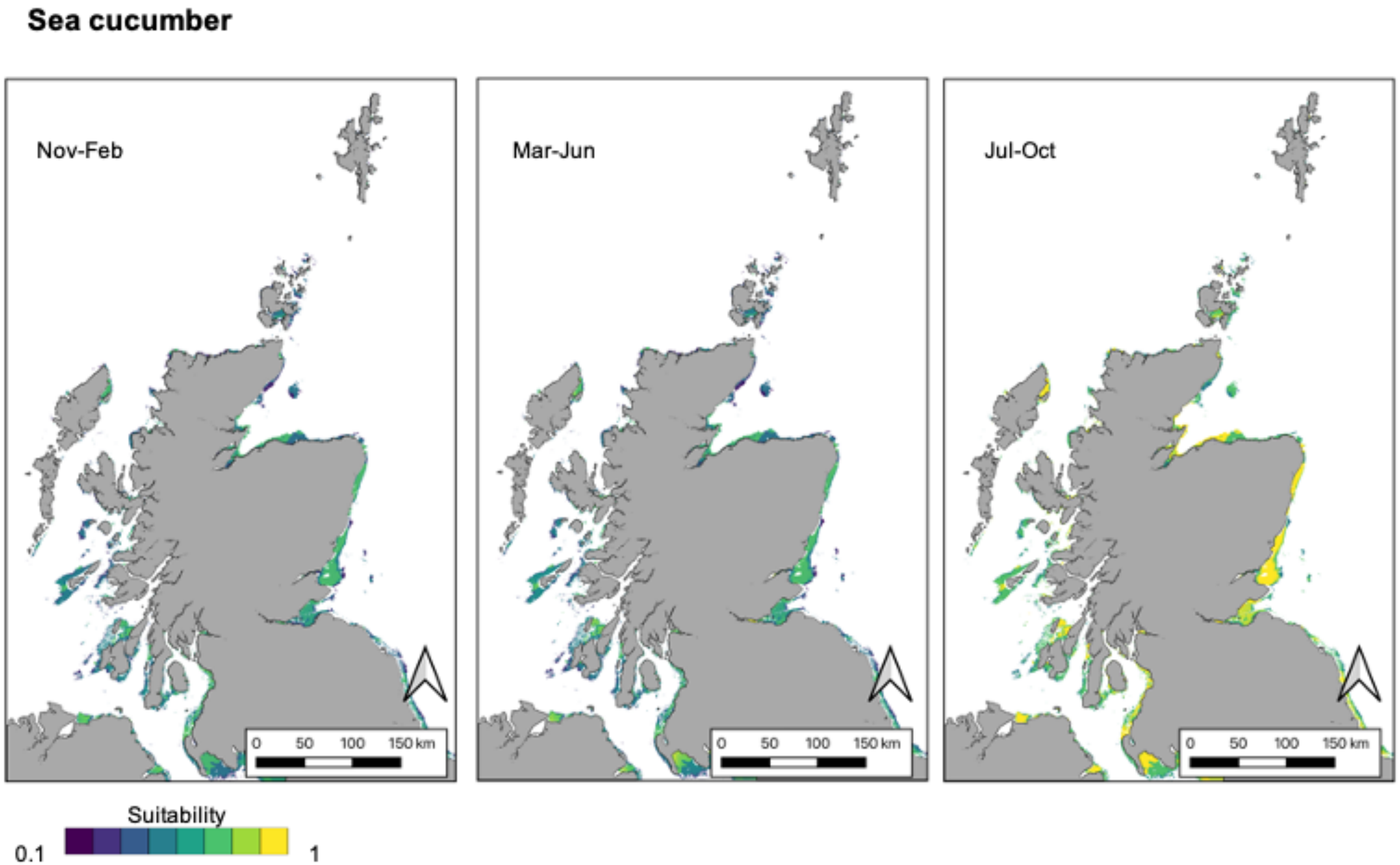

Figure 2-12: Map illustrates the seasonal suitability results of the Sea cucumber model over the three seasons. Suitability is assessed on a continuous scale: 0.1 low suitability, 1 high suitability. White background indicates areas with a suitability score of 0

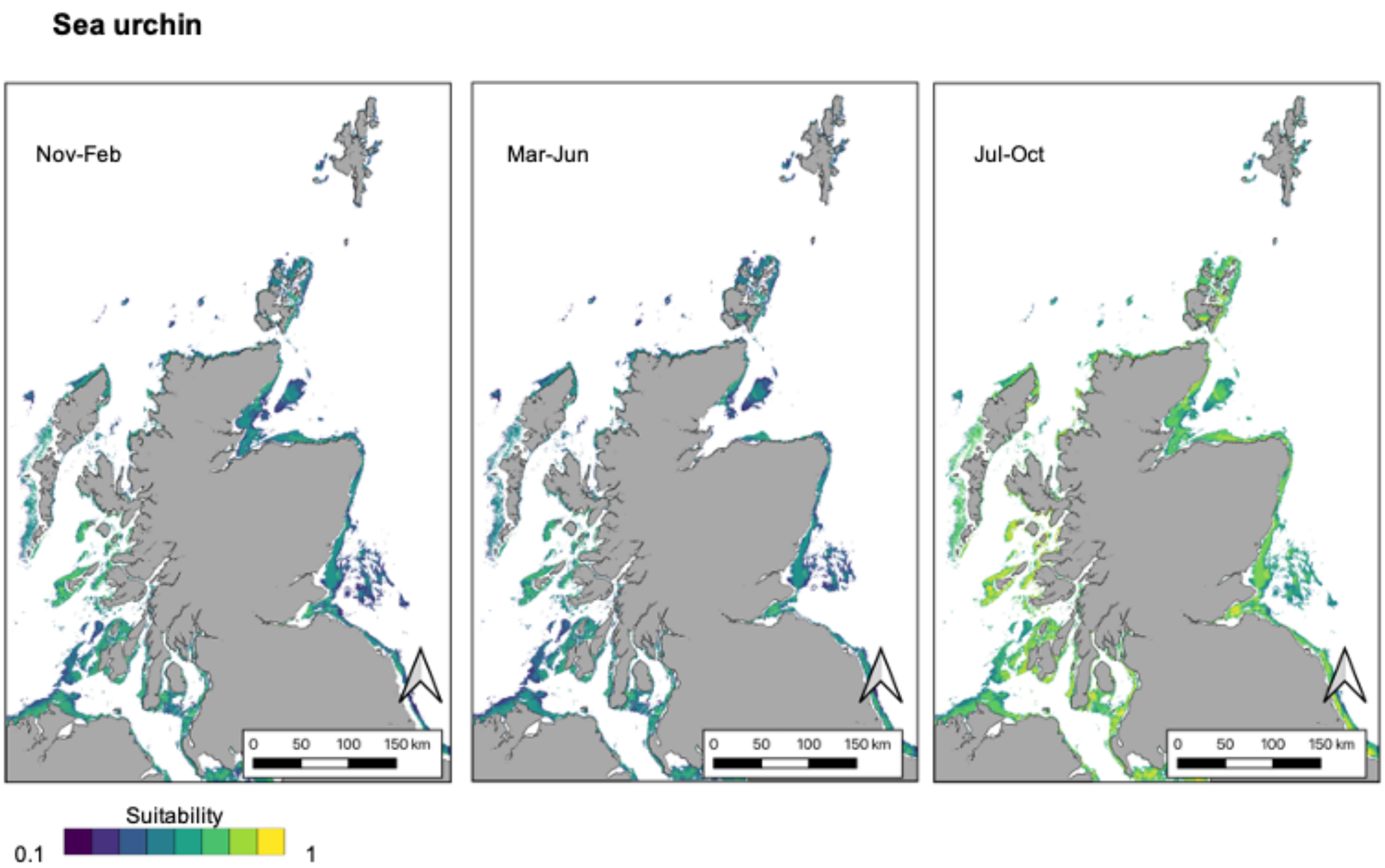

Figure 2-13: Map illustrates the seasonal suitability results of the Sea urchin model over the three seasons. Suitability is assessed on a continuous scale: 0.1 low suitability, 1 high suitability. White background indicates areas with a suitability score of 0

2.3.4 Final model output for each species

The final model output of each species indicated that there are potential development areas for all three species across Scotland (Figure 2-14). However, there were differences in the total amount of potential development areas for each species. The model output suggested there were more potential development areas for lobsters followed by sea urchins and then sea cucumbers (Table 2-7). For all three final species models an area with high probability of suitable locations for all three species was the South-West of Scotland

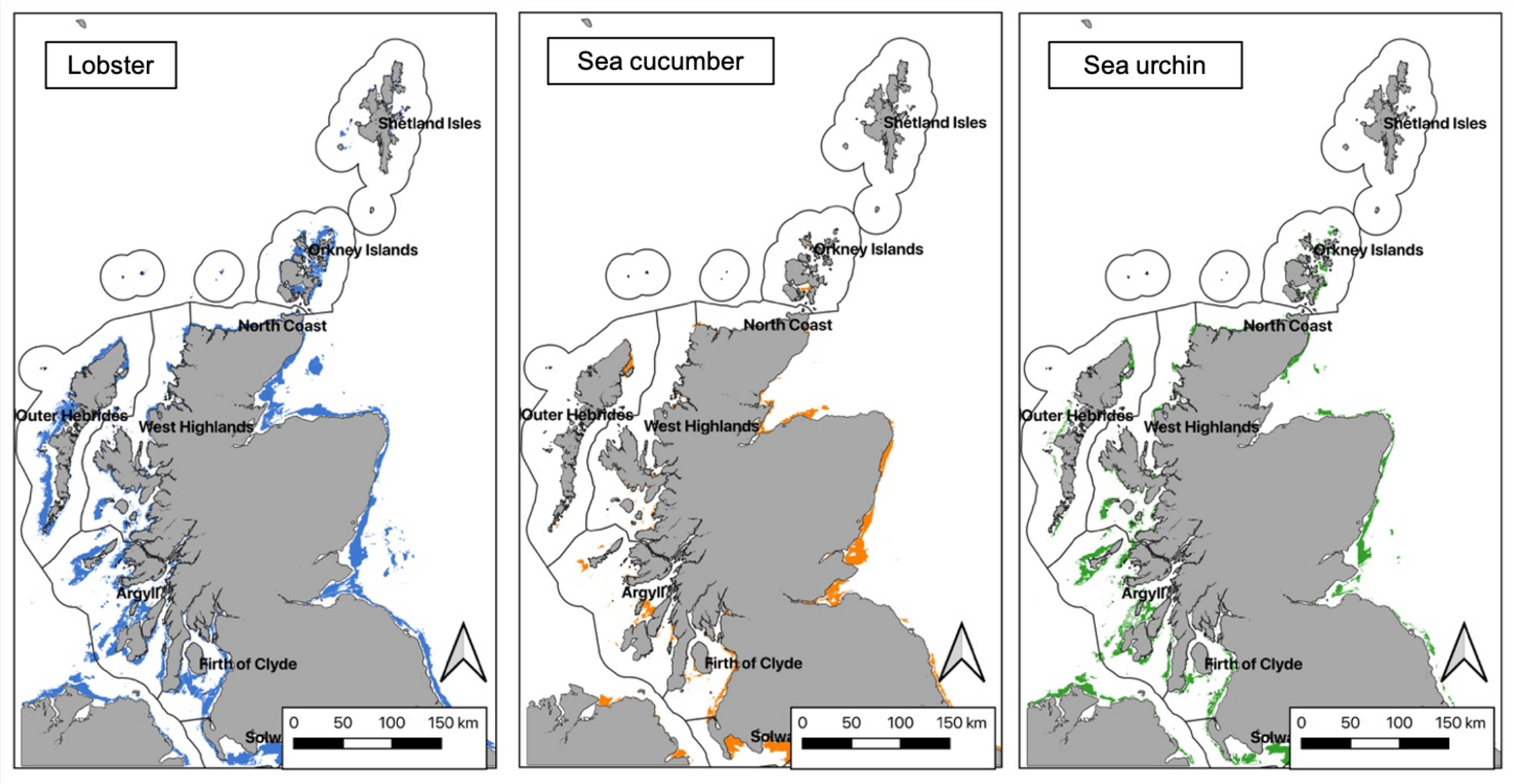

Figure 2-14 Final model output for each species indicating potential development areas across Scotland in relation to the Scottish Marine Regions. Blue colour = lobster, orange = sea cucumbers, and green = sea urchins

Table 2-7 Yearly suitability species model results, showing total area of model domain against areas where suitability score is >=0.6 and classified as high suitable and the rest as not high suitable

	Lobster	Sea cucumber	Sea urchin
Total model area km^2	405,520,142	405,201,420	405,201,420
No development area km^2	390,753,360	400,743,450	399,523,140
Development areas km^2	14,448,060	4,457,970	5,678,280

Salmon aquaculture is in eight of the Scottish Marine regions (Argyll, Firth of Clyde, North Coast, Orkney Islands, Outer Hebrides, Shetland Isles, Solway, West Highlands). The results indicated there were suitable locations for each species across all eight regions, however there were differences between regions (Figure 2-15). In each marine region, the model output for lobster had the highest coverage of potential development areas compared to the other two species. Potential development areas for the model output for sea urchins were identified on Argyll, Solway, West Highlands, and Firth of Clyde. Finally, the model output for sea cucumbers was the least suitable across all the regions with potential development areas only identified on Solway, Firth of Clyde, and Argyll.

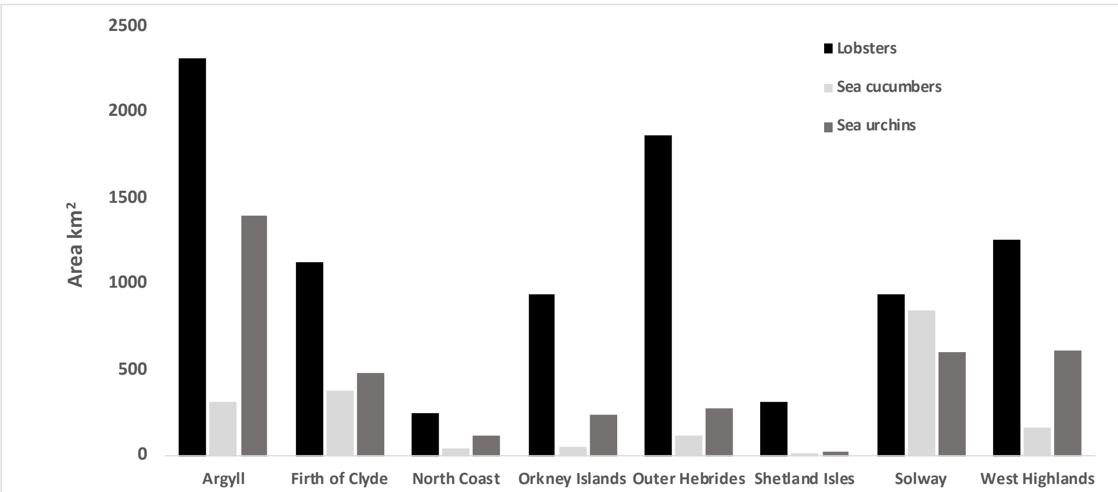

Figure 2-15 Suitable area covered by each yearly species suitability model in eight Marine Regions of Scotland

As seen in Figure 2-15 the potential areas for development were large, however, the actual percentage cover of those areas in relation to the total area of the marine regions was negligible (Table 2-8). Maximum percentage cover for lobsters was identified in the West Highlands (49%), for sea urchins on Solway (16%) and for sea cucumbers in Solway as well (23%). Solway had high percentage cover for all three species, because it was the smallest marine region with 3274 km^2. On the other hand, even though West Highlands percentage cover was smaller there was still much larger area available compared to Solway, as demonstrated for Sea urchins with 601 km^2 for Solway and 612 km^2 for the West Highlands.

Table 2-8 Percentage cover of high suitability areas versus total area of each Scottish Marine Regions.

Marine Regions	Area Km^2	Lobster	Sea cucumbers	Sea urchin
Argyll	12048	19%	12%	3%
Firth of Clyde	4279	26%	11%	9%
North Coast	2444	10%	5%	2%
Orkney Islands	9258	10%	3%	1%
Outer Hebrides	20849	9%	1%	1%
Shetland Isles	13308	3%	0%	0%
Solway	3274	25%	16%	23%
West Highlands	10419	49%	6%	2%

2.3.5 Comparison of species suitability models with active salmon farms

Based on the results of the final model output, areas within three marine regions (Firth of Clyde, Argyll, and West Highlands) were selected for comparison with existing salmon farm locations. The purpose was to address if suitability scores would vary between farm locations. Maps demonstrate the differences within coastal areas selected for demonstration, and located in Firth of Clyde (Figure 2-16), Argyll (Figure 2-17) and West Highlands (Figure 2-18) The results show that within and between sea lochs there is spatial variation in the availability of potential development areas across the three IMTA species. In Loch Striven (Figure 2-16) the model outputs suggest there are only priority development areas for lobster and there are no potential development areas for the other two species. In Loch Fyne, the model shows there are potential development areas for all three species, however in the north of the Loch there are only areas suitable for lobster, while in the south there are also potential development areas for sea cucumber and sea urchin. Overall, the location and extent of the potential development areas for sea cucumber and sea urchin are very similar in the Firth of Clyde region.

The Argyll marine region has a large number of salmon farms in Loch Linnhe (Figure 2-17). As shown in the map, lobsters have again the greatest extent of potential development areas. However, the two other species models had zero potential areas for development. Even though the sea urchins and sea cucumbers species models had a large extent of potential development areas in Argyll as shown in Table (2-8). It is noteworthy to see that none of these were located in Loch Linnhe where majority of salmon aquaculture takes place in Argyll.

Finally, in the Western Highlands marine region there are more suitable areas for lobsters in Loch Caron, Loch Ainort and Loch Alsh as shown in (Figure 2-18). However, in Loch Alsh and the neighbouring loch systems there are potential areas for development for the sea urchins and sea cucumbers as well, having very similar locations and extent. The results show that within a system characterized by numerous lochs there is still spatial variability between the species suitable areas.

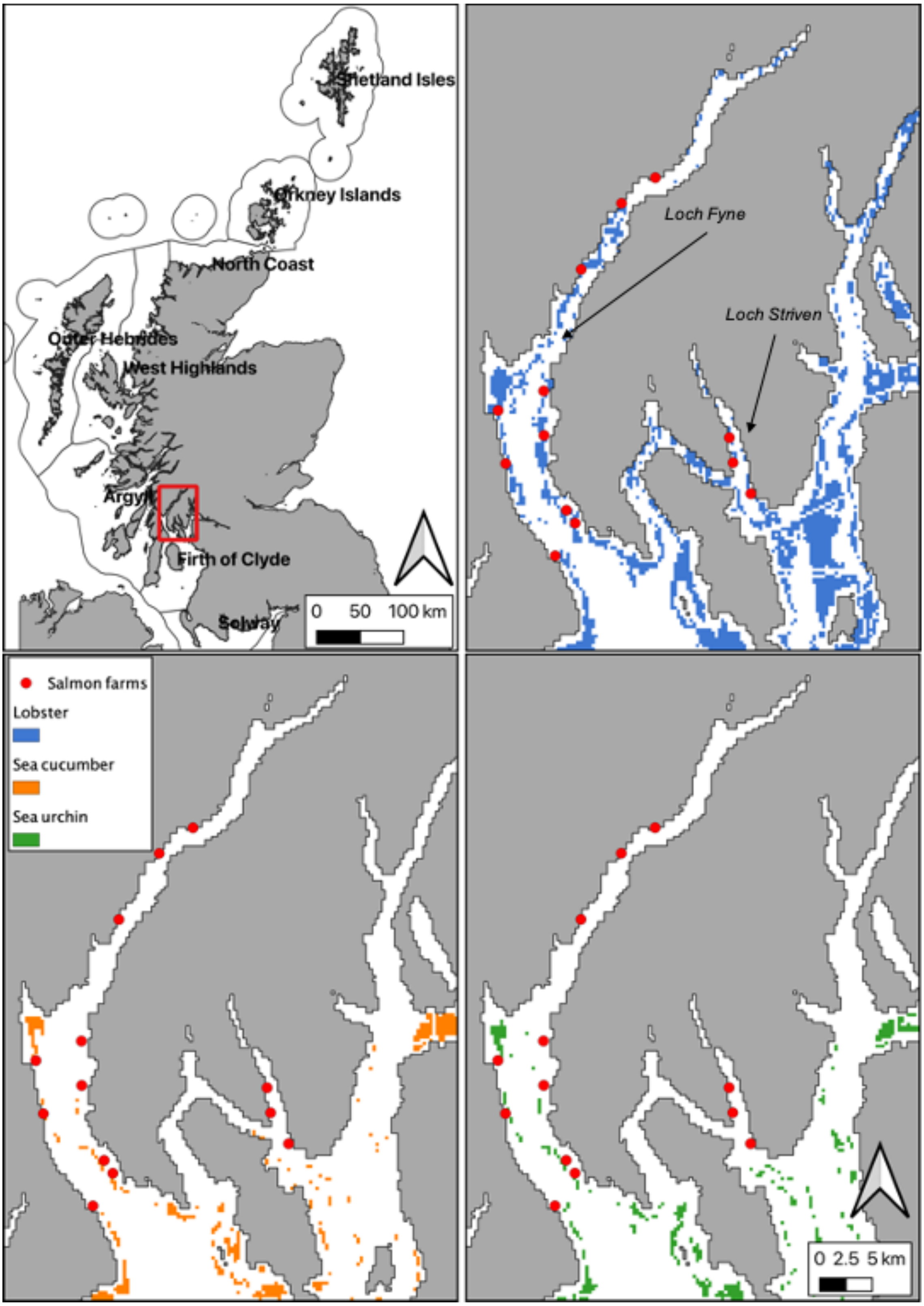

Figure 2-16 Map illustrates the Firth of Clyde marine region and the areas suitable for the three species models.

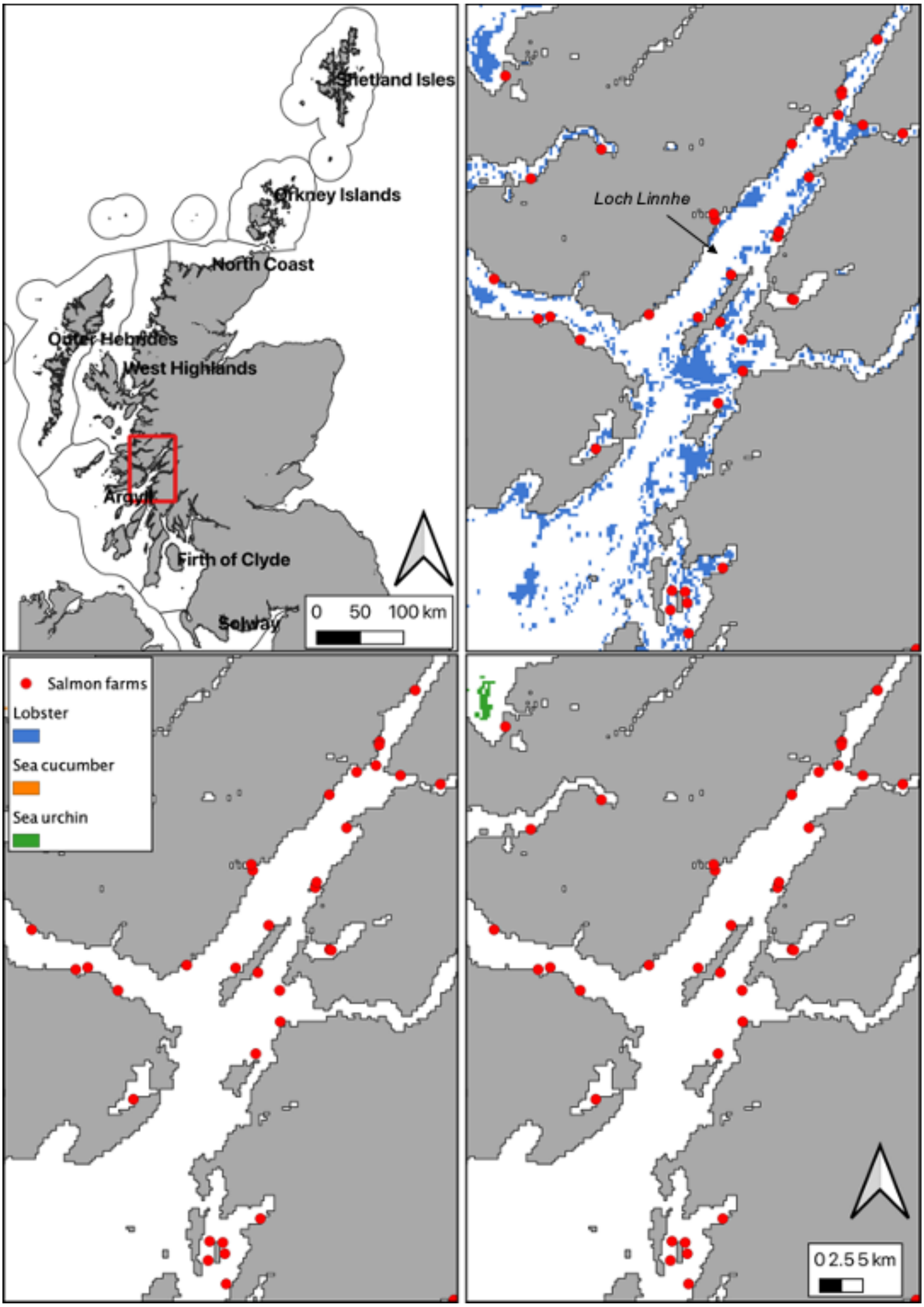

Figure 2-17 Map illustrates the Argyll marine region and the areas suitable for the three species models.

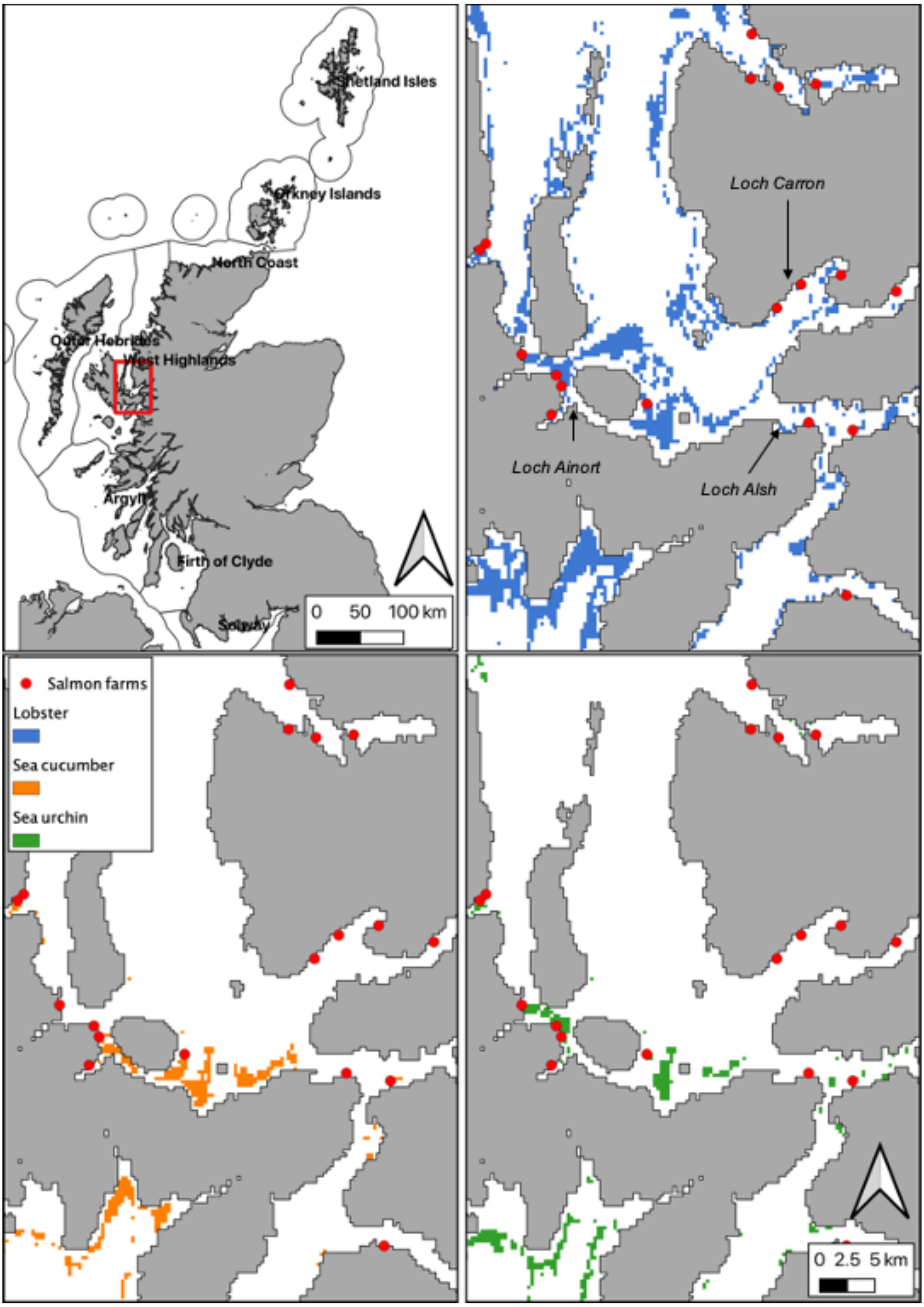

Figure 2-18 Map illustrates the West Highlands marine region and the areas suitable for the three species models.

2.4 Discussion

Competition for maritime space along with the need for sustainable increase in mariculture production is an issue regarding mariculture development (Elliot 2011). One of the potential ways to achieve that goal is through integrated culture and therefore establishment of IMTA systems (Christie et al. 2013). To establish an IMTA system, it is important to identify suitable locations initially based on the physical carrying capacity in order to assist in its development. This study constructed a site suitability model for three IMTA candidate species by using GIS and spatial analysis methodologies. Available data sets (factors) were sourced and processed covering the biological ranges of the species with temperature data as well as other factors responsible for the rearing design of the system such as sediment and bathymetry. The purpose of this chapter was to identify the level of spatial variability between the three different species in terms of suitability as well as temporal variability between each species models. This would allow better understanding when planning an IMTA system in coastal and open water conditions.

The results of the species suitability models suggest that results were influenced by the element of seasonality. The lobster seasonal site suitability model had high levels of suitable areas across all three seasons, whereas the sea urchin and sea cucumber seasonal models exhibited a higher level of temporal variability, with levels of suitability increasing towards the summer season. Furthermore, all three reclassified temperature species model outputs indicated that the level of temporal variability also differs spatially, with areas towards the South-East of Scotland experiencing less differences between seasons. The element of seasonal suitability and therefore temporal variation is very important for planning and development of aquaculture in general, as suitability of sites and areas can have differences across a year (Ross et al. 2011). This element is of particular importance for IMTA since, different species will experience different temperature ranges, not only affecting growth and welfare of the candidate species but also bio-mitigation capacity. Even though IMTA candidate species can cope with large temperature variations, temperature alterations can inhibit their physiological performance and alter their bio-mitigation capacity. Sea cucumbers even though they have a great adaptation capacity, extreme high and low temperature values have been suggested to alter the physiological responses such as enhancement of the aestivation period, not feeding on particulate matter (Dong et al. 2006; Ji et al. 2008; Hannah et al. 2012). These seasonal components need to be taken in account when calculating their bio mitigation performance (Zamora et al. 2016). The same is suggested for lobster where increased temperatures and also low temperatures can result into lower metabolic rates, used as a coping mechanism (Albalat et al. 2019). Temperature has also been shown to affect the total growth as total weight and growing of gonads for human consumption in sea urchins *Paracentrotus lividus* (Albano et al. 2018) The advantage of including the seasonal component on this model highlighted how the suitability of the species can vary throughout the seasons, indicating different species will encounter different variations and also how different location will encounter different variations. This would offer better understanding on candidate species capacities or even inform which areas could be used for longer growing periods when other areas may not be suitable, which has been highlighted as of great importance for farmers (Alexander and Hughes et al. 2017).

The results of the final model for each species suggest that suitability scores vary spatially as well with suitable locations predominantly identified on the South-Western part of Scotland. The model suggests that marine regions towards the South such as Argyll, Firth of Clyde, Solway had suitable locations for all three species, with more suitable locations for lobster. However, when looking at the ratio of a marine regions total area against the total suitable area of the species it is observed that in West highlands, even if the ratios are smaller, there is actually a much larger area covered by the three species totally. The results suggest that different regions will have different capacities for IMTA and this could be a valuable information in terms of Regional Marine Plans (RPM) offering information on Marine Planning Partnerships (MPPs) support regional management of IMTA.

Development plans for mariculture firstly identify priority areas at national level, the next step is the detailed plans at regional level (FAO and World Bank. 2015).

Moving close to the fjordic inlets, the model suggests that not all farms were suitable for IMTA neither for all the species. As an example, from Loch Fyne, the north part was suitable for lobsters whereas when moving south there were also suitable locations for the other two species. That highlights the importance of the farm scale conditions and suggest that not all salmon farm sites were suitable for any type of IMTA. It also enhances the understanding that even if specific regions had a high capacity of suitable locations for IMTA, specific locations which host the majority of cage farming activities were not suitable for some species. A more detailed assessment could enhance the understanding of the site characteristics and assess the suitability of the species suggested under more information. This approach highlights the importance of a suitability study before the establishment of a commercial scale IMTA system in order to characterize its capacity to withstand any IMTA, ensuring the appropriate conditions. That would be helpful on supporting decisions for farmers, research institutions and regulators for the development test IMTA sites under commercial conditions.

This chapter highlights that the differences between the suitability scores originates from both the different species biological design and the subsequent rearing design incorporated. Part of the reason, except the temperature element which was discussed above was the technical design of the IMTA systems influencing the suitability of the remaining factors of bathymetry and sediment type. Bathymetry is an important component for any aquaculture activity since it is a limiting factor both for the structures but also for the biological tolerances of the species. However, as suggested by the bathymetry reclassified layer, the suspended design of sea urchins and lobsters had larger areas with increased suitability scores, opposed to the deployed sea cucumbers to the benthos. Deployed crates require regular maintenance through direct diving inspections. In IMTA depth of the structures is a very important component since it mediates the appropriate transfer of nutrients to the secondary organism (Sanz-Lazaro et al. 2018). For sea cucumbers, particulate organic matter tents to disperse over greater distances with increasing depth whereas shallower systems tend to ensure better connectivity (Filgueira et al. 2017). In addition, depth needs to be considerably shallower in order to allow regular diving operations. For the case of suspended structures, depth was not a huge limiting factor since the reclassification was based only on the technical design. The ability of suspended culture systems to be self-adjustable based on the requirements of each location offers a great level of flexibility and therefore a wider range of depths. This highlights the importance that when discussing and choosing depth as a factor for site suitability of IMTA the type of structure deployed in very important. Furthermore, another factor which increased the suitability scores of the lobsters and sea urchins against the sea cucumbers was the sediment type. Sediment type was a limiting factor on both designs, suspended and benthic but for different reasons. Sediment type was limiting for the suspended structures only in terms of the technical design, focusing on the type of sediment optimal for the deployment of concrete blocks holding the structure still. On the other hand, the sediment type requirements of the sea cucumbers require specific sediment type in order to allow better deployment of the benthic cages and ensure optimal feeding efficiency of the sea cucumbers (Han et al. 2016).

The model presented in this chapter was based on data retrieved from Scottish territorial sea data sets. Site suitability modelling is greatly influenced by the availability of data sources and the quality of the datasets (Ross et al. 2013). This is difficult when collecting data for national scale assessment as resolution of the available data as well as the variety of data needed for the development of the model vary, making the process very challenging (Ross et al. 2013). Retrieving bathymetric and sediment type data was assisted through European joint projects through either EMODNET and JNCC in relatively good resolution and quality. Constant values are easier to collect opposed to values with the element of temporality such as temperature and current speeds. Therefore, technological advancement is offering high resolution temporal data retrieved either from satellite imagery or 3D hydrodynamic models. Generally, site suitability assessments tend to rely on either 3D models or satellite imagery, such as oyster aquaculture site selection (Snyder et al. 2017) and site suitability for marine fin-fish (Porporato et al. 2020). These type of temporal observations in the case of Scotland were created due to growing interest aquaculture site selection and offshore development has. Even though, this chapter does not include other important factors for the biological tolerances of the species along with physical conditions, established and operational farm sites would be expected to already be optimal for a specific fin-fish species, consequently eliminating the uncertainty regarding extreme weather and water quality conditions (e.g waves, salinity, oxygen).

The MCE approach is clear that it has some advantages and disadvantages. The key advantages of MCE is that it is a well-established technique in aquaculture site suitability and it could in this chapter combine different biological and technical considerations of the IMTA systems. On the contrary, the MCE approach introduced a number of user-define input within the model which in turn creates uncertainty for the quality of the model output. The species models and the final model outputs in this chapter relied a lot on the selection of the key variables, where in a scenario with inclusion of more or less variables or different quality would have a different outcome. One of the issues with the reclassification of the selected factors is that even though they are based on scientific reasoning and experience, majority of the reclassification and weighting values with often rely on individual or group biases. This has been demonstrated on a study where changing of reclassification techniques and scenarios, results were different (Falconer et al. 2016). The opinion of the experts on the matter is relative with conclusions on reclassification values varying substantially (Nath et al. 2000). Even though this might seem as an advantage in terms of flexibility to different scenarios, that might create a sense of confusion to regulators. These uncertainties are even larger when assessing site suitability of IMTA where little information is known regarding the physiological and physical requirements of the species and the rearing designs respectively. However, the purpose of this chapter was to map variability of suitability scores of IMTA in general but also between the extractive organisms selected and this has been achieved.

2.5 Conclusion

The purpose of the chapter was to evaluate the level of spatial variability of suitable locations for IMTA as well as the level of temporal variability within each species suitability scores, through the use of GIS, site suitability methodologies. The results suggest that the variability between each species' biological and technical requirements had an impact on the suitability scores, suggesting that not all marine regions are suitable for IMTA with these species, neither are all salmon farm sites. Therefore, for the development and planning of IMTA careful considerations must be made concerning the type of the system applied and the biological tolerances of the extractive organism chosen. This chapter could further enhance the discussion regarding planning and management of IMTA at a national scale level.

Chapter 3 Investigating variability between farm conditions affecting performance of an IMTA system

3.1 Introduction

Most waste released from an open water coastal marine fish cage is in the form of particulate organic matter (Strain and Hargrave 2005). With vertical fluxes dominating the waste release pattern, particles settle within hundreds of meters of the farm site (Filgueira et al. 2017). The amount and distribution of waste is a combination of multiple factors, dependent on the production biomass of the fish farm system (Giles et al. 2008), the characteristics of the sinking particles (Reid et al. 2009) and the hydrodynamic profile of the area (Bravo and Grant 2018). Delivery of particulate organic matter to the secondary extractive organism is of paramount importance to IMTA, influencing the design of an open water IMTA system. Consequently, dispersal models which spatially identify waste distribution patterns and amount of waste deposited are applicable to open water IMTA. Dispersal models are used to predict nutrient waste dispersal and deposition (Cromey et al. 2002; Gillibrand et al. 2002; Corner et al. 2006; Brigolin et al. 2009; Cromey et al. 2009; Cromey et al.2012), often required for regulatory compliance (Chang et al. 2013). Depositional models require information of the settling velocity of the of the uneaten feed and faeces to calculate settling time, horizontal current speed and direction. The outputs of the models are over a defined period of time in the form of a mass of material (e.g. carbon) deposited per unit area. The output is often visualized with contour plots (Cromey et al. 2002). These models have been also incorporated to open water IMTA scenarios, to ensure physical connectivity between co-cultured organisms (Filgueira et al. 2017), as well as to estimate the amount of organic material potentially available for ingestion by benthic feeders (Cubillo et al. 2016).

A second essential element for the performance of an open water IMTA system is the selection of the extractive organisms. Sea cucumbers have been used extensively for IMTA studies. They were first used for IMTA in China in 1980s where they were suspended in lanterns along with filter feeding scallops (Zhang et al. 1990), followed by a near shore integration of the sea cumber *Apostichopus. japonicus* with Pacific oysters (Yuan et al. 2008; 2012; 2013). At individual animal scale, tank-based studies have evaluated the ability of sea cucumbers to absorb particulate wastes and examined growth rates and mortality over time (Nelson et al. 2012; MacDonald et al. 2013; Robinson et al. 2019). In pilot-scale open water experiments, sea cucumbers cultured in association with finfish and bivalve cages have shown good growth and survival in relation to control sites (Hannah et al. 2012; Yokoyama et al. 2013), and high assimilation efficiency at the farm site compared to control locations (Yu et al. 2011; Neofitou et al. 2019). Sea cucumbers in open water experiments are either suspended in pearl nets (Paltzat et al. 2008, Hannah et al. 2012) or through direct placement of metal and plastic crates to the seabed (Navarro et al. 2013; Neofitou et al. 2019). Tentative results have shown that sea cucumbers feeding activity is influenced by stocking density (Yang et al. 1999; Yuan et al. 2014), where slow growth rates have been observed when animal density exceeds diet availability (Namukose et al. 2016). However, for use in IMTA more information is needed in understanding the relationship of food availability and waste impacts to the placement of sea cucumbers in relation to distance from a fin-fish sites. Lab based and in-situ physiology experiments have indicated an acute physiological response of sea cucumbers when experiencing low oxygen concentrations leading to hypoxia stress (Newell and Courtney 1964; Astall and Jones 1991; Huo et al. 2018), whilst in the field under open water fin-fish cages serious mortality events have been documented due to high amounts of organic load deposited leading to anoxia (Yu et al. 2012). Furthermore, stocking density effect is important when supplementation of feed is lower than the demand experiencing slow growth or even mortalities when supplementary feed is less than demand (Dong et al. 2009; Hannah et al. 2013).

Several studies have used models to investigate the use of sea cucumbers in IMTA systems (Cubillo et al. 2016; Ren et al. 2012; Watanabe et al. 2015; Zhang and Kitazawa 2016; Chary et al. 2019;). Modelling studies from Cubillo et al. (2016) and Ren et al. (2012) suggested that sea cucumbers have the capacity to mitigate 70 % of the benthic particulate organic carbon originating from Atlantic salmon (*Salmo salar)*. Other studies using a combination of in-situ IMTA observations and mathematical models, suggested that sea cucumber would remove 4.3 % of the total particulate nitrogen from milkfish, but only if the sea cucumber density was approximately 200 times that typically employed (Watanabe et al. 2015). In addition, a hypothetical life cycle assessment modelling study from Chary et al. 2019 indicated that sea cucumbers cultured underneath a fin-fish farm would only be able to extract 0.73 % of the total waste released. Even though there is a contradiction of modelling results regarding the bioremediation efficiency of an IMTA system with sea cucumbers, direct comparison between modelling studies is not appropriate, since there are different species involved, ration between fed organisms and extractives as well as assumptions made. However, there are some core parameters which should be considered when investigating an IMTA system from a farm scale perspective. The present study extends this research by modelling environmental conditions from actual commercial salmon farm sites to allow a realistic assessment of bio mitigation potential of an open water IMTA system. It also allows for physiological limitations of the species, to be taken into account at these sites, in relation to the variable amount and spatial extent of the waste settled.

The specific aims of the present chapter were to compare the spatial variability of waste dispersion at different salmon farm sites and how that can affect performance of extractive species in an IMTA system. The investigation used the farm scale boundaries of an open water system using the sea cucumber, *Holothuria forskali,* and Atlantic salmon, *Salmo salar*. A waste dispersion model was used to estimate deposition of particulate organic waste around six commercial salmon farms and to calculate suitable areas for the deployment of the sea cucumbers based on each farm's total organic nutrient footprint and sediment oxygen conditions. Subsequent bio-mitigation efficiency was assessed for different stocking densities of sea cucumbers at the different locations. Finally, the performance of each system was evaluated by assessing the remediation cost efficiency as a function of waste removed against operational costs.

3.2 Methodology

3.2.1 Modelling farm sites

Six Atlantic salmon farm sites in Scotland were selected, which occupied a range of different hydrographic conditions (Table 3-1). Information on production biomass for a year of production in tonnes were sourced from Scotland environment web (Scotland's Aquaculture 2019). Site layout such as number of cages, distance between cages and orientation were retrieved by using Google Earth™. Hydrography (current flow speed and direction) measured at 20-minute intervals over a 15-day period at 3 depths (near surface, mid water and near seabed) for each site was obtained from the respective companies. All sites were anonymised.

Table 3-1 Modelled farm sites, site specific conditions and production biomass.

Parameters	Modelled farm site					
	1	2	3	4	5	6
Water column current speeds (cm s^{-1})	9.1	6.0	7.9	9.0	9.9	3.7
Mean seabed current speeds (cm s^{-1})	8.8	2.9	6.5	3.3	9.6	3.8
Maximum biomass stocked (tonnes)	1767	1450	1648	1455	1057	716
Depth (m)	20	22	29	24	19	16

3.2.2 Waste dispersion and deposition modelling

The hydrographic (15 days), production and depth data were used to parameterize a spreadsheet-based particulate waste dispersion model; the Cage Aquaculture Particulate Output and Transport (CAPOT) model (Telfer et al. in prep). CAPOT predicts the output and dispersion of particulate organic waste from the farm site. The CAPOT model operates in two phases. The first phase is the calculation of the amount of waste being organic carbon or suspended solids, entering the environment in the form of uneaten feed or faeces. The second phase of the model is the calculation of the spatial dimension of the dispersion of the wastes in two dimensions. The type of the waste and quantity are calculated through mass balance equations (Beveridge 2004; Perez et al. 2002). Biomass change over a period of time (normally a year) is used to calculate the usage of feed through a feed conversion ratio (FCR). The model makes a number of assumptions:

- Concentration of carbon in the feed is approximately 50% (Chen et al. 1999)
- Uneaten feed accounts for 3% of the total feed used (Hargrave 1994, Cromey 2002)
- Flesh of fish contains 14.6% carbon w/w (Chen et al. 1999)
- 50% of the carbon consumed is respired as CO_2 (Gowen et al. 1988) and 10% is excreted as urea (Perez et al. 2002)

The distance of the horizontal dispersion of food and faeces at any given point in time is calculated using the equations developed by Gowen et al. (1988). Initial settlement of waste is calculated (no resuspension after settlement) based on measured current speeds and directions over 3 sigma layers, and empirically derived settlements velocities for feed and faeces (Chen et al, 1999; Chen et al, 2003). Measured depth at the cage site (see Table 3.1) used was assumed to be flat and keep the same value.

The model was run for an average year of production with an output representing g C/ m^{-2}/y^{-1} over the modelled seabed area, using a 10 m grid within 500 m of the fish farm. The contour plot model output from the spreadsheet was plotted using Surfer^{TM} [Golden Software, Colorado, USA]. The software was then used to calculate the total areal cover of deposition for each farm in m^2 and total deposition within this area in tonnes. CAPOT model was used over other particulate depositional models due to its flexibility and freedom to use without paying any fees.

3.2.3 *Modelling areas for sea cucumber culture*

Based on the particulate depositional modelling of six salmon farm sites in Scotland. Depositional footprint of each farm site was divided into three spatial components allowing the physiological limitations and stocking density of the species to be taken into account at these sites. Characterising areas based on their suitability for the deployment of the extractive organisms.

The three components modelled were. Areas:

A. An area unsuitable for sea cucumber growth due to nutrient levels resulting on low oxygen conditions. Increased particulate organic deposition has been shown to induce anoxic events in the benthic sediments. A relationship between particulate organic deposition and oxygen supply provided through current speeds was used to identify these areas.

B. An area suitable with sufficient food supply within the IMTA containers. This area would allow good replenishment of oxygen supplementation and sufficient provisioning of waste feed for the extractive organisms.

C. An area less suitable for the deployment of sea cucumbers due to low nutrient deposition levels and limited food supply.

In Figure 3-1 a conceptual design of the three components approach used for the characterisation of the depositional footprint (contour plot) in relation to sea cucumber suitable areas for deployment area being shown.

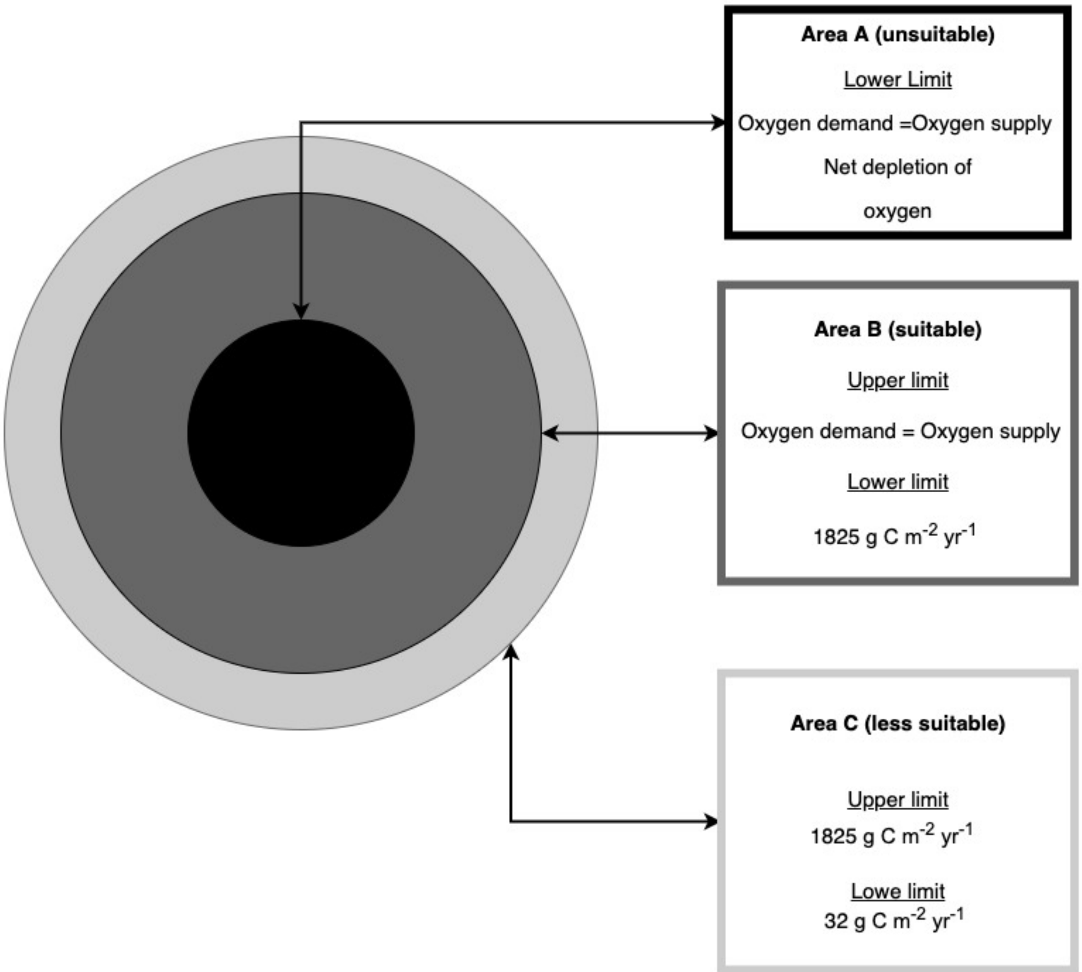

Figure 3-1 A hypothetical plot of a depositional footprint (g C m^{-2} yr^{-1}) of a single salmon cage with different colours showing Area A, B and C along the subsequent limits

3.2.3.1 Calculation of Area A

High levels of deposition of particulate organic matter dominates the sea bed close to the farm site, which can lead to areas of oxygen depletion due to elevated bacterial oxygen consumption (Findlay and Watling 1998; Karakassis 2000; Keeley at al.2012), resulting in mortalities on benthic organisms (Yu et al. 2012). Dissolved oxygen is supplied to benthic sediments from the water column, with current speed playing a crucial role in the rate of flux (Findlay and Watling 1998). The ratio of oxygen supply to oxygen demand can be used as a predictor for oxygen depletion of benthic sediments (Findlay and Watling 1998). Oxygen depleted conditions in lab-based experiments (Huo et al. 2018) and in experimental IMTA systems (Yu et al. 2012) have been shown to cause sea cucumber mortality. In this model it was assumed that areas where oxygen demand was higher than oxygen supply were considered unsuitable for culture of sea cucumbers.

The sediment oxygen demand can be calculated as a function of the particulate carbon flux to the seabed sediments (Findlay and Watling 1998), using Equation 3.1. Where:

$$Oxygen\ Demand = 1.7Carbon\ Flux - 32.6) \qquad \text{Equation 3-1}$$

Where Oxygen Demand is in mmol m^{-2} yr^{-1} and carbon flux is in mol m^{-2} yr^{-1}.

Oxygen Supply can be modelled from Maximum Fickian diffusion of oxygen as a function of flow velocity of the water layers near the seabed (Findlay and Watling 1998), using Equation 3-2. Where:

$$Oxygen\ Supply = 736.3 + 672.6log(Current\ Flow) \qquad \text{Equation 3-2}$$

Where Oxygen Supply is in (mmol m^{-2} yr^{-1}) and Current Flow velocity (cm/s).

As the area unsuitable for sea cucumber culture was where Sediment Oxygen Demand = Oxygen Supply, then the lower limit of Area A was where Sediment Oxygen Demand = Oxygen Supply. The lower limit of area A could be modelled as equivalent Carbon Flux by combining Equations 3.1 and 3.2 as Equation 3.3. Where for each modelled farm site:

$$Carbon\ flux\ = \frac{787.9+672.6\log(Mean\ Current\ Flow)}{1.7} \qquad \text{Equation 3-3}$$

The contour line for the equivalent calculated boundary carbon flux (Equation 3.3) was highlighted on the output of the CAPOT model for each farm site on the Surfer^{TM} output. The CALCULATE function in Surfer^{TM} was used to calculate the area of Area A in km^2, and the total amount of carbon deposited to Area A in tonnes yr^{-1}. See Figure 3.1.

3.2.3.2 Calculation of Area B

Area B for the modelled farm sites was the area suitable for sea cucumbers, this is areas where there is sufficient oxygen supply and enough food supplied by farm waste. The upper limit for the designation of Area B is the lower limit of Area A (Section 3.2.3.1). The lower limit of Area B was calculated based on carbon flux at a minimum constant feeding rate for the sea cucumber and so not be limiting. In tank trials, *H. forskali* individuals consume 0.5 g C day^{-1} (MacDonald et al. 2013). Consequently, based on this value and a standard stocking design of 10 individuals per m^2 (MacDonald et al. 2013), the minimum annual carbon flux to adequately support sea cucumbers would be 1825 g C m^{-2} yr^{-1}. This was used as the lower limit of Area B.

Surfer^{TM} was used to calculate the total area (km^2) and total carbon load (tonnes yr^{-1}) within this lower limit contour for the CAPOT outputs. Carbon loading and area specifically for Area B was then the differences between values for Area A (Section 3.2.3.1) and the totals to the 1825 g C m^{-2} yr^{-1} contour (See Figure 3.1).

3.2.3.3 Calculation of Area C

Area C was established in the outer region of waste dispersion where the supply of food is not sufficiently supplemented by waste to maintain growth in sea cucumbers. This area was bounded by a lower contour of 32 g C m^{-2} y^{-1} and an upper limit of 1825 g C m^{-2} yr^{-1} (see Section 3.2.3.2). The extent (km^2) and total carbon loading (tonnes yr^{-1}) of Area C was calculated in Surface^{TM} as the area and loading within the 32 g C m^{-2} y^{-1} contour, after subtracting the combined area and loading for Areas A and B. See Figure 3.1.

3.2.4 Statistical analysis

A multiple regression analysis (Jobson 1991) was used to test the site specific relationship between depth of the cages (m), production biomass (tonnes), and average water body current speeds (cm s^{-1}) and the suitable Areas B at each farm site. The analysis was performed using MINITAB software (Minitab LLC, Pennsylvania, USA).

3.2.5 Assessment of mitigation potential using different sea cucumber production scenarios for Area B

It was assumed that only Area B would be suitable area for deployment of the sea cucumbers, as Area A is limited by potential mortalities occurred and Area C is limited by food supply. The bio-mitigation potential of sea cucumbers within Area B was explored using three hypothetical sea cucumber production scenarios. These were:

- **Scenario 1** – stocking the whole of Area B with sea cucumbers at 10 ind m^{-2} (non-contained)
- **Scenario 2** - deployment in Area B of 500 crates (each 1 m^2), each crate having a stocking density of 10 ind m^{-2}
- **Scenario 3** - deployment in Area B of 200 crates (each 1 m^2), each crate having a stocking density of 10 ind m^{-2}

The deployment design of crates for Scenarios 2 and 3 used 25 crates per system, as given in Figure 3.2. This means there were 20 culture systems deployed within Area B for Scenario 2 and 8 culture systems deployed within Area B for Scenario 3.

Mitigation potential was for each fish farm site calculated as the total amount of deposited carbon per year to the culture areas in Area B, less the amount eaten by the number of individuals within the area based on the stocking densities. At a 10 ind m^{-2} for all scenarios, sea cucumbers were assumed to remove 1825 g C m^{-2} yr^{-1} (MacDonald et al. 2013) over the culture area. For Scenario 1 the culture area modelled was the whole of Area B. For Scenario 2 the culture area modelled was 500 m^2 within Area B. For Scenario 3 the culture area modelled was 200 m^2 within Area B. The total removal per year for each scenario was then calculated as a fraction of the total carbon deposited in Areas A, B and C to calculate the bioremediation at each site.

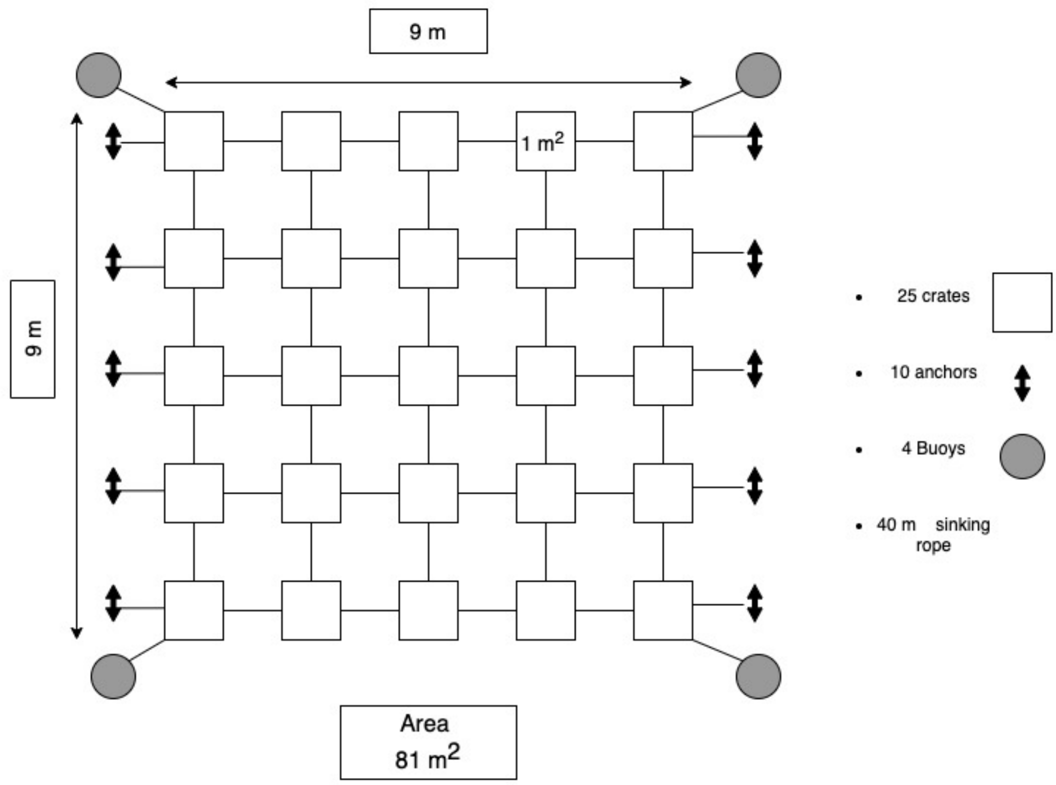

Figure 3.2 The hypothetical rearing desing for sea cucumbers. Legend depicits each part of the system

3.2.6 Remediation cost efficiency

The monetary cost of bioremediation/removal of 1 kg of carbon waste was used as an indicator of provision of ecosystem services. These were based on overall production costs and how this related to the amount of waste removed for each scenario as described in Section 3.2.5. The calculation of sea cucumber production costs included seed, the culture systems, their deployment on the seabed and their maintenance for each scenario. The production cost for to scenario 1 Table 3-2 where sea cucumbers were released into Area B without containment was related to the price of sea cucumber seed and SCUBA diving costs for maintenance and harvesting for a year's production. The production costs for Scenarios 2 and 3 were related to the price of seed, and the costs of SCUBA diving maintenance and the production equipment used. The consequent costs for a year's production are show in Table 3.3 for Scenario 2 and Table 3.4 for Scenario 3.

Table 3-2 Scenario 1 , including price of the seeds and diving labour. The number of sea cucumber seed (X) is dependent the size of Area B at each farm site.

Equipment	No of units	Unit	Unit price (£)	Annual Price (£ y^{-1})
Seed	X	individual	0.2	X
Diving labour	20	hours	187.50	9,000
Total				10,757

Table 3-3 Scenario 2 cost of deployment and maintenance for 500 crates.

Equipment	No of units	Unit	Unit price (£)	Total price 500 crate system (£ y^{-1})
Oyster crates	500	crate	3	1500
Riser rope	2	200 m roll	10.50	21
Sinking groundlines	2	550m roll	297	594
Buoys	80	buoy	12.50	1000
Seed	5000	individual	0.2	1,000
Anchor	200	Anchor	70	14,000
Diving labour	150	hours	187.50	28,125
Total				46,240

Table 3-4 Scenario 3 cost for deployment and maintenance for 200 crates.

Equipment	No of units	Unit	Unit price (£)	Total price 200 crate system (£ y^{-1})
Oyster crates	200	crate	3	600
Riser rope	1	200 m roll	10.50	10.50
Sinking groundlines	1	550m roll	297	297
Buoys	32	buoy	12.50	400
Seed	2000	individual	0.2	400
Anchor	80	Anchor	70	5,600
Diving labour	60	hours	187.50	11,250
Total				18,557

Finally, based on the assumptions that there were no mortalities or escapes and no replacement of the equipment over the year, the remediation cost efficiency of the three scenarios was estimated by dividing the expenditure (£ y^{-1}) by the amount of carbon removal (kg y^{-1}) for each scenario at each farm site to give a cost per kg of carbon removal.

3.3 Results

3.3.1 Total area and deposition for Areas A, B and C at each modelled farm site

The contour plots of carbon deposition in the three Areas A, B and C for each farm site are given in Figure 3.3. This shows the different extent of distribution of particulate waste and the relative areas suitable for culture of sea cucumbers over the range of farm sites, with varying environmental conditions and levels of production.

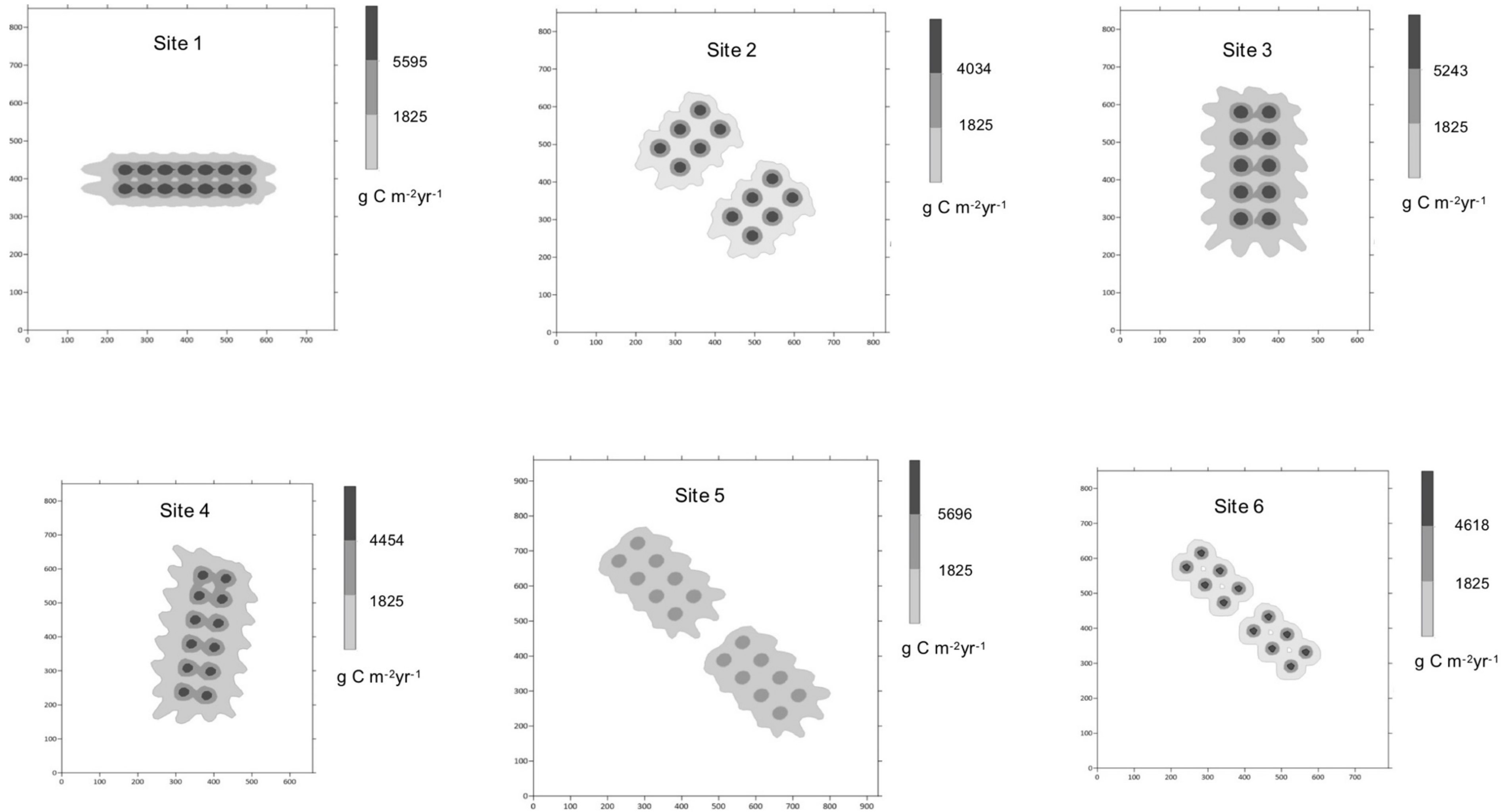

Figure 3-3 Contour plots of the depositional extent of the six modelled farm sites for each area; Area A (dark grey), Area B (medium grey) and Area C (light grey)The results for the total extent for Areas A, B and C are given in Figure 3.4 for each modelled farm site. All three designated Areas A, B and C varied between the different sites. Axes as given in metres.

The deposition extents and loading for each site and the different Areas in relation to sea cucumber production are presented in more detail in Figures 3-4 and 3-5. Site 5 had the largest overall depositional area with 115320 m^2. Site 5 was a well flushed location with the highest water body current speeds (mean = 9.9 cm s^{-1}) and the second lowest maximum biomass (1057 tonnes), dispersing the smaller amounts of waste over greater distances. The location with the lowest depositional extent was Site 6 which had the slowest current speeds (mean = 3.7 cm^{-2}) and the lowest production biomass (716 tonnes).

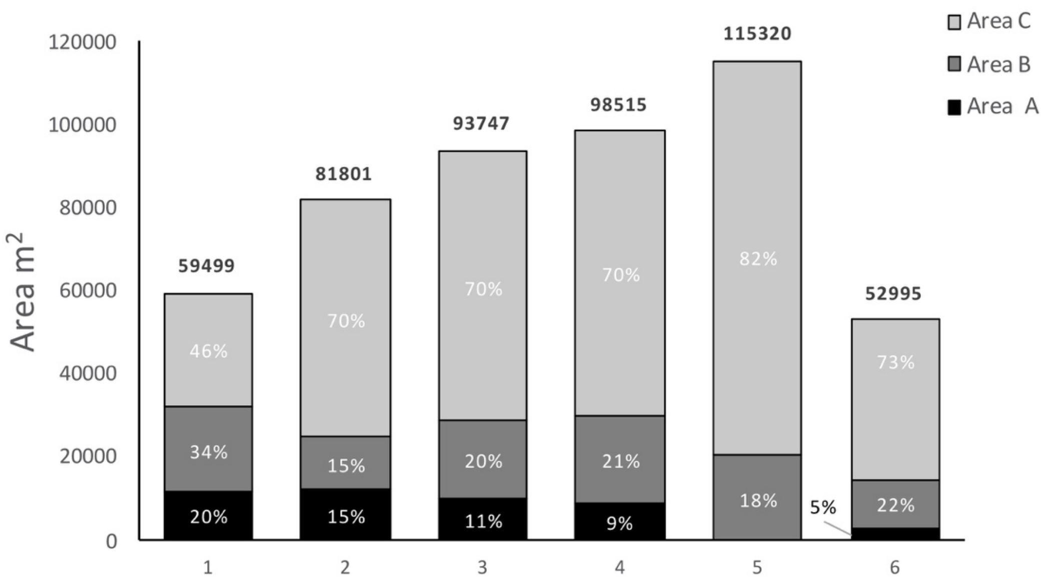

Figure 3-4 Depositional area extent in m2 for each of Areas A, B and C in relation to the total depositional area of each modelled farm site.

The results for total amount of carbon settled per year to the seabed over the deposition areas are given in Figure 3-5. Here the highest is at Site 1 and lowest at Site 6. The relative amounts of carbon deposited per year in each of the areas are given as percentages.

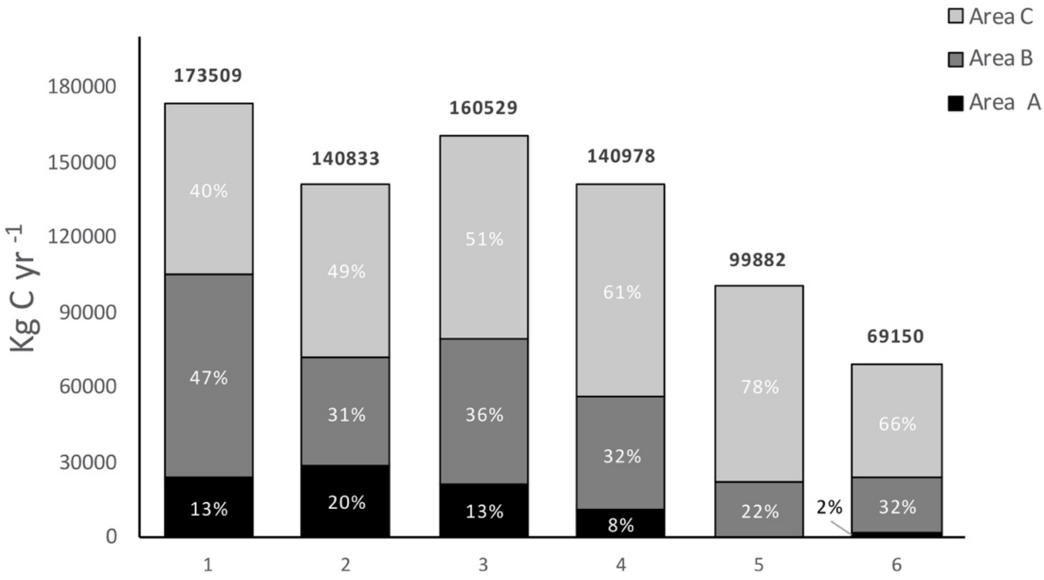

Figure 3-5 Percentage carbon deposition (kg C/yr) for each of Areas A, B and C in relation to the total depositional area of each modelled farm site.

The relationships between individual environmental factors and the depositional areas and amounts of carbon deposited in Area B for each site was compared in a series of regression plots, given in Figure 3.6. Here there is a clear correlation between carbon deposition in Area B and biomass (See Figure 3-6B) and between the extent of Area B at each site and current flow (See Figure 3-6C). The regression equations for the relationships are given in the Figures. Consequently, we should be able to approximate the area for optimal growth of any suitable site using:

$$Area\ for\ optimal\ growth = (1739 \times Mean\ current\ speed) - 4177 \quad \text{Equation 3-4}$$

Where Current Speed is in cm/s and Area is in m^2

The amount deposited in this area is:

$$Carbon\ deposited = (52 \times Max\ biomass) - 24878 \quad \text{Equation 3-5}$$

Where carbon deposited is in kg C/yr and max biomass is in tonnes

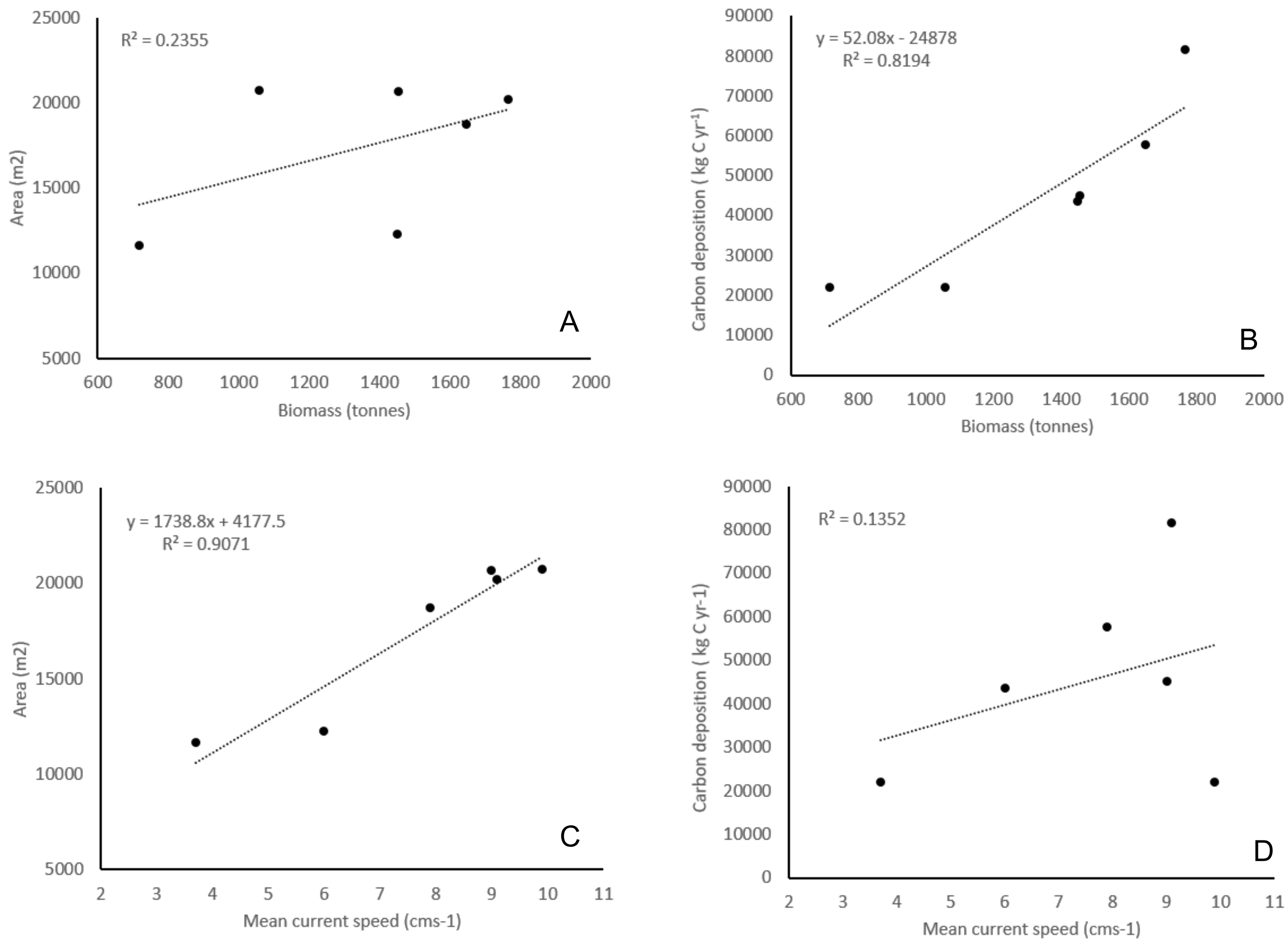

Figure 3.6 Areas (m^2) and carbon deposition ($gC\ m^{-2}\ yr^{-1}$) for different biomasses (A, B) and different current speeds (C, D). Regression equations are given.

Here we see that there is a relationship in the optimal areas near the cages for sea cucumber growth and the amount of additional food (carbon source) available and site-specific current flow and biomass respectively. This relationship can be easily calculated for any site as using the equations and has potential for use as a first estimator of economic and bioremediation potential by calculating the potential number of sea cucumbers grown.

3.3.2 Bio-mitigation capacity of sea cucumbers

Bio-mitigation capacity was compared for the three scenarios presented in Section 3-2-5. Comparison was made between scenarios by calculation of cost for sea cucumbers removing 1 kg of deposited waste carbon in Area B.

The results of the bio-mitigation capacity of the IMTA system based on Scenario 1 is shown in Table 3-5. This scenario where sea cucumbers were evenly spaced over the whole of Area B gave varying results. At Site 1, 200530 sea cucumbers were estimated to remove 55 % of the waste deposited within Area B which is 26 % of the overall carbon waste deposited. At Site 5 and Site 6, the amount of carbon waste deposited within Area B would not be enough to satisfy feed requirements of the sea cucumbers, resulting in 100 % consumption, and growth conditions would be sub-optimal. The same occurs for Site 5 and Site 6 when looking at the waste removed against the total waste settled across all three zones.

Table 3-5 Number of individuals in Area B on six modelled farmed sites and the relative bio-mitigation capacity of each site.

	Modelled farm sites					
	1	2	3	4	5	6
Number of individuals in Area B	200530	123950	186570	206800	204030	117900
Proportion of waste in Area B removed %	55	48	41	16	100	100
Proportion of total waste removed %	26	15	15	5	100	100

Scenarios 2 and 3 results, presented in Table 3.6, showing the waste deposited on and removed by a single cage. This is then used in calculating removal of waste by the sea cucumbers for each individual scenario.

Site with the highest load of organic matter settling on each crate within the Area B was Site 1 with 4067 g C m^{-2} yr^{-1}, so based on a constant feeding rate of each create being 1825 g C m^{-2} yr^{-1} ,there would be potential to remove 45 % of the waste settled on the crate. Conversely, for Site 5 the average organic matter deposition on each crate was 1054 g C m^{-2} yr^{-1} meaning that sea cucumbers would remove 100 % of the waste settled on each crate.

Table 3-6 Amount of waste deposited in one crate across the six modelled farmed locations in relation to the constant waste removed from each crate, shown as % waste per crate removed.

	Modelled farm sites					
	1	2	3	4	5	6
Waste entering one crate (gC m^{-2} yr^{-1})	4067	3519	3092	2172	1054	1867
Waste removed from each crate (gC m^{-2} yr^{-1})	1825	1825	1825	1825	1825	1825
Remediation potential per crate (%)	45	52	59	84	100	98

For Scenario 2, using a total of 500 crates, the removal potential for each site is shown in Table 3-8. Removal potential ranged from 0.53 %, at Sites 1, to 1.27 %, at Site 6.

Table 3-7 Scenario 3 showing % of total area covered by 500 crates along with % proportion of total amount of waste removed in each location

	Modelled farm site					
	1	2	3	4	5	6
Proportion of area covered by 500 crates (%)	0.84	0.61	0.53	0.51	0.43	0.94
Proportion of total waste removed by 500 crates (%)	0.53	0.65	0.57	0.65	0.53	1.32

For Scenario 3, using a total of 200 crates, the removal potential for each site is shown in Table 3-7. Removal potential ranged from 0.21 %, at Sites 1 and 5, to 0.53 %, at Site 6.

Table 3-8 Scenario 2 showing % of total area covered by 200 crates along with % proportion of total amount of waste removed in each location.

	Modelled farm site					
	1	2	3	4	5	6
Proportion of area covered by 200 crates (%)	0.34	0.24	0.21	0.20	0.17	0.38
Proportion of total waste removed by 200 crates (%)	0.21	0.26	0.23	0.26	0.21	0.53

Using these values within calculations for the cost efficiency for remediation, results are shown for all six modelled farm sites in Table 3-9. Scenario 1 appeared to be the most cost effective, ranging from £0.001 kg^{-1} to £0.06 kg^{-1}. The only negative value was in Site 5 indicating that the system was consuming more feed than available. Concerning Scenario 1 and 2 all sites had the same value of £51 per kg removed. The only difference was in Site 5 with the value reaching £88 per kg removed. This originated from the amount of waste deposited in each crate as shown in Table 3-6 with each crate consuming less than 1825 gC m^{-2} yr^{-1}

Table 3-9 Remediation cost efficiency as cost (£) of removal for 1 kg of carbon waste at all modelled sites for each scenario.

Scenarios	Modelled farm site					
	1	2	3	4	5	6
1 (No crates)	0.001	0.001	0.001	0.007	-0.03	0.06
2 (200 crates)	51	51	51	51	88	51
3 (500 crates)	51	51	51	51	88	51

3.4 Discussion

The performance of an open water coastal IMTA system in relation to bio-mitigation is one of the most important pillars of IMTA studies and a matter of a great discussion (Troell 2003; Neori et al. 2004; Chopin et al. 2012). The physical characteristics of the natural environment along with the production biomass of a fed species, influences the amount of waste deposited, dispersion pattern and so the area of influence of that waste (Cubillo et al. 2016). Equally, the extractive species selected and its physiological capacity to absorb waste, the system design and stocking density of the extractive species influences the amount of bioremediation of that waste (Cubillo et al. 2016; Chary et el. 2019). The purpose of this chapter was to investigate how the performance of extractive species in an IMTA system can be affected by farm scale factors, such as local environmental parameters, and how they vary between sites and local conditions. Here, the spatial variability of waste was assessed using dispersion models and how this affected environmental conditions for rearing the extractive species and its bio-mitigation potential.

The results suggest that not all locations around a farm cage are suitable for the deployment of sea cucumbers based on the assumption that sea cucumbers will not survive in locations where high waste loading will result in anoxic conditions. The depositional area and amount of carbon deposited suitable for sea cucumber culture varied between locations and were highly dependent on environmental (current speed) and production (biomass) characteristics. This agrees with several other studies looking at site specific characteristics influencing depositional extent and cumulative waste load in multiple red drum farms in Mayotte, Indian ocean (Chary et al 2019b), salmon and sablefish farms in Canada (Brager et al. 2015), salmon farms in Norway (Carvajalino-Fernández et al. 2020). The areas suitable for sea cucumber cultivation (Area B) ranged from 15% to 34% of the total depositional area of the waste from the fish cages, with areas of up to 20% being highly unsuitable due to depleted sediment oxygen levels and their effect of sea cucumbers (Area A). In addition, statistical analysis indicated that there is a significant relationship of the optimal areas near the cages and the amount of additional food available with site specific current flow and biomass respectively.

Studies have shown that particularly epifauna organisms subjected to less than < 0.5 ml^{-1} endured high mortality events from severe hypoxia to early anoxia, in this particular study all polychaetas, decapods and echinoderms died (Riedel et al. 1012). Except mortality events, low oxygen conditions can also alter the physiology of the organisms and trigger different immunological responses (Huo et al. 2019). This study took into consideration this limitation showed particularly in lab studies in combination with empirical observations from Yu et al. (2012). The predictive model for oxygen exchange with sediments (Findlay and Watling, 1998) was validated for a series of farm sites in Canada and has also been confirmed by a study developed in New Zeeland using the same predictive modelling approach. The study indicated good agreement of modelling values with actual values suggesting the model's ability to perform in other locations, beyond the local conditions for which it was originally developed (Morrisey et al. 2000).

Another modelled area (Area C) not considered highly suitable for the deployment of sea cucumbers was where limited food supply was not be enough to fulfil sea cucumber nutritional needs. The depositional area and amount of carbon deposited within this area across the six modelled farm sites ranged from 58 % to 68% of the total depositional extent. Again, there was variation between locations due to the environmental characteristics of each site. Wild sea cucumbers have been shown to perform well under low total organic matter sediment concentration, due to their foraging behaviour (Roberts 2000). However, the nature of the IMTA design proposed in this study requires deployment of sea cucumbers in cages, limiting movement, ultimately increasing competition for resources such as food and oxygen (Zamora et al. 2016). Based on the recommendations of Zhang et al 2016, sea cucumbers need to be located in areas where there is efficient feed supply to sustain the welfare of the species and meet its feeding demands. Therefore, this chapter suggests that when looking at suitable locations for IMTA with benthic deposited organisms, stocking design and density plays an important role for the requirement of the species and has an impact on the available area and amount of waste provided for culture. This agrees with other studies showing a large effect of stocking density on sea cucumber growth (Lavitra et al. 2010; Hannah et al 2013) with better growth performance at low densities (Slater and Carton 2007; Yu et al. 2014). Importance of stocking density considerations in bioremediation performance of an IMTA system with sea cucumbers has also been highlighted from Chary et al. 2019 as a realistic insight over the capacity of the system. It is important to note however that in this particular chapter, mortalities were not taken in account, whilst feeding rates were assumed to be constant during the whole year.

This chapter argues that not all waste and area influenced is available for consumption and settlement of the co-cultured organism. This comes in contradiction with other conceptual models which assumed that 100 % of the waste is available for consumption, whilst through DEB equations they calculated the capacity of the sea cucumbers to feed and mitigate the whole waste provided by a farm site (Watanabe et al. 2015, Cubillo et al. 2019). Spatial management of the co-cultured organisms is often overlooked while in this chapter it is suggested that is very crucial affecting the performance capacity of the system to mitigate waste. This approach could aid in decision making in terms of the most efficient spatial arrangement for the extractive organisms. In addition, it suggests that prior to the establishment of an IMTA farm scale set-up, conditions of the site should be modelled to take in account suitable areas for deployment. The derived regression equations indicating a relationship between current speed and biomass with area and loading respectively, suggest that they could potentially be used for assessing sites prior to establishment of the system. The same spatial approach has been created using salmon and seaweeds (Fossberg et al. 2019) and (Broch et al. 2019), by calculating the relative potential growth of seaweeds in relation to the dispersion footprint of the dissolved waste, after the name of spatial index.

Bio mitigation capacity for the three stocking density scenarios differed between Scenario 1 with Scenario 2 and 3. Scenario 1, which included the open distribution of sea cucumbers, results in a relatively high bio-mitigation potential ranging from 5% to 100% of the total carbon waste released. However, open distribution of sea cucumbers as a technique poses many questions. The number of sea cucumber released beneath the farm site averaged 167850 individuals, the supply of which would be unsustainable under full commercial conditions. *H forskali* is a difficult species to maintain in captivity and breeding and hatchery rearing of this species is still in the early stages of development (Laguerre et al., 2020). Equally, it is not possible to access such numbers sustainability from wild sources. In addition, ranching of sea cucumbers has been associated with impacts on the wider ecosystem, particularly with genetic contamination with the wild populations, increased bio-turbation and degradation of the sediments (Purcell et al. 2016). This scenario was used as a reference observation in order to show that for such levels of mitigation by the IMTA system, unrealistic number of individuals is required. On the contrary, Scenarios 2 and 3 with deployed metal crates revealed a limited capacity to mitigate waste effects averaging 0.28 %. The location with the highest bio-mitigation potential was the site with the slowest current speeds and lowest biomass.

Furthermore, it is important to note, that growth and therefore food consumption was assumed to be consistent throughout the year (MacDonald et al. 2013) and not following typical seasonal patterns described by Hannah et al. (2013). It is important to note that in this study consumption rate was underestimated with *H. forskali* absorbing 0.18 Kg C yr^{-1}, opposed to other studies where sea cucumbers fed with finfish waste demonstrated an approximate removal of 1kg solids yr^{-1} for *H.Forskali* (MacDonald et al. 2013) and 5 kg solids yr^{-1} for *P. californicus* (Cubillo et al. 2016) and 0.879 kg sediment yr^{-1} (Chary et al. 2019). However, it is important to note that the model did not taken in account seasonal effects (slow growth over winter (Dec-Feb), weight increase in the spring season (Mar-Jun), negative scope for growth due to aestivation in summer (Jul- mid Sep), and feeding cessation due to seasonal visceral atrophy (mid Sep- Nov)) (Hannah et al. 2013).

The last part of this chapter explored costs related to the establishment of a rearing design under all three hypothetical scenarios, whilst comparing that versus the amount of carbon removed. This analysis suggests that under Scenario 1 the costs related compared to the carbon removed were negligible ranging from 0.001 £kg^{-1} and 0.06 £kg^{-1} whereas under Scenarios 2 and 3 the cost is considerably higher at 80 £kg^{-1}. The same approach has been used to examine the cost-efficiency of an IMTA system with seaweeds and mussels developed by Holdt et. al (2014), indicating that mussel effectiveness as a bio-filter required from 11 to 30 € kg^{-1} and 61 € kg^{-1} for different systems, whilst seaweeds *Saccharina latissimi* was the most expensive reaching 1012 € kg^{-1}. These values were extracted by calculating the nitrogen content of seaweeds and mussels through a growing cycle, whilst cost related to the establishment of these two systems was based on a year's estimation cost of *Laminaria digitata* production from Edwards and Watson (2011) and experience from a full-size mussel compensation cultivation in Denmark from Petersen et al. (2013). Furthermore, another study which included cost effectiveness of mussels, suggested the value of 162 € kg^{-1} for one location with high biomass and 4.018 € kg^{-1} for another location with low biomass. In addition, due to lack of commercial examples costs attributed to the operation of the sea cucumber production systems may be inaccurate. However, this was only investigated to show cost effectiveness of ecosystem provided in comparison to other IMTA species design. There are important steps to be taken in order to close the information gap of growing sea cucumbers in the West as opposed to Asia where they are commercial examples available (Suryono et al. 2019)

3.5 Conclusion

The findings of this chapter suggest that fate and amount of particulate organic waste is influenced by site specific farm conditions, having an effect on the areal and depositional extent of each farm site. The results indicated that not all areas and not all particulate organic matter is available to the co-cultured organism originating from extractive organisms biological and stocking design limitations, influencing the performance of each site location. Bio-mitigation efficiency of the farm level IMTA system investigated (salmon with sea cucumbers), appears to be limited.

Chapter 4 Investigating IMTA at farm scale: a field trial.

4.1 Introduction

Mariculture is crucial for the future supply of seafood, but environmental impacts associated with increased production is impeding its growth. Therefore, mariculture is taking steps in order to become influential on supporting ecosystem services beyond the purpose of just producing goods, through providing regulating services, habitat formation or even restocking of wild populations (Alleway et al. 2018). An example can be seen in the United states of America where hatchery produced oysters were used for natural oyster reef restorations (Brumbaugh et al. 2000). Despites IMTA most important precondition being bioremediation it has also been suggested as a measure to provide ecosystem services (Giangrande et al. 2021). Under this new approach the establishment of new candidate species has emerged. However, assessing the candidate species ability to be considered in an open water IMTA system requires considerations at the individual spatial scale.

The capacity of an organism to utilize wastes depends on trophic connectivity between the different components of the system, where a sufficient amount of waste material reaches the secondary extractive organism (Barrington et al. 2009). Established physical connectivity between the components of the system will increase the likelihood of a trophic connectivity between wastes and the extractive species which can be dependent on the environmental conditions and production methods at the site location (Filgueira et al. 2017). This will have obvious implications for the design of an open water system, since it will influence the designation of appropriate locations for the deployment of the co-cultured species and ultimately affect the performance of the candidate extractive organism.

Trophic connectivity of IMTA can be measured empirically by comparing growth or measuring nutrient uptake by the extractive species between the farm site and a reference location. This approach has been used to assess shellfish growth

(Lander et al. 2013; Sara-Zenone and Tomasello 2009) and nutrient uptake and growth of seaweeds (Abreu et al. 2009; Blouin et al. 2007). Assessment of growth in relation to the cages has also been observed for sea cucumbers (Yuan et al. 2008; Yokoyama et al. 2013; Yokohama et al. 2015), sea urchins (Cook and Kelly 2007). Capacity of an IMTA candidate extractive species to consume wastes to establish trophic connectivity has been mainly performed in lab based controlled environments through stable isotopes and fatty acids in tissue of mussels (Redmond et al. 2010), faecal organic content in sea cucumbers (Nelso et al. 2012), absorption efficiency in mussels (MacDonald et al. 2011). However, in the natural environment where there is a majority of food sources, it is difficult to assess if any physiological response such as growth owes to the utilization of fish feed waste (Irisarri et al 2015), therefore more robust molecular indicators are being used like fatty acids and stable isotopes. Fatty acids and stable isotopes have been used effectively in the past to investigate seasonal and spatial changes in the diets of the consumer species (Allan et al. 2010; Zhao et al. 2013). The use of biological tracers has been extensively used in the past primarily to assess environmental implications of particulate organic deposition originating from farms to wild populations altering their trophic status. (e.g. Fernandez-Jover et al. 2009, 2011; White et al. 2017, 2019; Woodcock et al. 2018).

The use of fatty acids has been applied to open water IMTA studies to investigate trophic connectivity between the fed and the extractive species, as demonstrated for particulate waste uptake from mussels (Handa et al. 2012; Irrisari et al. 2015), particulate organic matter for sea urchins (Cook and Kelly 2007). Fatty acids provide a qualitative assessment of a trophic relationship, having the advantage of having high residence times without undergoing major changes (Kelly and Scheibling 2012). However, assessing the performance of an IMTA system with fatty acids is dependent on the target species physiological status and the turnover rate of each sampled tissue, as there have been variable results using mussels (*Mytilus edulis*) (Redmond et al. 2010; Irisarri et al. 2015), though more consistency found for zooplankton (Fernandez-Jover et al. 2009), shrimp (Olsen et al. 2012) and sea urchins (George and Parrish 2015).

Stable isotopes of carbon and nitrogen have been used widely to assess the flow of nutrients through food webs, and also to trace waste products from aquaculture (Marin-Leal et al. 2007; Deudero et al. 2012). Stable isotopes assimilate within the tissues of consumers, with heavier isotopes, ^{13}C and ^{15}N, remaining longer in animal tissues than lighter ones, ^{12}C and ^{14}N, which are rapidly utilised during metabolism (Marin-Leal et al. 2007). Consequently, the tissues of organisms tend to adopt the same stable isotopic signature as their food source (Paulet et al. 2006). The ratios $\delta^{13}C$,$\delta^{15}N$ have been applied for nutrient tracking within IMTA systems with fish and mussels in the Bay of Fundy, Canada (Irisarri et al. 2015), fish with oyster and mussels (Navarrete-Mier et al. 2010) and in multiple farms with fish and mussels in the Western Mediterranean (Sanz-Lazaro and Sanchez-Jerez 2017).

Species, such as echinoderms and bivalves, have been already demonstrated their capacity to feed on fin-fish waste. This study though investigates another potential species for use within a finfish IMTA system, the European lobsters, and how this affects its early life stage placement in the marine environment. The European lobster, *Homarus gammarus* (Linnaeus.1758), is an important economic decapod crustacean, with a natural distribution ranging from Morocco to northern Norway (Wilson 2008). Global demand for lobsters as food are increasing, but over the past few decades wild populations are on the decline (Drengstig and Bergheim 2013; Ellis et al. 2014; Nillos Kleiven et al. 2019) prompting research into commercial hatchery production of larvae for restocking purposes (Addison et al. 1994; Schmalenbach et al. 2011). Lobsters grown in land-based hatcheries and directly released into the environment are known to be vulnerable to immediate predation as they have limited exposure to environmental stimuli (Agnalt et al. 2017). To improve survivability, sea-based containers have been used successfully to acclimatise juvenile lobsters to environmental conditions before final release (Beal et al. 2002; Perez Benavente et al. 2010; Beal and Protopopescu 2012; Daniels et al. 2015; Halswell et al. 2016). At the release stage juvenile lobsters form small burrows in sediment and feed on zooplankton and organic matter particles suspended in the water column, using currents created by their pleopods (swimmerets). The particle sizes consumed at this stage are normally between 60 and 100 µm (Lavalli et al. 1989), suggesting that they are able to consume fine particulate wastes from the fish farm.

The aim of this study was to investigate IMTA at farm scale in the field and assess whether there is trophic transfer between fish and European lobsters. In addition, it investigated whether there nutritional and locational benefits in using lobsters within such IMTA systems.

4.2 Methodology

4.2.1 Experimental site

The study was conducted at the Lehanagh Pool Marine Research Site, a small scale experimental IMTA site in Bertraghboy Bay, Connemara, Co. Galway (Figure 4-1), between January 2018 and March 2019. Salmon production at the site comprised of two 50 m circumference and 8 m deep polar circle cages at a water depth of 21 m. These were stocked in April 2018 with 7660 Atlantic salmon post-smolts (5360 and 2300 in the two cages), averaging 90 to 100 g. In addition, 400 lumpfish (*Cyclopetrus lumpus*), averaging 40 - 50 g were also stocked into each cage to control sea lice as per commercial standards for cleaner fish. All fish were hand-fed a maintenance diet according to manufacturer’s feeding tables. The site was managed without the use of any chemical treatments, with no prescription medicines or antifoulants used. A control site for the study was set up approximately 300 m west of the cages.

Figure 4-1Map showing the location of the experimental pilot scale IMTA site in Lehanagh pool. The positions of the salmon cages, lobster deployment and sampling locations are given in Figure 4.3.

Figure 4-2 The photographs show the SBCC structures on the cage site. The photos below show the SBCC structures in water and off water. Photo on the right corner shows the fouling organisms inside an SBCC compartment.

4.2.2 In-situ environmental sampling

During a 5-day sampling campaign between 02/07/2018 and 06/07/2018 water samples were collected at three different stations with locations shown in Figure 4-3 and Table 4-1, at two different depths (surface and 5 m) on three different stages of the tidal cycle with height of the tides and times of collection shown in Figure 4-4.

Figure 4-3 Map displaying the location of cages, Control station (Reference). Legends indicate the location of data collection points in relation to the cages and the control site. The wide positioning of this location is given in Figure 4.1

Table 4-1 GPS (longitude and latitude) coordinates of the water quality sampling locations

Sampling stations	Northings	Westings
C1	53° 24.043'N	9° 49.186'W
C2	53° 24.054'N	9° 49.150'W
Control (Reference)	53° 23.976'N	9° 49.424'W

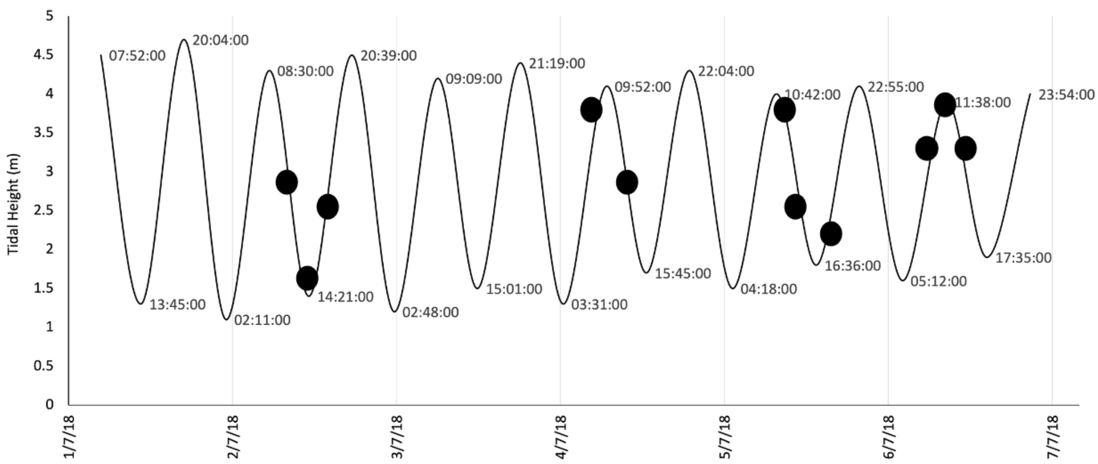

Figure 4-4 Water quality sampling times shown as black circles in relation to the tidal height across the in-situ water quality collection.

During every sampling occasion, water quality parameters were measured in order to characterize the surrounding environment, assess any potential externalities, but also to compare the reference stations versus the cage farm locations. Dissolved oxygen and temperature were measured by deploying a Handy Polaris 2 (OxyGuard International, Denmark). Water was collected for the surface and 5 m depth using a Van Dorn water sampler from which 3 sub-samples were taken; 1) a small sample was used to measure salinity (in ppt) using a refractometer, 2) a sub-sample was poured into acid washed, pre-labelled 500 ml polypropylene bottles for measurement of Total Ammoniacal Nitrogen and Chlorophyll. Position and time of every samples was recorded using a GPS watch (Ambit Peak 3, Suunto, Finland).

On site 250 ml of the sampled water was filtered through a FisherBrand Glass Microfibre paper ‘MF200’ in a Millipore vacuum filter. The residual water and the filter papers were then frozen (-20°C) prior to transportation in cool boxes to the Institute of Aquaculture for analysis.

The CAPOT model (Telfer et al., in prep), was used to determine if the lobsters would have access to the wastes from the cage. To characterise water flow at the farm and for the parameterization of the CAPOT model, hydrographic and farm production data was collected. Current speeds and directions at the cage site were measured at 10 min intervals by a MIDAS ECM self-recording electromagnetic current meters (Valeport Ltd. Totnes, UK) deployed, 50 m of the cages, at two depths (9 m and 14 m) between 1 and 15 July 2018. During the same period of current meter deployment, three sediment traps were positioned to assess the numerical and distributional accuracy of the model, by comparing modelled particulate depositional values ($gC/m^2/15days$) versus actual particulate depositional values into the environment. Two sediment traps were placed at a depth of 19 m, one at 0 m south of the south-west cage and the second 5 m north-east from the south-west cage, the latter was positioned between the two cages. The third sediment trap was deployed at the control site at 5 m depth. Each sediment trap had four cylindrical collectors with a height: diameter ratio 7.5:1 (60 cm:8 cm) and were secured vertically on a gimballed stainless-steel frame. A clear container (plastic fix pot) was screwed onto the bottom of every cylinder. The design of the sediment traps was based on specifications in Blomqvist and Hakanson (1981). All the samples were stored in -20 °C freezer in the Marine Institute, Ireland and they were transported back to Scotland in an ice box.

4.2.3 Lab Analysis

After thawing overnight at room temperature, the Chlorophyll was extracted by immersing the filter papers into 13 ml of 90% acetone solution and centrifuged for at least 10 min at 2000 RPM. The clear supernatant was transferred to a cuvette and absorbance measured on a Perking Elmer Spectrophotometer at wavelengths 663 nm and 750 nm (see Richards and Thomson, 1952, Parsons and Strickland, 1963). Corrections for turbidity were made by subtracting values recorded at 750 nm from those at 663 nm. Chlorophyll-a in µg/L was the calculated using the equations of Jeffrey and Humphrey (1975).

After thawing overnight at room temperature, water samples were analysed for ammonia, nitrite, nitrate components using a Digital Calorimeter (Bran and Luebbe, auto analyzer).

Significant differences in individual water quality parameters - Total Ammonium Nitrogen (TAN), Dissolved Oxygen and Chlorophyll-a - over time and between locations were tested using two-way ANOVA., using statistical software [Mintab Ltd, Coventry, UK]. When significant differences were found, a post- hoc Tukey HDS test was performed to determine which of the independent variable was responsible.

4.2.4 Lobster growth

Lobsters were deployed in SBCCs at the cage site at the beginning of April 2018, and growth measurements commenced after a period of one month. Growth of lobsters was assessed by measuring carapace length on nine occasions over a 319-day period, between May 2018 and March 2019. Due to variable weather conditions during sampling, between 20 and 60 lobsters were randomly subsampled from the cage and control sites on each occasion. Each lobster was removed from the SBCC unit and placed on gridded paper. A digital image was taken at 90 degrees directly above to each specimen. The lobster was then returned to its container and to the water. The carapace length was measured (in mm) from the images using Digimizer image analysis software [MedCalc Software, Ostend, Belgium]. Carapace length gain (CLG) was used as an indicator of growth at the cage and control site. Growth curves for carapace length and age of lobsters were fitted using the Von Bertalanffy model as in Equation 4.1 (Von Bertalanffy 1938).

$$L(t) = L\infty(1 - \exp\left(-K(t - t_0)\right)) \qquad \text{Equation 4-1}$$

Where L(t) is carapace length (mm) as a function of time (t), L∞ is the largest lobster carapace length over the sampling period, K is the growth coefficient (yr^{-1}).

Growth of lobsters was compared between the two locations over time, using a Repeated Measures ANOVA, using SPSS Ver 26 software (IBM Corp) on the fitted growth curves. As sphericity is an important assumption of a repeated-measures ANOVA (where the variances of the differences between all possible pairs of within-subject conditions are equal), the growth data was tested using Mauchly's test of sphericity.

4.2.5 Lipid and fatty acids analysis

At the end of the trial 20 juvenile lobsters were sampled and stored in a freezer (-20C°) overnight. The next day, samples were shipped on ice to the University of Stirling for analysis. Ten individual juvenile lobsters from the control and 10 from the cage location, and a sample of the salmon aquafeed used at the cage site were individually homogenized and subjected to lipid extraction. Samples were checked to ensure they were still frozen on receipt. Total lipids were extracted from 0.5-1.0 g of feed and lobster tissue in ice-cold chloroform: methanol solution (ratio 2:1, v/v). Extraction of lobster tissue was achieved using 20 ml of solution and feed using 36 ml of solution. The samples were homogenised in an Ultra-Turrax tissue disruptor (Fisher Scientific, Loughborough, UK). Lipid content was determined gravimetrically (Folch et al. 1957).

Fatty acid methyl esters (FAME) were separated from total lipids by acid-catalysed transmethylation at 50^{o}C for 16 hours using 2 ml of 1% (v/v) sulphuric acid (95 %, Aristar®, BDH Chemicals, Poole, UK) in methanol and 1 ml toluene (Christie, 1993). FAME (6 ml) were extracted and purified by adsorption chromatography using 500 mg sorbent acid washed solid-phase extraction cartridges (Clean-up® silica extraction columns; UCT, Bristol, Pennsylvania, USA). Cartridges were pre-conditioned with 5 ml of iso-hexane before the sample was added and the FAME eluted with 10 ml isohexane : diethyl ether (95:5, v/v) and separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m x 0.32 mm i.d x 0.25 µm ZB- wax column (Phenomenex, Cheshire, UK). Hydrogen was used as the carrier gas with an initial oven gradient 50°C to 150°C at 40°C min^{-1} to a final temperature of 230°C at 2°C min^{-1}. Individual FAMES were identified by comparison to standards (SupelcoTM 37- FAME mix; Sigma-Aldrich Ltd., Poole, UK). All data were collected and processed using Chrom-CardTM for Windows (Version 1.19; Thermoquest Italia S.p.A Milan, Italy) software. 17:0 heptadecanoic acid was used as internal standard to calculate fatty acid content per g of tissue.

Predominant fatty acids measured within the salmon feed were used as tracers. The five selected were oleic acid (18:1n-9; OA), linoleic acid (18:2n-6; LA) and α-linolenic acid (18:3n-3; ALA) based on the increased 'terrestrial' fatty acids derived from an increasing inclusion level of vegetable oil used within salmon feed (Sprague et al. 2016). In addition, cetoleic acid (22:1n-11) and eicosenoic acid (20:1n-9) were also chosen as they tend to be typically found in higher quantities within salmon feed and have consequently been observed in fish farm waste (Henderson et al. 1997).

Fatty acids percentages for each sample were arcsine transformed prior to statistical analysis to correct for the binomial distribution of proportional data (Sokal and Rohlf 1995). Fatty acids occupying more than 0.1% of the feed fatty acid profile were compared between the cage and the control site, using a two sample Student T-test. Statistical tests performed using Minitab^{TM} statistical software (Minitab Ltd., UK). Principal component analysis (PCA) was used to classify and discriminate between the fatty acids profile of the lobster samples at different locations. PCA creates two orthogonal values known as principal components (PCs) which are representative of the original variables. The higher the PC value the more representative of the data set it is. PCA was performed using MVSP statistical software (KCS Ltd, UK). A non- parametric multi-variate analysis of similarity (ANOSIM) was performed with PRIMER 5 (Clarke and Gorley 2001) to detect significant differences between *a priori* source of variation for the cage site and control site results (as defined factors) using a Bray-Curtis similarity matrix.

4.2.6 Stable isotope analysis

Stable isotopes analysis was used to assess any influence of the feed signatures on the lobsters between the cage site and the control site. Furthermore, samples of naturally fouling kelp on the cage site and control site were also collected and subjected to stable isotope analysis. Stable isotopes analysis requires a long-term relationship for lobsters whereas signatures in kelps require a shorter period of time for a signal to occur (Fossberg et. al. 2018). Five samples of lobster leg muscle and fouling kelp from the control and cage site (n=5 Control, n=5 cage site) and 2 samples of salmon feed were subjected to stable isotopes analysis. Each sample was frozen at - 20°C prior to lyophilisation in a Christ Alpha 1-4 LSC freeze-drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). Samples were prepared for the analysis by weighing 0.7 mg of lobster leg muscle, 1.5 - 2.0 g of kelp sample and 1.5 mg of feed into 3 x 5 mm tin capsules. The tin capsules were sent to Scottish Universities Environmental Research Centre, Glasgow for the analysis using an Elementar (Hanau, Germany) Procure analyser, which converted organic N and C in the samples to N_2 and CO_2 for measurement of $\delta^{15}N$ and $\delta^{13}C$ respectively on a Thermo-Fisher-Scientific (Bremen, Germany) Delta XP Plus IRMS. Units of Isotope rations were expressed in δ15N and δ13C according to equation 4-2:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000 \qquad \text{Equation 4-2}$$

Where X is 13C or 15N, and R is either 13C:12C ratio or 15N :14N ratio. In-house reference materials used were: GEL (gelatine solution), ALAGEL (alanine-gelatine solution spiked with 13C-alanine), and GLYGEL (glycine-gelatine solution), each dried for 2 hours at 70 °C. Four USGS 40 glutamic acid standards (Qi et al. 2003; Coplen et al. 2006) were used as independent checks of accuracy.

The relative contribution of nitrogen from natural sources and from salmon feed in kelp was calculated according to the method of Fossberg et al. (2018) adapted from Phillips and Gregg (2001), Equations 4-3 and 4-4:

$$f_A = (\delta^{15}N_M - \delta^{15}N_B)/(\delta^{15}N_A - \delta^{15}N_B) \quad \text{Equation 4-3}$$

$$f_B = 1 - f_A \quad \text{Equation 4-4}$$

Where $\delta^{15}N_M$, $\delta^{15}N_A$, and $\delta^{15}N_B$ are the mean nitrogen signatures of the mixture M and for sources A (salmon feed) and B (natural seston) respectively. Where f_A and f_B are the proportions of A and B in M. As $\delta^{15}N_B$ for the seston was not measured a representative value for temperate coastal systems in July of 9.7‰ was used (Sommer 2004). A two sample T-Test, by Minitab Software (Minitab Ltd. UK), was used to assess whether the farm site had any effect on the stable isotope signatures in lobster leg muscle or kelp by comparing the farm site and control site isotopic values.

4.3 Results

4.3.1 Water quality

Total Ammonia-N (NH_3 + NH_4^+), Total Nitrogen, Dissolved Oxygen and Chlorophyll-a results for all sites and sampling dates are shown in Figures 4-5, 4-6 and Table 4.2. Mean values and standard deviations of total ammonium for the duration of five days are presented in Figure (4-5). The results suggest that the surrounding environment was not affected by the presence of the farm, with two sample T-test indicating that both location and date of sampling having no statistical differences ($P<0.005$). Total ammonia values were withing acceptable limits ranging and no externalities were found. The same was observed for Total Nitrogen (Figure 4-6) with location and date of sampling having no significant statistical differences ($P<0.005$).

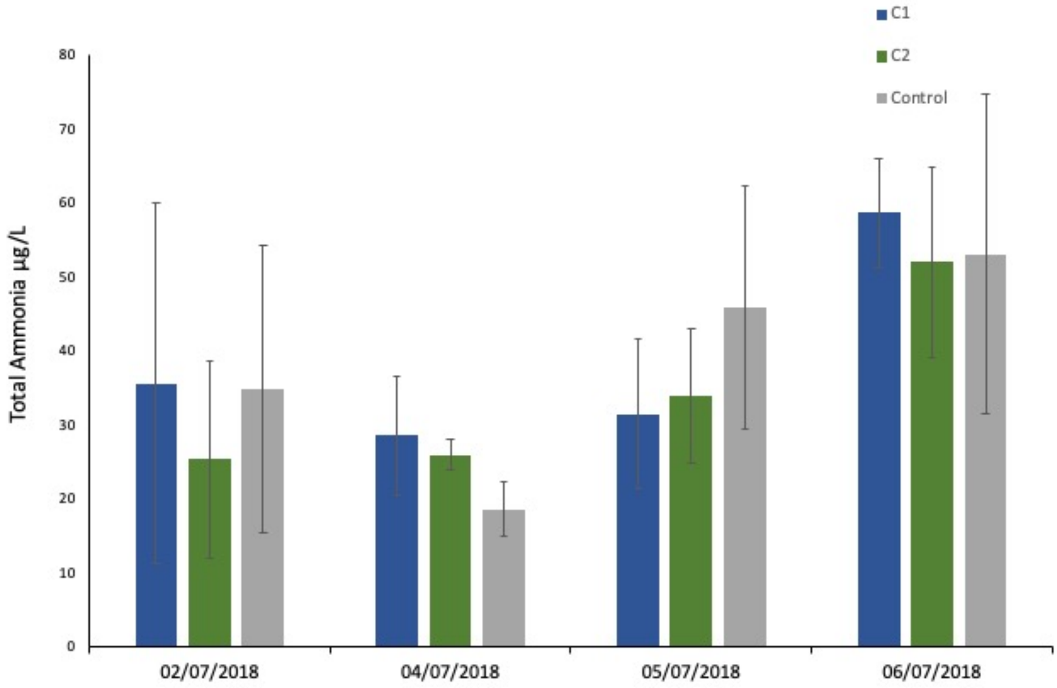

Figure 4-5 Cumulative concentrations of surface and five meter water Total Ammonia-N values in Leanagh pool for sampling station near to the fish cages (C1 C2) and Control three sampling over four sampling occasions. Error bars are standard deviations of the mean.

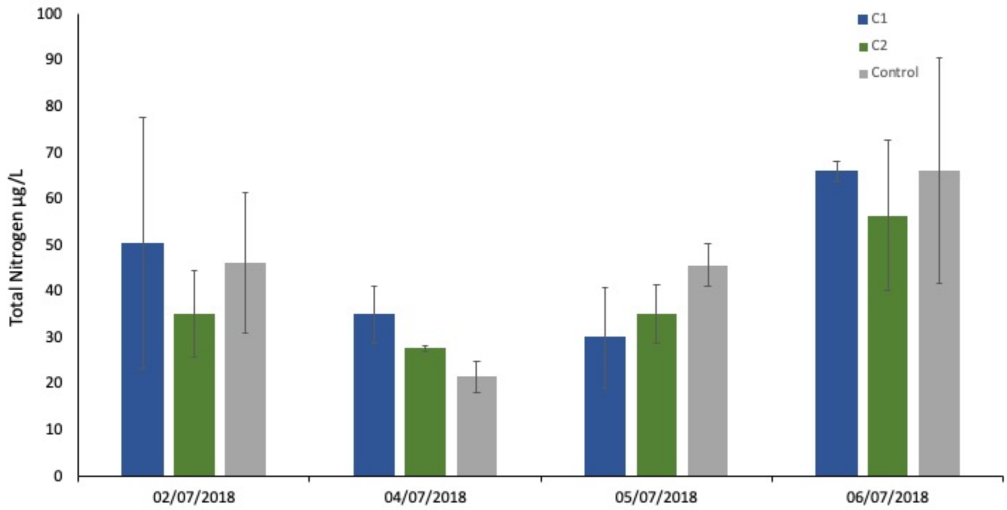

Figure 4-6 Cumulative concentrations of surface and five meter water Total Nitrogen values in Leanagh Pool bay across the three sampling stations through the in-situ sampling campaign. Error bars are standard deviations of the mean.

Table 4-2 Comparison of mean values (± sd) of temperature (°C) and dissolved oxygen (DO) in the form of saturation (%) and concentration (µg/L). Non-detectable values (<1 µg/L) = n.d.

Date	Stations	Temp	DO		Chl-a
		°C	Saturation %	µg/L	µg/L
02/07/2018	C1	17.5 ± 0.9	105.0 ± 6.6	8.2 ± 0.4	2.3 ±2.3
	C2	17.2 ± 0.8	108.5 ± 7.7	8.5 ± 0.4	2.2 ± 1.8
	Reference	17.6 ± 0.7	114.7 ± 8.5	8.9 ± 0.7	1.5 ± n.d
04/07/2018	C1	18.1 ± 1.1	108.5 ± 6.1	8.3 ± 0.3	1.1 ± 0.05
	C2	17.5 ± 0.6	110.8 ± 4.9	8.5 ± 0.4	n.d
	Reference	17.5 ± 0.5	90.7 ± 54.8	9.1 ± 0.4	1.43 ± 0.3
05/07/2018	C1	17.8 ± 0.5	116.5 ± 2.6	9.0 ± 0.2	n.d
	C2	17.9 ± 0.5	125.7 ± 19.6	9.1 ± 0.1	n.d
	Reference	17.8 ± 0.3	118.2 ± 6.2	9.2 ± 0.5	n.d
06/07/2018	C1	17.7 ± 0.5	108.5 ± 2.7	8.4 ± 0.2	1.5 ± 0.75
	C2	17.7 ± 0.3	109.2 ± 1.7	8.5 ± 0.3	1.5 ± 0.65
	Reference	17.9 ± 0.3	111.2 ± 8	8.6 ± 0.6	n.d

4.3.2 Waste dispersion and current flow at the cage site

Scatterplots showing tidal ellipse and cumulative vector plots for mid water and seabed are given in Figure 4-7. Mean current speeds in the area were 0.040 and 0.028 m/s for mid water and seabed respectively. Whilst, the residual current speeds and directions were 0.028 m/s following a southerly direction of 171^0N and 0.015 m/s at 180^0N for mid water and seabed respectively. These results were indicative of a location with a poor current flow and with limited dispersive capacity from the fish cage and shows that the lobsters SBCCs were situated in the path of predominant current flow and waste discharge.

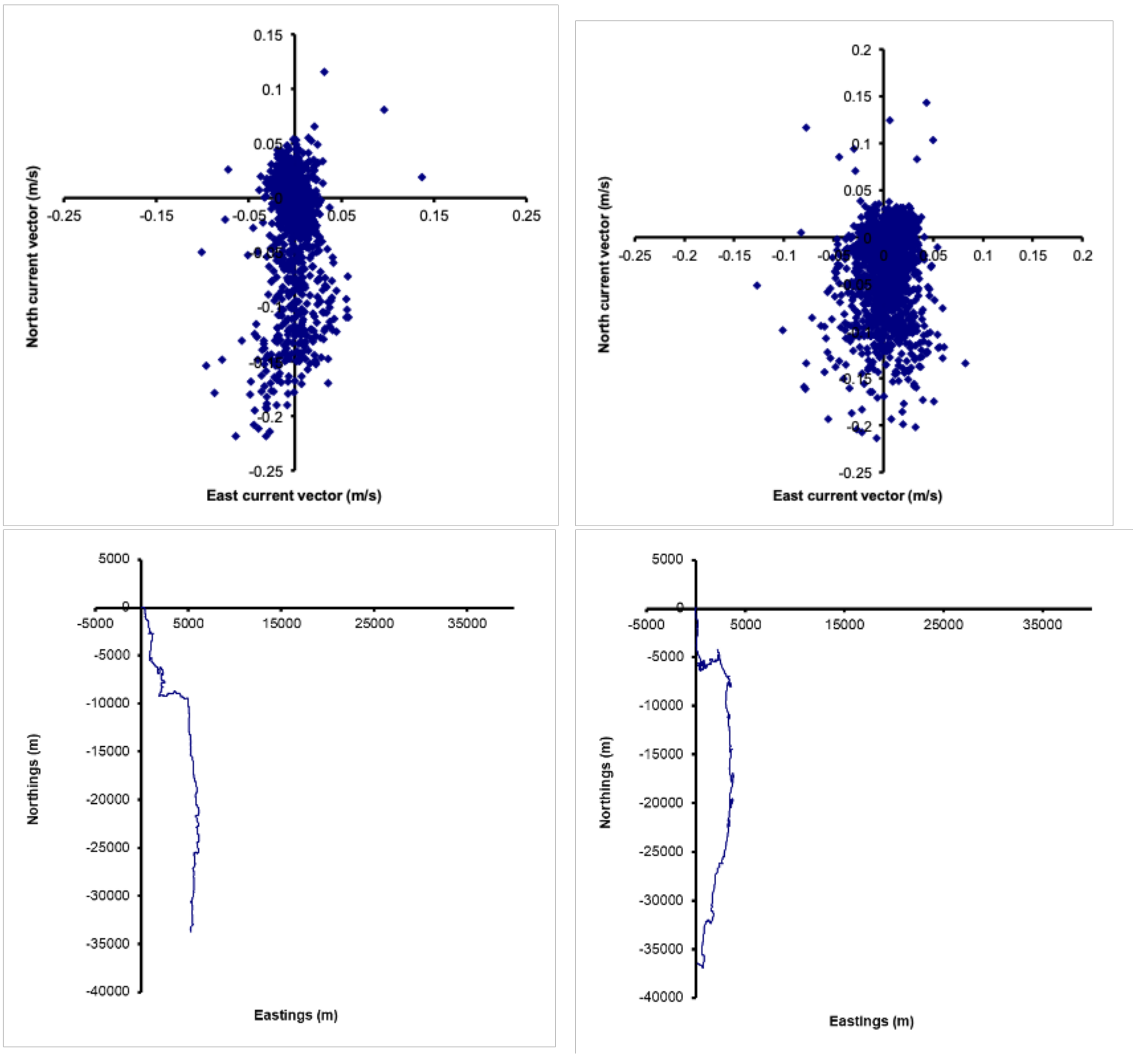

Figure 4-7 Surface water and Mid-water current direction plots shown on the top of the figure. Residual water flow plot of surface water and mid-water

Distribution of the particulate waste from the cages over the trial period, as predicted by the CAPOT model, was very local to the fish cages with only low amounts of particulate waste travelling beyond 20 m from the cage edge (Figure 4-8). The model also showed there was little possibility that particulate waste from the cages would reach to the control lobster site approximately 300 m to the west in meaningful quantities. However, even if fine particles have a higher residence time in the water column, travelling greater distances, the residual currents indicated that the majority of fine particles would follow a southerly direction and not towards the control site.

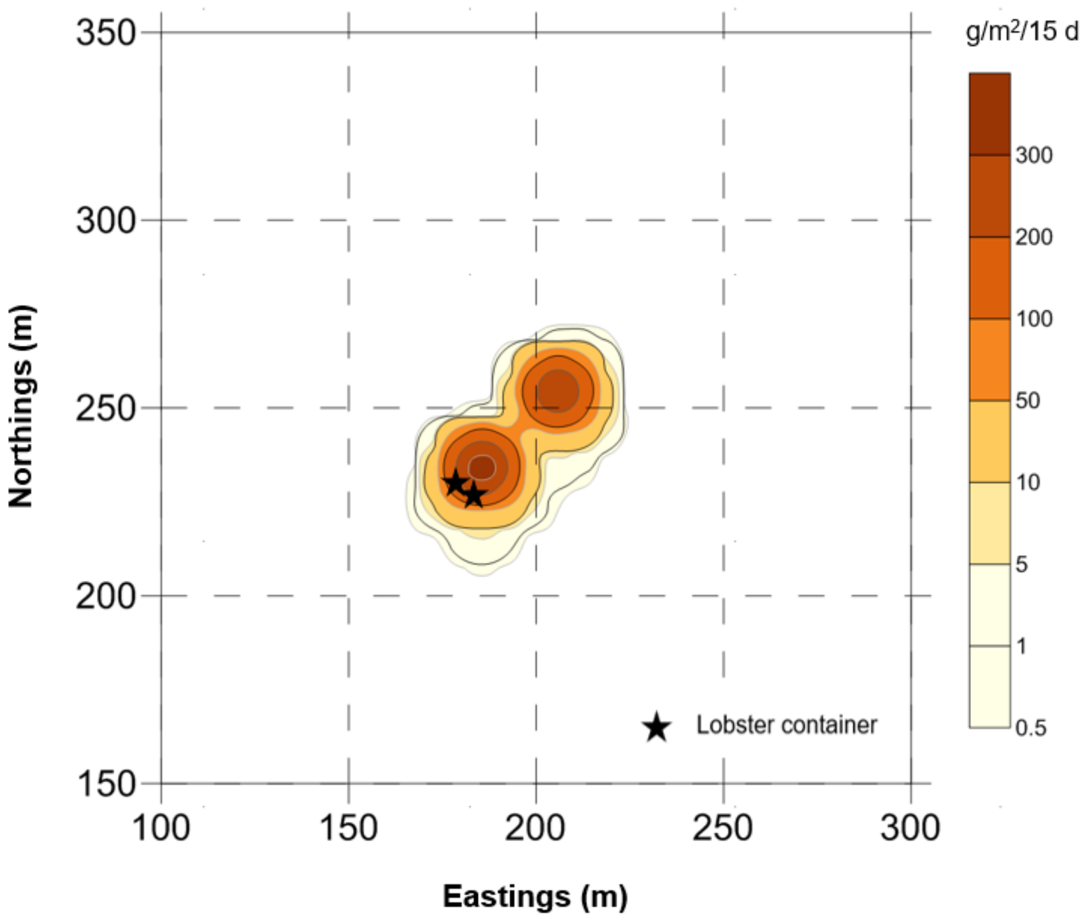

Figure 4-8 Contour plot of spatial distribution of total solids (g m^{-2} over 15 days) settled to the seabed for the IMTA site.

4.3.3 Fatty acids

The fatty acid profile of lobster tissues varied with location as the amount of fatty acid tracers (given as % of total lipid) showed significant differences ($P < 0.05$) between the cages and control stations (Table 4-4). Lobsters located near to the cages had a significantly higher total lipid content (1.22 ± 0.34 mg g^{-1} DW) than those at the control site (0.93 ± 0.19 mg g^{-1} DW) ($P < 0.05$). The fatty acid profile of the supplemented salmon feed was largely characterised by 18:1n-9 (24.4 %), 20:1n-9 (7.2%), 22:1n-11 (12.8 %), 18:2n-6 (11.6 %) and 18:3n-3 (4 %), confirming that these are indicative and appropriate for use as tracers.

Table 4-3 Fatty acid profile (% fatty acid of total lipid) of lobster muscle. Means (± SD) in the same row. Last column indicates level of significance between the two locations, (*** when P<0.001, ** when P<0.01, * when P<0.05). Selected tracer fatty acids are g

	Feed	Lobsters		Significance
		Cages	Control	
Total lipid (%)	**24.16**	**1.22 ± 0.34**	**0.93 ± 0.18**	
Fatty acids				
14:0	4.6	1.2 ± 0.3	0.5 ± 0.1	***
16:0	10.6	12.1 ± 0.5	11.8 ± 0.5	NA
18:0	1.5	4.6 ± 0.6	6.8 ± 0.4	***
20:0	0.3	0.4 ± 0.0	0.5 ± 0.1	***
Total saturated[1]	**17.4**	**19.3 ± 0.8**	**20.8 ± 0.5**	***
16:1n-7	3.4	3.1 ± 0.5	2.7 ± 0.4	*
18:1n-9	24.0	14.6 ± 1.6	8.4 ± 0.8	***
18:1n-7	2.0	5.2 ± 0.4	6.3 ± 0.6	***
20:1n-9	7.2	3.4 ± 0.5	1.5 ± 0.2	***
22:1n-11	12.7	3.9 ± 1.2	0.1 ± 0.1	***
24:1n-9	0.7	0.4 ± 0.1	0.1 ± 0.1	***
Total monoenes[2]	**51.9**	**33.8 ± 3.6**	**21.6 ± 1.3**	***
18:2n-6	11.5	6.4 ± 1.4	0.9 ± 0.1	***
20:2n-6	0.2	1.2 ± 0.2	1.4 ± 0.1	*
20:4n-6	0.3	3.6 ± 0.7	6.6 ± 0.9	***
Total n-6 PUFA[3]	**12.0**	**12.2 ± 0.8**	**10.3 ± 0.7**	***
18:3n-3	4.0	0.8 ± 0.1	0.5 ± 0.1	***
18:4n-3	2.0	0.6 ± 0.2	0.4 ± 0.1	**
20:3n-3	0.1	0.4 ± 0.0	0.5 ± 0.0	***
20:4n-3	0.3	0.5 ± 0.1	0.5 ± 0.1	NA
20:5n-3	5.0	14.9 ± 2.0	22.8 ± 1.0	***
22:5n-3	0.6	1.2 ± 0.2	1.4 ± 0.8	NA
22:6n-3	5.5	13.8 ± 1.5	17.3 ± 1.5	***
Total n-3 PUFA[4]	**17.8**	**32.3 ± 3.0**	**43.3 ± 1.4**	***
Total PUFA[5]	**30.7**	**44.7 ± 2.6**	**54.1 ± 1.4**	***
Total DMA[6]	**0.0**	**2.2 ± 0.5**	**3.4 ± 0.5**	***

The relative percentage of these tracer fatty acids were significantly higher (*P*< 0.05) in lobsters located near the cage site (see Figure 4-9) than those at the control site. Additionally, n-6 PUFAs levels were found to be significantly higher at the cage site (12.24 ± 0.77 %) compared to the control (10.27 ± 0.65 %) (*P* < 0.05), with 18:2n-6 being higher in the cage (6.3 ±1.4%) compared to the control

(0.9 ±0.1%) ($P < 0.05$) (see Table 4.4). The tracer fatty acids accounted for 29.08 % of the total lipid content of the cage station which was significantly higher when compared to the control station (11.36 %). Conversely, total n-3 PUFA levels were significantly higher ($P < 0.05$) at the control site (43.27 ± 1.38 %) compared to the cage site (32.3± 3 %). Fatty acids contributing to the difference between the control and the cage site were EPA (22.7 ± 1% and 14.8 ± 0.9%) and DHA (17.2 ± 1.46% and 13.8 ±1.4%), respectively.

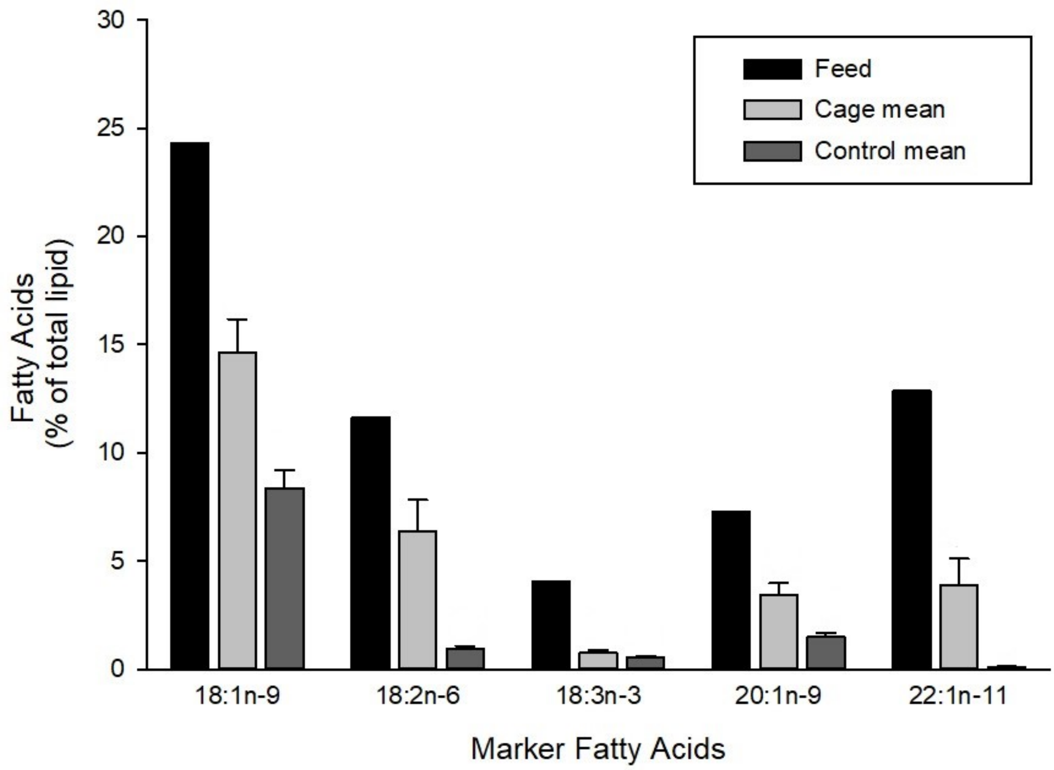

Figure 4-9 Contribution of selected fatty acids (% of total lipid, mean ± SD) to the total fatty acid content in the lobster tissue at the end of the experiment March 2019, at the control site (dark grey) and the cage site (light grey).

The PCA plot (Figure 4-10 indicated clear differences between fatty acid profiles of lobster leg muscle from the cage site and from the control site along PC-1, which accounted for 92.8 % of the total variance in the data. Post hoc ANOSIM confirmed the groups from control and cage sites were significantly different (*P<0.05*) with an R statistic value of 0.994. The fatty acids primarily driving this difference at the cage site were the marker fatty acids, 18:1n-9 and 18:2n-6, along with 20:1n-9 and 22:1n-11, confirming observations in Figure 4.9. Conversely, EPA (20:5n-3) and DHA (22:6n-3) together with arachidonic acid (20:4n-6) were more dominant in defining the fatty acid profile at the control site, making it likely that the diet of the lobsters here was primarily influenced by naturally derived marine oils, accounting for 40.4 % of total fatty acids against 28.7 % for lobsters at the cages (Table 4-4).

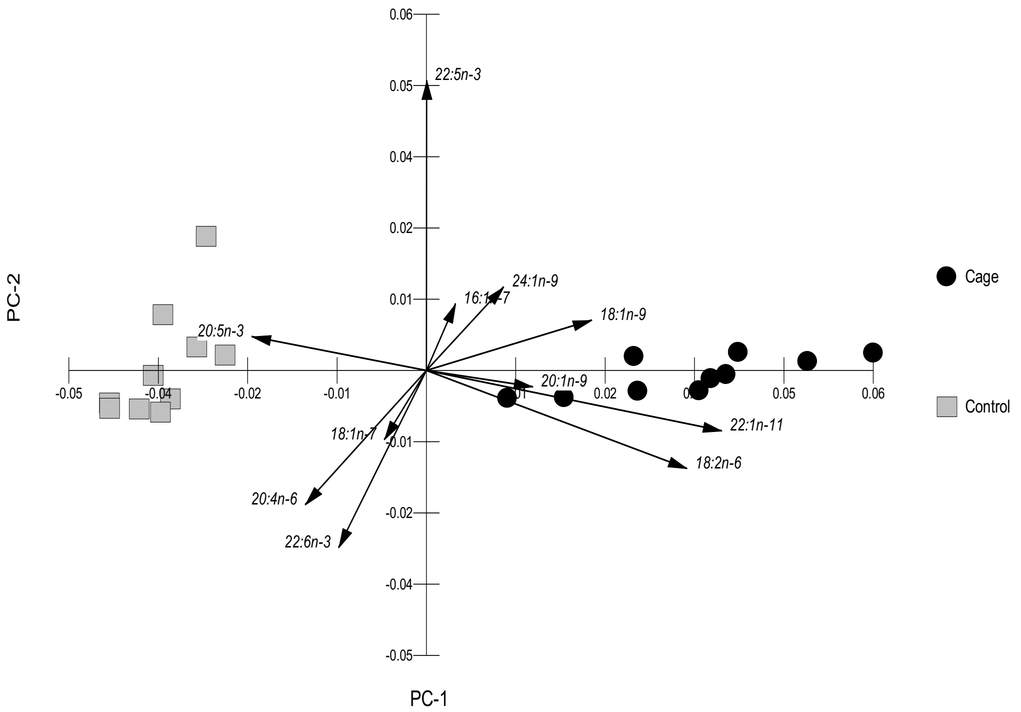

Figure 4-10 Principal Component Analysis for fatty acid profiles (over 0.1 %) from the leg muscle of lobsters taken from the cage and control sites. Vectors for key fatty acids responsible for the grouping pattern are displayed.

4.3.4 Stable isotopes

Both the $\delta^{13}C$ and $\delta^{15}N$ signatures for lobster tissue and kelp at the control and cage sites are similar, as shown by their close positioning in the plot (Figure 4-11). The signature for salmon feed though was different to those found for lobsters. Two sample Student T-test indicated no significant differences for $\delta^{13}C$ or $\delta^{15}N$ between cage and control sites for lobster (*T = 1.33; df = 5; P = 0.240*), and neither showed any similarity to the signatures for the salmon feed.

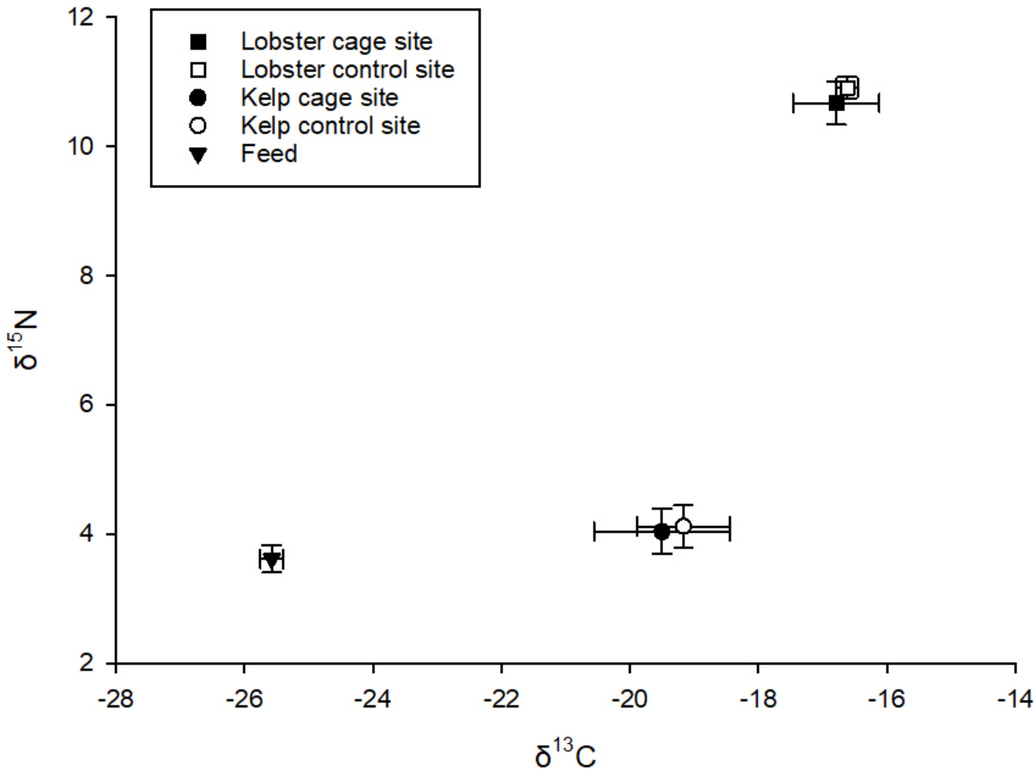

Figure 4-11 Biplot of δ15N (‰) and δ13C (‰) (mean ± SD) stable isotopes of Lobster leg muscle and supplemented feed between cage and control station at the end of the trial in March 2019.

The contribution of nitrogen in kelp tissues of salmon farm origin was relatively high, estimated at 92.9 % for kelp at the farm site and 91.6 % at the control site. Suggesting that soluble nitrogen released from the farm site was reaching the control station.

4.3.5 Growth of Lobsters

An increase in carapace length was observed over the trial period at both cages and control sites. These were fitted to Von Bertalanffy growth curves (Figure 4-12) using calculated values of K = 0.0048, t_0 = -32.08 for the cage site and K = 0.0044 and t_0= -0.4318 for the control site. After Mauchly's test showing the data conformed to sphericity (*W = 0.0224, P = 0.150*), the repeated measures ANOVA showed there was significant growth in lobsters over the trial period (*F = 77.5; P*

< 0.001) at both farm and control sites, though there was no significant difference in lobster growth between the sites (*F = 20.9; P = 0.149*).

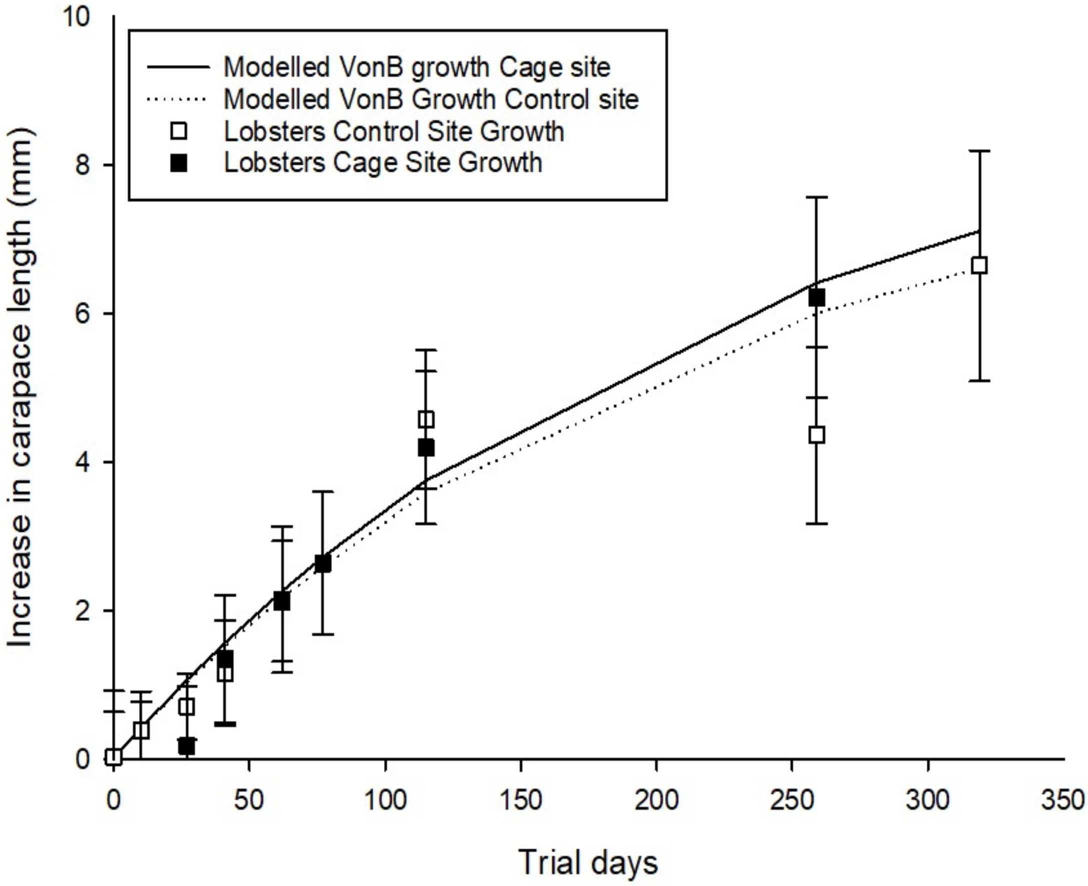

Figure 4-12 Growth curves of lobsters between control (green) and cages (blue), based on measured values (mean +_ SD). Von Bertalanffy growth functions plotted by trial day and increase in carapace length for 319 days of trial. Parameters estimated for the cage site were $L\infty$ = 9.43, K = 0.0044, t0 = -0.4318, and control site $L\infty$ = 8.44, K = 0.0048, t0 = -0.3208.

4.4 Discussion

One of the most important aspects of open water IMTA is for the secondary extractive organism to have the capacity to include parts of its diet from the waste of the fed organism ensuring a trophic connectivity. The assessment of trophic connection on IMTA follows an individual spatial scale assessment of IMTA. Taking a closer look on IMTA compared to the previous experimental chapters in this thesis. Nutrient transfer has been used in the past to evaluate candidacy of a species to be considered as IMTA, initially through lab-based studies. Conclusions from lab-based studies however are inconclusive since organisms are only exposed to a particular set of feeds under a controlled environment following starvation. However, when establishing a nutrient connection between the different parts of IMTA in an open water environment, more parameters have to be considered, looking at location of the extractive species on the prevailing stream of waste to ensure physical connectivity. Ability to trace the nutrient exchange in an environment where extractive organisms tend to have the ability to feed on other sources and finally whether these organisms benefit from that trophic relationship.

The results of the current speeds and directions and consequently the direction of the waste showed that allocation of the SBCC structures was on the right side of the cages, within the prevalent stream of waste. That was particular important since assessment of these conditions ensured that a sufficient amount of nutrients was being received by the extractive organism ensuring physical connectivity. In this case, the scale of the experimental site meant there were limited options to position the SBCC within the waste stream of the fish cage. This study took all this into consideration following suggestions by other authors regarding the importance of the environment conditions and their consequent impact on IMTA extractive organisms spatial arrangement (Filgueira et al. 2017; Reid et al. 2018) In addition, particulate depositional modelling indicated that majority of the wastes would be deposited within the 20 meters from the cage site following a southerly direction. That reenforces the idea that particulate organic fluxes are being dominated by a vertical trend (Sanz-Lazaro and Sanchez-Jerez et al. 2017). However, that brings questions regarding the approximate distance of the lobster in relation to the cages. Lobsters located further away from the farm site would have limited access to heavy particles and would consequently rely on lighter particles, limiting their access to food waste. The same issue was raised from Filgueira et al. 2017 on IMTA with mussels, suggesting that distance from the cages influences trophic connectivity, depending on the type of waste and organism selected. It also raises issues with interference on farm management practises under a commercial scenario with accessibility being an issue.

The results from the fatty acid analysis showed that the content of oleic acid (18:1n-9), linoleic acid (18:2n-6), α-linoleic acid (18:3n-3), cetoleic acid (22:1n-11) and eicosenoic acid (20:1n-9), which were characteristic of the salmon feed used, were each significantly higher in the tissues of the lobsters located near to the cages than those at the control location. The PCA performed further confirms these results as the lobsters at the cage and control sites showed distinctly different fatty acid profiles, which suggests different food sources. The fatty acid profile of lobsters at the cage site was clearly influenced by the salmon feed, but at the control site lobster nutrition was dominated by natural marine oils. Fatty acids are considered a robust method for identification of nutrient transfer, even within short term. Furthermore, even though the production biomass of the system was small reflecting a small-scale pilot study, nutrient transfer was still established between the two components and that was reflected on the diets of the extractive organism close to the farm cages. Similar results were achieved for blue mussels (Redmond et al. 2010), for great scallops (Bervik et al. 2019) and for northern shrimp (Olsen et al. 2012) located near to fish farms. A full scale IMTA production system, that includes lobsters and salmon would have also to consider a number of technical, logistical and regulatory factors that have not been taken into account here. In such a system, there would also be greater potential for lobsters to be exposed to higher amounts of waste and consequently increased levels of terrestrial fatty acids. The nutritional implications of this are unclear since that its has been shown that supplementation of greater amount of fish waste have affected growth as diets with greater than 50% of soya bean meal (rich in Linoleic Acid - the main PUFA in soya bean meal and taken up in significant quantities in the present study) resulted in stage IV lobsters having lower growth than a natural diet (Floreto et al. 2000). While substitution of conventional marine oils with diets of terrestrial origin did not inhibit food consumption or alter digestive conditions of *Nephrops norvegicus* (Karapanagiotidis et al. 2015)

In contrast, stable isotope analysis of lobster leg muscle taken at the cage and control sties, showed that there was little difference in $\delta^{15}N$ ratio between locations, nor was there a similarity between the signature ration for lobster tissue and salmon feed. There may be several reasons for the differences between the fatty acid tracers and the stable isotope analysis. Bethoney et al (2011) demonstrated that $\delta^{15}N$ values in lobster tissue reflect their long-term diet so the time-period of this study may not have been long enough. Another consideration is that there may have been an insufficient amount of waste consumed to establish an isotopic signal as lobsters are a slow and periodic feeder (Bordner and Conklin 1981). Conversely, tracing nutrient sources through stable isotope analysis of seaweed is a reliable method in the short term (Fossberg et al. 2018). Here the results show clearly that there is a close relationship between the $\delta^{15}N$ ratios in kelp at the research and the reference locations, both of which are similar for that of the salmon feed. For both locations, high levels of nitrogen in the kelp tissues where sourced from the fish waste (92.9% at the research and 91.6% at the reference station) showing that the soluble nitrogen discharged from the fish at the research site, distributed in the water column evenly to more than 300 m to the west. These results are comparable to the trends in dissolution of soluble nitrogen contained in kelp from fish farms in Norway (Fossberg et al. 2018). These results also suggest that seaweeds up to 300 m from the fish may be effective at uptake and thus removal of soluble nitrogenous wastes.

Evidently, based on the fatty acid profiles, the juvenile lobsters at the cage site consumed fish farm nutrient waste, although it was not clear if this was direct or indirect. The size of waste feed and faecal particles in the water column from the salmon cages were not measured in this experiment, but other studies have shown that suspended particulate organic matter originating from cages are often between 1 and 10 µm (Lander et al. 2013) and up to 300 µm (Law et al. 2014). As the internal mesh size of the SBCC structures were 2.5 x 2.5 mm (Daniels et al. 2015), particles of waste this size could have entered the SBCC and been within the size range eaten directly by juvenile lobsters (Lavalli and Barshaw. 1989). Studies have demonstrated direct uptake of waste particles by consumer IMTA species (Handå et al. 2012; Bergvik et al. 2019). However, in the marine environment there will be a wider dietary choice and a complex food web. An *in-situ* study showed that mussels did not directly assimilate wastes and have a selective diet, preferring other sources of food (Sanz-Lazaro and Sanchez-Jerez. 2017). Even within the SBCC system there would be a dietary choice (Daniels et al. 2015), and the uptake of wastes by lobsters could have been indirect via fouling organisms.

Despite the differences in the fatty acid profiles of the juvenile lobsters at the cage site and the reference site, there was no significant difference in growth. Information on growth rates of wild juvenile European lobsters is scarce (Mercer et al. 2001; Wahle et al. 2013), which prevents comparison to natural conditions. Stage IV is the first post larval stage of the life cycle, and juvenile lobsters are transitioning to benthic organisms (Charmantier et al. 1991). Therefore, the position of the SBCC may not have been deep enough in the water column for their feeding behaviour and could have influenced the results, as other studies have shown that depth can be an important factor in IMTA systems (Sanz-Lazaro et al. 2018). There will always be multiple factors to consider in designing the optimal setup and there will often be trade-offs (Halswell et al. 2018).

Previous studies have demonstrated the advantage of acclimatising juvenile lobsters *using in-situ* containers as a way of improving survivability (Beal et al. 2002; Perez Benavente et al. 2010; Beal & Protopopescu 2012; Daniels et al. 2015; Halswell et al. 2016), and locating an SBCC next to salmon cages might offer additional shelter from storm events. However, it is also important to note that fish medication can be harmful to juvenile lobsters and is often used to treat fish diseases (Burridge et al. 2014; Cresci et al. 2018). The effect of routine operations, such as disease treatment, on the different components of an IMTA system, is not only an issue for lobsters and salmon, but is an essential consideration for any combination of species for an IMTA system. In this study no medical treatment has been used for lice treatment, one of the reasons being the small produced biomass of salmon whereas under commercial salmon farming standards medicine for treatment of sea lice is still in use in some cases. Generally, one of the key limitations is the information gap regarding the effect of medicinal use to the physiology of the extractive organisms with limited information on different residues and contaminants on different species, but also the effects on food safety (Rosa et al. 2020).

4.5 Conclusion

This chapter investigated nutrient transfer IMTA from the perspective of an individual candidate organism in a pilot scale IMTA environment. The chapter suggests that when establishing a trophic connection for a new species, considerations of the hydrographic conditions need to be made along with the fate of the waste released, ensuring connectivity between the IMTA system. Furthermore, the results clearly demonstrate that for the case of particulate organic matter the use of fatty acids is a robust biological tracer in order to trace a trophic connection since it can be traced in shorter time, whilst effect of fatty acids are seen within the depositional footprint of the farm site. On the other hand, the use of stable isotopes did not find any influence by soluble nutrients released from the farm on the lobsters but showed that these were taken up by seaweeds at both the cage site and control site. That suggests that for deposit feeders which feed on particulates, fatty acids are a more robust methodology whereas for seaweeds influence of stable isotopes can be traced further, covering greater distances not necessarily being constrained by small distances from the farm site. Establishing a trophic connectivity between the species is very important, however consideration the effect of diet substitution on the physiological, metabolically, immunological mechanisms. The trophic connectivity did not have a clear effect on growth of the lobsters. Therefore, there is a need to be further explored since it will affect the fate of the organism once released influencing the restocking initiatives. IMTA can also been seen as a mechanism of providing ecosystem services on the surrounding environment by offering candidate species designated for restocking purposes a sufficient amount of feed and shelter prior to release.

Chapter 5 Investigating waterbody scale nutrient budgets of Regional Multi-Trophic Aquaculture (RIMTA)

5.1 Introduction

The three previous experimental chapters presented in this thesis investigated IMTA across different spatial scales (national level, farm level, and species level). These investigations were based on closely integrated systems with different extractive organisms suspended or positioned close to the fed farm sites. For coastal IMTA systems, bivalves and macroalgae have been extensively studied as a means to reduce particulate and dissolved waste respectively (Ferreira et al. 2012; Sara et al. 2012). In mesocosm and lab-based studies bivalves and macroalgae have been shown to incorporate waste from fish farms in their diet by using fatty acids and daily water turnover nutrient exchange (Handa et al. 2012, Samocha et al. 2015). However, in field-based studies use of fatty acids and stable isotopes on bivalves and macroalgae has shown that fin-fish waste constitutes only a small fraction of their diet (Navarrete-Mier et al. 2010; Aguado-Gimenez et al. 2014; Irrisari et al. 2014; Sanz-Lazaro and Sanchez-Jerez 2017). The reason behind this difference is the connectivity between the waste from the fed and extractive species within the IMTA systems. In the field the majority of particulate organic wastes fall within few metres from the farm site, at times related to fish feeding (Oppedal et al. 2012), therefore release of particulate matter occurs in abrupt pulses and not constantly through the day. Furthermore, location of farm sites are generally areas with high hydrodynamic environments, flushing suspended solid wastes, having a low persistence in the water column (Sanz-Lazaro and Marin 2008). Secondly for the case of bivalves, it has been shown that they have a diet preference for living organisms rather than non-living particles, such as fish waste (Sanz-Lazaro and Sanchez-Jerez 2017). Dissolved nutrient wastes have been shown to have limited effect on primary production and water quality close to the fish cages (Price et al. 2015) suggesting the discharge of multiple farms is more likely to lead to increased overall primary production over a coastal region.

It has been suggested that in terms of mitigating potential environmental impacts there needs to be a holistic approach into account the relative spatial scale in relation to the dispersion patterns of the targeted waste (Costa-Pierce and Page 2013). Dissolved and particulate organic waste have different dispersion dynamic and patterns, therefore different spatial scales needs to be taken in consideration based on the type of waste targeted by extractive species within the IMTA system (Sanz-Lazaro et al. 2011; Jansen et al. 2018). For the case of bioremediation of particulate organic materials, a near farm scale approach is needed since these waste settle to the sea bed close to the farm (Sanz-Lazaro et al. 2011). A suitable extractive organism would be deposit feeders, such as sea cucumbers, urchins and polychaetes, since they feed directly on benthic sediments (Cook and Kelly 2007; Cubillo et al. 2016; Jeronimo et al. 2020). Dissolved waste on the other hand is diluted and dispersed widely in the water column, consequently culture which can remove soluble nutrients or the consequences of any increase in productivity caused such as bivalves or seaweeds may be better approach (Sanz-Lazaro et al. 2017). These secondary organisms do not need to be close to the vicinity of the farm sites.but still they should be physically connected within the same body of water.

Bivalves can indirectly regulate the pelagic environment through incorporation of N and P in their tissues and shells which in turn is harvested. (Rose et al. 2014; Rose et al. 2015; Reitsma et al. 2017; Clements et al. 2019). One of the biotic factors which play a considerable role on N removal through bivalve harvesting is the density and production biomass of the system (Burkholder and Shumway 2011). Abiotic factors can also play a crucial role such as the effect of local hydrodynamics conditions in the nutrient removal potential. If the location is characterised by high energy current speeds and consequently short residence times, nutrients and particularly phytoplankton can be removed from the area before it reaches the seabed and be consumed. In dynamic systems removal of N and P by bivalves through harvesting might be outcompeted by the N and P input through bio deposits. In contrast areas with longer residence times, phytoplankton has also longer residence times, bivalve farming might significantly help removing N and P through harvesting playing a significant ecological benefit by regulating phytoplankton biomass.

The IMTA concept is constraint by spatially linked organisms in relation to the stream of waste under a farm scale management approach, requiring secondary organisms to be located close to the vicinity of the farm cages. The restricted feasibility of mitigating dissolved waste using macroalgae and bivalves is constrained only when looking it through a typical IMTA system linked to the farm scale. Therefore, IMTA can be considered over a wider scale than in the immediate vicinity of the farm and investigated through ecosystem interactions rather than absolute distances (Chopin et al. 2012; Sanz-Lazaro and Sanchez-Jerez 2020). Consequently, bivalves and macroalgae can serve an ecosystem function even when indirectly linked with the farm wastes, as long as they are within the same body of water interconnected by the hydrodynamics of the area. This introduces the concept of Regional Integrated Multi-Trophic Aquaculture (RIMTA) which is defined as "the culture of low trophic level species such as macroalgae and bivalves molluscs to mitigate the ecological negative effects derived from the input of dissolved nutrients from fed aquaculture" (Sanz-Lazaro and Sanchez-Jerez 2020). This treats a whole body of water, such as fjords and bays, containing multiple aquaculture activities as a single system, with nutrients wastes originating from fin-fish activities as sources and extractive organisms as sinks. As a concept it is similar to integrated coastal approaches attempting to regulate the nutrient budgets of the system through measuring the amount of nutrients going in and out of the system

The aims of this study were to investigate nutrient budgets of a RIMTA system using a case study a location with multiple finfish and bivalve production units. The nutrient budget incorporated dissolved N and P entering the system through the salmon farms and their subsequent removal through bivalve harvesting. Recommendations of the efficiency of RIMTA in relation to environmental conditions were given.

5.2 Methodology

5.2.1 Study area

Mulroy Bay is a fjordic inlet situated on the Northern Coast of Co. Donegal, Ireland. The bay is highly convoluted and consists of a number of deep basins, separated by shallow sills. These form three main sub-regions; Broadwater, Northwater and the Narrows (Figure 5-1). These sub-regions have different volumes, hydrodynamic conditions and consequently, residence times of the waters which might affect the capacity of the RIMTA system as a whole.

Mulroy Bay is known for its rich aquaculture industry, primarily containing Atlantic salmon and mussel aquaculture, and the introduction of Pacific oyster farms is also under consideration (Marine Institute, 2018). Allocation of aquaculture production is variable within the system and each sub-region would be expected to receive different amounts of wastes. The surrounding areas of Mulroy Bay are characterised by low rural development with the effect of nutrient run-off being considered minimal. Aquaculture production within Mulroy Bay was based on real values from two different time points. First time point is the salmon aquaculture on 2003 (Telfer and Robinson. 2003) and for bivalves from 2018 (Marine Institute 2018). Therefore, it is important to mention that these values represent a hypothetical scenario of production even though based on published reports.

Table 5-1 Production of salmon farms and area of bivalve farms in Mulroy Bay and its subregions

Sub-regions	Salmon Biomass (tonnes)	Bivalves Area (m^2)	
		Mussels	Oysters
Narrows	2882	NA	450000
Northwater	811	NA	110000
Broadwater	1997	597800	NA

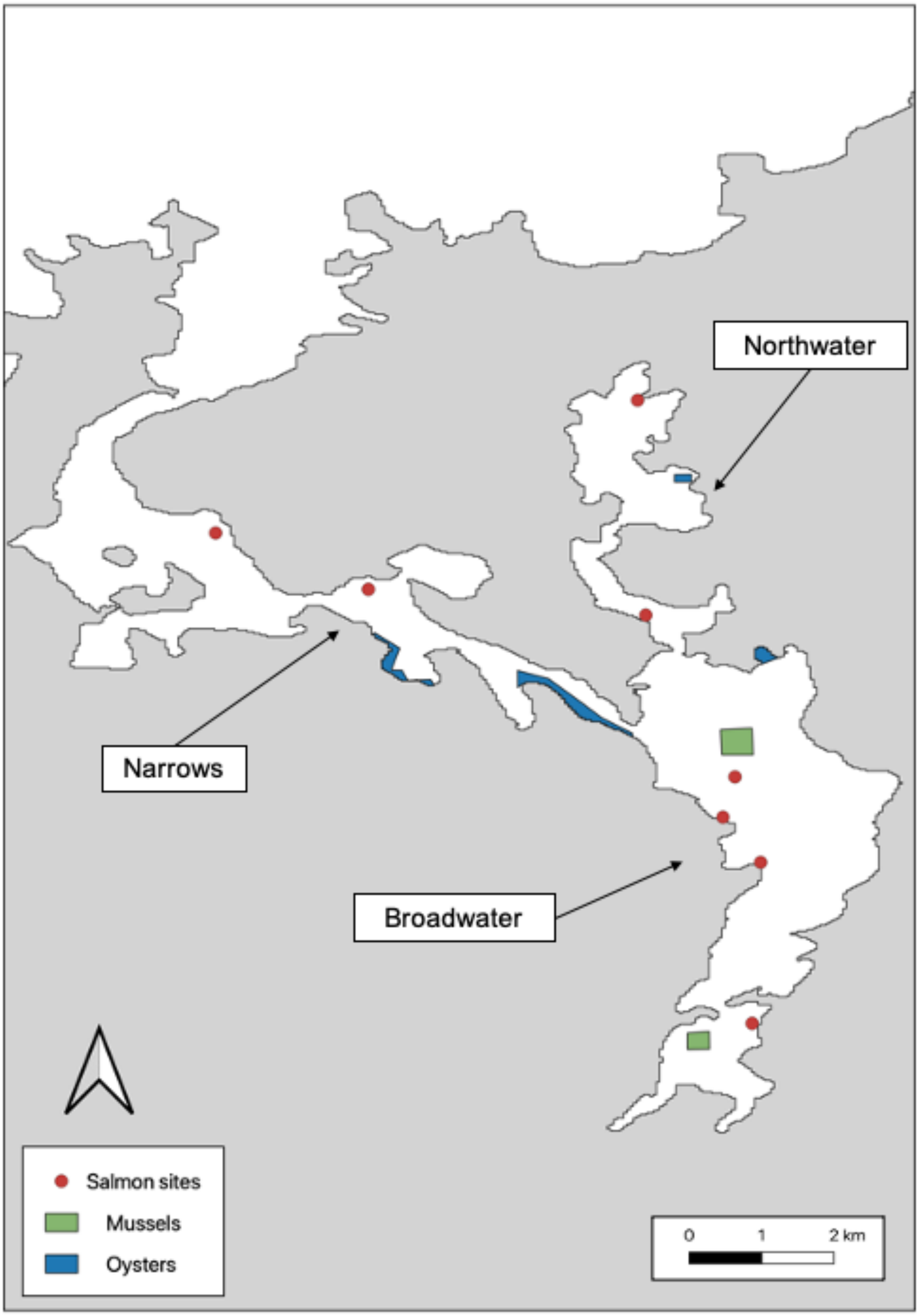

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Figure 5-1 Map of the region of Mulroy Bay with the name of the sub-regions. Green colours indicated location of mussel culture and blue colour indicates location of Oyster culture. Salmon locations based on (Telfer and Robinson 2003) and bivalves locations based on (Marine Institute 2018)

5.2.1.1 Physical environment

The hydrodynamic conditions of the waterbody have been sourced from an environmental quality and carrying capacity assessment for aquaculture installations in Mulroy Bay on April 2003 as part of a commissioned study by the Marine Institute to Stirling Institute of Aquaculture on behalf of Marine Harvest Ireland 1986 (Telfer and Robinson 2003). The consequent hydrographic conditions were sourced from this report in order to assess the background physical profile of the area. Current flows within the Northwater sub-region are primarily very slow with average current speeds over a spring/neap tide reaching 0.004 m/s at the surface and 0.001 m/s at the seabed. This might be considered as a location with quiescent waters with most of the waste following a southerly direction towards Broadwater. Current speeds at Broadwater have a more complex hydrodynamic profile with the northern part of Broadwater characterised by a moderate dispersive capacity with current speeds reaching 0.08 and 0.06 m/s, direction of the currents are following a south-eastern direction, emptying the Northwater sub-region. In the middle of Broadwater, currents are following a north-west direction leading to the Narrows suggesting the potential wastes would also follow this pattern. Current speeds are relatively slow with average speeds being 0.04 and 0.01 m/s at surface and seabed respectively. Finally, the southern part of Broadwater is characterised by a southerly direction of the residual currents with speeds being 0.03 and 0.02 m/s respectively for surface and seabed. This suggests that cages settled there would have a limited dispersive capacity and waste would most likely remain locally. The Narrows is the mouth of the system where water from Northwater and Broadwater is passing through to the Atlantic Ocean. Due to its narrow passage the subregion is characterised by high current speeds being 0.10 and 0.09 m/s for surface and seabed respectively, being a highly dispersive site, with the residual direction of the currents following a north direction. The same hydrographic conditions were highlighted by (Moreno Navas et al. 2011a, 2011b) as part of two studies based on spatial particle modelling and 3D hydrodynamic modelling assessing the carrying capacity of aquaculture activities in Mulroy Bay.

Based on the different hydrodynamic condition within each sub-region, different areas would have different flushing times exchange rates. These values are important to calculate the residence times of the dissolved nutrients. Areas of the system and tidal volumes have been sourced from Telfer and Robinson (2003) and shown in Table 5-1.

Table 5-2 Tidal volumes, exchange and flushing rates for the sub-regions within Mulroy Bay (Telfer and Robinson 2003).

	Northwater	Broadwater	Narrows
Area (m^2)	5255609	19671653	17240178
Volume LW (m^3)	43709546	125429368	41950899
Volume tide (m^3)	9913060	38639700	24075200
Exchange (m^3/day)	19826120	77279400	48150400
Flushing time (no. of tides)	4.41	3.25	1.74

5.2.1.2 Water quality

The background nutrient and chlorophyll-a profile is important for the characterisation of the level of influence of aquaculture on each sub-region. The overall nutrients and chlorophyll -a levels in Mulroy bay vary spatially within sub-regions but also temporally across different years of collection. Nitrate concentrations increased in 1994 in the Broadwater sub-region and 1999 in the Northwater. Similarly, concentrations of ammonia have been observed to peak in 1994 (Northwater) and 1998 (Broadwater), again values have shown that there wasn’t any overall increased of ammonia concentrations over the sampling period. Finally, chlorophyll-a levels were found to be low over the whole sampling campaign with only slight increases in the Northwater sub-region in 1995. Even though, these concentrations have not shown to increase over the sampling campaign time, they do identify the fact that some sub-regions such as Northwater and Broadwater might be prone to elevated concentrations from time to time, primarily due to reduce flushing rates compared to the locations such as the Narrows close to the mouth of the system.

5.2.2 Nutrient budgets

5.2.2.1 Salmon aquaculture and waste release

The purpose was to calculate the amount of dissolved nutrients from each salmon farm site respectively and each sub-region cumulatively using a mass balance approach. Mulroy Bay based (Telfer and Robinson 2003) had eight operating salmon farm sites, distributed over all three basins (Figure 5-1), where; Northwater hosts two salmon farm sites, with Broadwater four salmon and Narrows the remaining two. The stocking biomass for a year of production for each site is shown in Table 5-2. Mass balance approach used was based on Wang et al. (2012). The data required to calculate overall particulate and dissolved N and P released was the yearly feed used across each farm site. Feed usage was calculated from the yearly production biomass of each farm site (Telfer and Robinson 2003) multiplied by an FCR of 1.2 (Tacon and Metian 2008).

Table 5-3 Sub-regions hosting salmon farm and the subsequent production biomass (t) over a year (Telfer and Robinson 2003)

Subregions	Farm sites	Biomass (tonnes)	Feed (tonnes)
Narrows	Millstone	1765	2118
	Glinsk	1117	1340
Northwater	Moross 1	29	34
	Moross 2	449	539
	Kindrum	333	400
Broadwater	Cranford C	29	35
	Cranford A	1888	2265
	Milford	80	96
Total		5691	6829

The annual release of N and P were calculated at farm scale annual production level for each farm respectively based on mass balance principles developed by Wang et al. (2012). Feed loss was assumed to be 3 % as suggested by Corner et al. (2006) and Reid et al. (2009). N and P intake through feeding was equal to the total feed intake times the feed content of the N and P elements. The N content was 7.2 (%DW) (Gillibrand et al. 2002), and P content was 1.2 (%DW) (Petersen et al. 2005). The dissolved inorganic nitrogen and phosphorous (DIN and DIP) released from each farm site were then added to form the DIP and DIN release on each subregion.

The mass balance outputs retrieved from Wang et al. 2012, suggested that out of the 100% of nitrogen supplemented through feed, 38% would be incorporated as fish biomass, with 45% of the total feed lost into the environment in dissolved form as dissolved inorganic nitrogen (DIN) and 15% as particulate organic nitrogen (PON) to the benthos. Approximately 15 of N of lost feed and faeces settled would be expected to leach after few minutes in water contributing to the DON (Chen et al. 2003). Even though, the values presented in Figure 5-2 do not constitute 100 % of the feed supplemented, this is due to the decision to keep percentages with no decimal points.

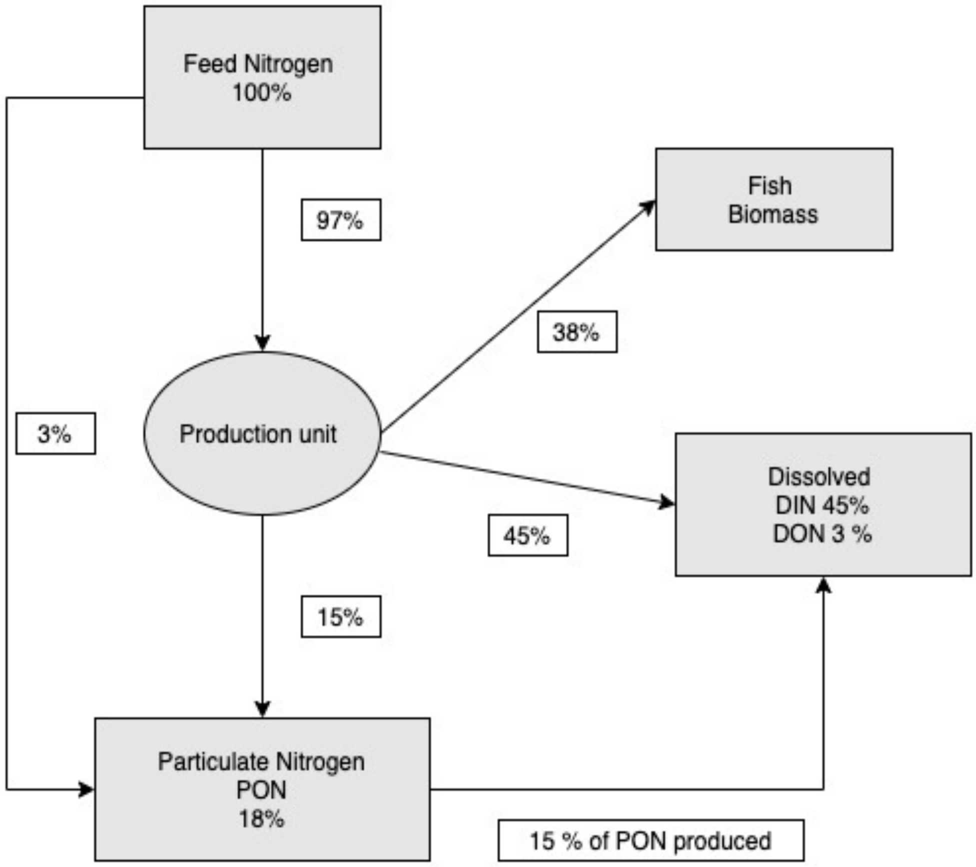

Figure 5-2 Flow chart of mass balance showing the annual fluxes of Nitrogen in Kg per tonne produced per year used for each salmon farm (Wang et al. 2012)

The phosphorous mass balance is shown in Figure 5-3, where, out of the total feed, 30% was incorporated as fish biomass, 18% was lost as dissolved inorganic phosphorus and 44% of the total feed was released to the bottom as particulate organic phosphorous (POP). The same approach as followed with N has been adopted with P with 15 % of lost feed and faeces expected to leach after few minutes or hours (Sigiura et al. 2006), contributing to the DOP pool.

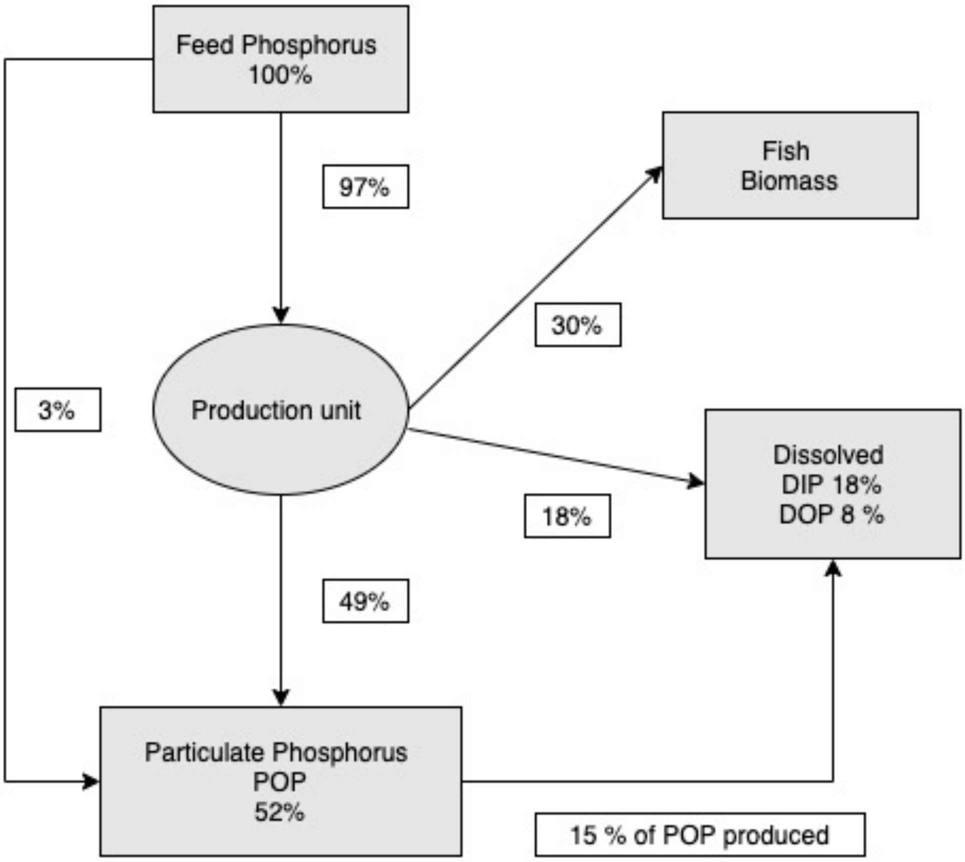

Figure 5-3 Flow chart of mass balance showing the annual fluxes of Phosphorous in Kg per tonne produced per year used for each salmon farm (Wang et al. 2012)

5.2.3 Nutrient removal by extractive farmed organisms

5.2.3.1 Removal of dissolved nutrients through mussels

Based on the suggestions of the report released from the Marine Institute (2018), production values for bivalves were used in this hypothetical production scenario. Therefore, it was assumed that mussel aquaculture (*Mytilus edulis)* in Mulroy Bay is located solely in Broadwater, covering an area of 59 ha (Marine Institute, 2018). Nutrient removal of the dissolved aquaculture wastes of N and P were measured only through the harvesting of mussels, by calculating the harvested dry weight of DIN and DIP stored in the tissues and shells, during a year of production. Equation used was sourced from Clements et al. (2019), presented as the Nitrogen removal potential (NRP in tonnes/yr) and Phosphorous removal potential (PRP in tonnes/yr), by adding the cumulative content of N and P of the tissue with the shell as Equations 5-1 and 5-2.

$$NRP = \left\{[(A \times I) \times (DMshell)] \times \left(\frac{\%\,Nshell}{100}\right)\right\} + \left\{[(A \times I) \times (DMtissue)] \times \left(\frac{\%\,Ntissue}{100}\right)\right\}$$

Equation 5-1

$$PRP = \left\{[(A \times I) \times (DMshell)] \times \left(\frac{\%\,Pshell}{100}\right)\right\} + \left\{[(A \times I) \times (PMtissue)] \times \left(\frac{\%\,Ptissue}{100}\right)\right\}$$

Equation 5-2

Where A is the areal coverage in ha, *I* is the number of individuals harvested ha $^{-1}$, assuming a constant of 987000 individuals per ha. DM is the individual dry mass of the shell and the tissue harvested (DM_{shell}, DM_{tissue}), for a typical longline mussel farm, we assumed the dry weight of the shell to be 5.95 g and tissue 0.49 g. Both for N and P, $\%P_{shell}$, $\%P_{tissue}$, $\%N_{shell}$ and $\%N_{tissue}$ were the content as percentage of N and P in the shell and tissue of the harvested mussels. Information on the coefficients is provided in Table 5-4.

Table 5-4 Coefficients, values and references used for the NPR and PRP in nutrient removal calculations for mussels.

Coefficients	Value	Reference
DM shell g/ind	5.95	Clements et al. 2019
DM tissue g/ind	0.49	Clements et al. 2019
% N in shell	1.13	Haamer et al. 1996
% N in tissue	10.64	Haamer et al. 1996
% P in shell	0.04	Bruer et al. 2020
% P in tissue	0.8	Bruer et al. 2020

5.2.3.2 Removal of dissolved nutrients through oysters

Based on plans developed from Mulroy Bay (Marine Institute 2018), recommendations for oyster farming (*Crassostrea gigas) will* cover an area of 56 ha. Particularly 45 ha would be in the Narrows and 11 ha in Northwater. The individuals per ha were assumed based on the stocking design, hosting 66 individuals per m^2, equating to 37,333,333 individuals. DM is the individual dry mass of the shell and the tissue harvested (DM_{shell}, DM_{tissue}), for a typical longline mussel farm, we assumed the dry weight of the shell to be 40 g and tissue 2 g. Both for N and P , $\%P_{shell}$, $\%P_{tissue}$, $\%N_{shell}$ and $\%N_{tissue}$ were the content as percentage of N and P in the shell and tissue of the harvested Oysters. The same constants were fed into equations 5-1 and 5-2 in order to calculate the removal potential of dissolved Nitrogen and dissolved Phosphorous in the Oysters. Information on the coefficients is provided in Table 5-5.

Table 5-5 Coefficients, values and references used for the NPR and PRP in Pacific oysters nutrient removal calculations.

Coefficients	Value	Reference
DM shell g/ind	40	Higgins et al. 2011
DM tissue g/ind	2.00	Higgins et al. 2011
% N in shell	0.16	Zhou et al. (2002)
% N in tissue	7.85	Zhou et al (2002)
% P in shell	0.04	Schatte Olivier et al. (2018)
% P in tissue	0.91	Schatte Olivier et al. (2018)

Finally, total NRP and PRP removed as harvestable material from the tissues and shells of the oysters and mussels was deducted from the total DIN and DIP entering the system through salmon farming in each sub-region respectively.

5.2.4 Bivalve ecosystem interactions

The ecosystem interaction of bivalve farming is evaluated through the food uptake in relation to the food available. In areas where the clearance times (CT) of the bivalves are longer than water residence times (RT) of the body of water, then it is suggested that bivalves do not dominate the food dynamics of the area (Smaal and Prins 1993; Dame and Prins 1998). There are also cases where clearance times are shorter than residence times (CT/RT<1). The CT/RT was used in order to assess whether production of bivalves would dominate any of the potential sub-regions within the RIMTA system, that could characterise a sub-region's necessity to accommodate bivalves for the regulation of phytoplankton communities or suggest that an area is flushing waste faster than it is filtered from the bivalves irrespective of production or not. If CR/RT > 1 then bivalves would have minor or no nutrient regulation at all, whereas, if CT/RT < 1 then bivalves would have a nutrient regulating effect, filtering the body of water faster than the hydrodynamic replenishment, potentially controlling pelagic processes through their grazing activity (Dame 1996; Dame and Prins 1998)

Clearance time calculations were based on the equation provided from Dame and Prins (1998) as shown in equation 5-3:

$$Clearance\ time = \ System\ volume\ /Clearance\ rate\ x\ mussel\ biomass$$

Equation 5-3

Volume of each sub-region holding mussel farms are shown in Table 5-6. Clearance time of the mussels was sourced from Widdows 1978 at 1.5 L g^{-1} h^{-1}. Clearance times of the oysters were based on Wheat and Ruesink (2013) at 0.35 L g^{-1} h^{-1}.

Table 5-6 Volume of each sub-region in (m^3) and bivalves production biomass per year

Sub-regions	Volume (m^3)	Mussel biomass (tonnes)	Oyster biomass (tonnes)
Narrows	51,720,534,000	376	0
Northwater	15,766,827,000	0	309
Broadwater	59,014,959,000	0	1266

The next factor for the assessment of bivalve ecosystem interaction was the residence times (RT) of each subregion shown in Table 5-2. The residence times were initially based on number of tides and converted in hours (two tides over approx. to 24 hours), retrieved from (Telfer and Robinson 2003).

5.3 Results

5.3.1 Salmon mass balance release of N and P elements

The total amount of feed used across Mulroy Bay along with the sub-regions within a year are shown in Table 5-7. The results suggest that the sub regions with the highest usage of feed was the Narrows, followed by Broadwater and Northwater. Highest release of feed sued was 3459 tonnes in the Narrows and cumulatively 6829 tonnes within Mulroy Bay.

Table 5-7 Feed used within each sub-regions

Sub-regions	Feed used (tonnes)
Narrows	3459
Northwater	973
Broadwater	2397
Total	6829

DIN was the type of waste with the highest amount dispersed as shown in Table 5-8. Cumulatively 222 tonnes of DIN were released in Mulroy Bay with the Narrows receiving most of the waste within a year. PON would settle to the seabed withing few meters from the farm sites having a local effect whereas DIN would follow the dispersion patterns of the surface layers at each sub-region. Strong dispersive capacity of DIN at Narrows meant that majority of the DIN within Mulroy bay would be dispersed in the Atlantic Ocean. Whereas DIN released from Northwater would have a higher residence time.

Table 5-8 Amount of Dissolved Organic Nitrogen (DON), Dissolved Inorganic Nitrogen (DIN) and Particulate Organic Nitrogen (PON) released back to a soluble state. Release of nutrients represent a year of production.

Sub-regions	DON (tonnes)	DIN (tonnes)	PON (tonnes)
Narrows	45	112	7
Northwater	13	32	2
Broadwater	31	78	5

Concerning the phosphorous release (Table 5-9), majority of the waste would have been released as particulate organic phosphorous within few meters from the farm sites with the Narrows again receiving most of the waste, followed by Broadwater and Northwater. In terms of DIP which is characterised by a higher dispersive nature following the water body directions and speed, DIP in Northwater would have a higher residence time in the system compared to the DIP released in the Narrows.

Table 5-9 Amount of Dissolved Organic Phosphorous (DOP), Dissolved Inorganic Phosphorous (DIP) and Particulate Organic Phosphorous (DOP) released back to a soluble state. Release of nutrients represent a year of production.

Sub-regions	POP (tonnes)	DIP (tonnes)	DOP (tonnes)
Narrows	22	7	3
Northwater	6	2	1
Broadwater	15	6	2

Regarding the volumetric loading, shown in Table 5-10, the results suggest that even though the Northwater sub-region considerable received the lowest DIN and DIP load across all three sub-region, volumetric loading was higher compared to the one of Broadwater, and very close to the Narrows. That is an important result as it suggests that loading of waste is relevant to the volume of the receiving sub-region.

Table 5-10 Volumetric loading of DIN and DIP in each Sub-region

Sub-regions	Volume m^3	DIN $t\ yr^{-1}$	DIP $t\ yr^{-1}$	DIN $mg\ m^{-3}\ yr^{-1}$	DIP $mg\ m^{-3}\ yr^{-1}$
Narrows	51720534	112	7.7	2165	149
Northwater	15766827	32	2.0	1841	127
Broadwater	59014959	78	5.0	1322	8

5.3.2 *Bivalves remediation*

5.3.2.1 Dissolved inorganic nitrogen removal

Results of the nitrogen removal potential (NPR in tonnes/yr) through harvesting are shown in Table 5-11. Results suggest that 59 ha of mussels within the sub-region of Broadwater incorporated 7 tonnes/yr of DIN, the other two sub-regions would have had no removal of DIN through mussels due to absence of production. Regarding the oyster production cumulatively 8.2 tonnes/yr of DIN at the whole Mulroy Bay, spreading to two subregions removing 6.6 tonnes/yr of DIN and 1.6 tonnes/yr of DIN from the Narrows and Northwater respectively. Cumulatively, within the whole bay mussels and oysters removed 15.2 tonnes/yr of DIN. The area with the greatest removal was Broadwater with 7 tonnes/yr of DIN followed by the Narrows 6.6. tonnes/yr od DIN and Northwater 1.6 tonnes/yr of DIN. Even though waste release in the area of the Narrows was significant, the combination with the water residence times that release withing the Narrows would be easily dispersed out to the Atlantic Ocean, whereas 8.6 tonnes/yr of DIN released from Broadwater and Northwater would have higher a higher impact.

Table 5-11 Dissolved inorganic Nitrogen removal through harvesting of mussels and oysters per year.

Subregions	Mussels (tonnes)	Oysters (tonnes)
Narrows	0.0	6.6
Northwater	0.0	1.6
Broadwater	7.0	0.0
	Total	15.2

5.3.2.2 Dissolved inorganic phosphorous removal

Results of the phosphorous removal potential shown in Table 5-12, suggests that mussels through harvesting removed 1.03 tonnes/yr of DIP only in the Broadwater area. Oysters however, again removed the greatest amount of dissolved phosphorus (1.2 tonnes/yr of DIP), with the majority being removed from the Narrows (1 tonnes/yr of DIP), where the largest production took place, and the rest from Northwater (0.2 tonnes/yr of DIP). Cumulatively, 1.6 tonnes/yr of DIP were removed from Mulroy Bay (both from mussels and oysters, with the highest removal taking place in the Narrows.

Table 5-12 Dissolved inorganic phosphorous removal tonnes/yr of DIP through harvesting of mussels and oysters

Subregions	Mussels	Oysters
Narrows	0.0	1.0
Northwater	0.0	0.2
Broadwater	0.3	0.0
	Total	1.6

5.3.3 Nutrient budget

Results of the nutrient budget of the Mulroy bay as a whole waterbody, and within each sub-region including nutrients sources from salmon and nutrient sinks of mussels and oysters cumulatively are shown in Table 5-13 for DIN and Table 5-14 for DIP. The overall nutrient budget for DIN was positive with 6.6 % removed by the bivalves. The largest effect was found in Broadwater with 9.0% removed where Northwater had the lowest removal at 5.0%. A similar trend was observed with DIP with 10.2 % of the total released DIP within the sub-region removed by the bivalves. However, percentage cover of waste regulated by bivalves was different within the Narrows having the highest percentage cover with 13.4% and Broadwater the lowest with 5.8%. That originates from the different species cultured within sub-regions, originating from different production biomass and DIN and DIP retention within the shells and tissue, having a different regulatory capacity of dissolved nitrogen and phosphorus.

Table 5-13 Nutrient budget of Dissolved inorganic Nitrogen along with % of DIN removed within a year of production .

Sub-regions	DIN in (tonnes)	DIN out (tonnes)	Net DIN	% of DIN removed
Narrows	112	6.0	105	5.4
Northwater	32	1.6	30	5.0
Broadwater	78	7.0	71	9.0
Total	222	14.6	206	6.6

Table 5-14 Nutrient budget of Dissolved inorganic phosphorous (DIP) along with % of DIP removed within a year of production.

Sub-regions	DIP in (tonnes)	DIP out (tonnes)	Net DIP (tonnes)	% of DIP removed
Narrows	7.47	1.0	6.44	13.4
Northwater	2.10	0.2	1.85	9.5
Broadwater	5.18	0.3	4.81	5.8
Total	14.70	1.5	13.10	10.2

5.3.4 Regulating potential of bivalves

The analysis of the CT/RT ration as an indication of positive and negative bivalve regulation is shown in Figure 5-4 and Table 5-15. The results suggest that clearance times of bivalves are higher in Northwater followed by Broadwater and the Narrows indicating that for better clearance times increase in biomass in this first two subregions is needed. The clearance times however have to be related to the residence times of the waters with Northwater again showing the highest residence times with 105 hours followed by Broadwater and the Narrows.

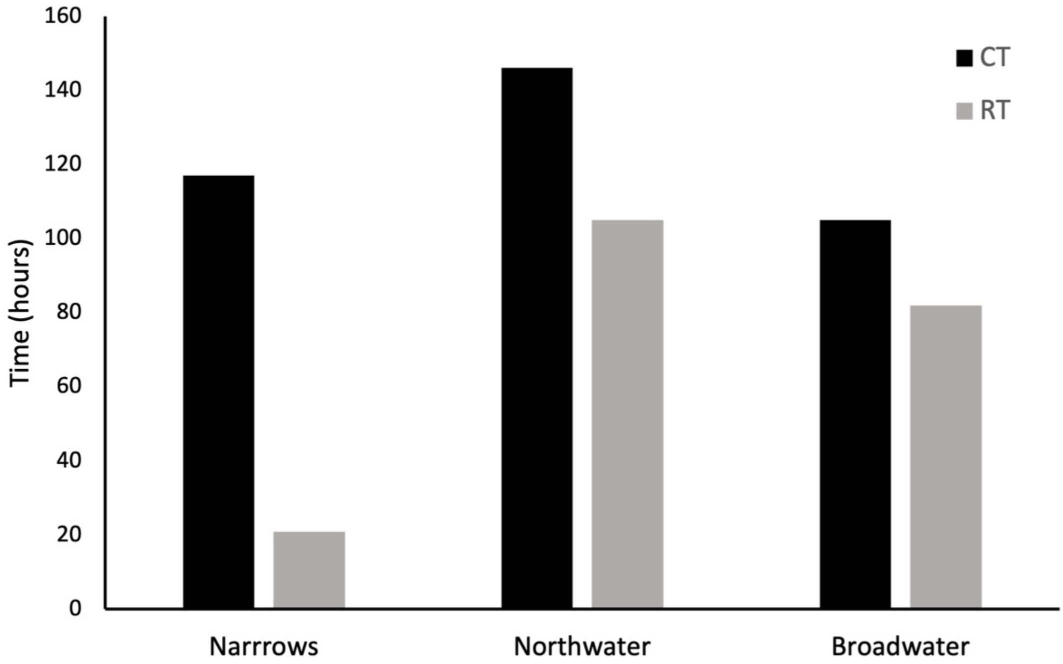

Figure 5-4 Clearance times (CT) of bivalves and residence times (RT) of each sub-region

The indicator of positive or negative regulating services from the bivalves are shown in Table 5-15. Across all three locations bivalves did not have a positive effect on regulating the nutrient budgets of all three sub-regions. The location with the highest ratio was Northwater, a sub-region with notable high clearance rates and high residence times. The other two locations where close to an CT/RT ratio of 1 with 1.4 for Northwater and 1.3 for Broadwater.

Table 5-15 Clearance times (CT) and residence times (RT) along with CT/RT ratio.

Sub-regions	CT	RT	CT/RT
Narrows	117	21	5.6
Northwater	146	105	1.4
Broadwater	105	82	1.3

5.4 Discussion

The results of this study identified locations within a waterbody scale where increase in bivalve biomass would have a regulating effect on ecosystem interactions and potential eutrophication events. This study calculated nutrient entering they system by using mass balance principles across a range of farm sites and sub-regions with different stocking biomass. In turn, the removal of nutrients through bivalve farming as harvestable material contained in shells and tissue was calculated. The regulating services of bivalves was assessed by looking at the clearance times of the bivalves in relation to the water residence times of each sub-region.

The mass balance calculation of nutrients entering the system revealed that cumulatively within Mulroy Bay 221 tonnes of DIN and 14.7 tonnes of DIP were released in the form of dissolved inorganic wastes. Mass balance equations for the calculation of wastes entering a bay or a system has been successfully replicated by other studies, considered as a simple but widely used method (Olsen and Olsen 2008; Verdegem et al. 2013, Qi et al. 2019). The same mass balance principles developed from Wang et al. 2012 have also been used from Qi et al. 2019 to calculate nutrient loading in Daya Bay, China. The effect of additional release of N has been shown to affect phytoplankton with accumulation close to the vicinity of the farm not being evident, however accumulation of wastes have been observed in greater distances (Tsagaraki et al. 2011). In this case, dispersal and accumulation of wastes is relying on the hydrodynamics of the area, where areas with high residence times are more susceptible to nutrification and high phytoplankton production rates (Cloem 2000; Wild-Allen et al. 2010). Management of salmon farming practises within Mulroy Bay based indicated that area with the highest loading of soluble waste would be the Narrows followed by Broadwater and Northwater. Looking at the volumetric loading rate of DIN and DIP, Northwater received relatively the same amount with the Narrows with 1841 mg m^{-3} yr^{-1} and 2165 mg m^{-3} yr^{-1} of DIN , the same pattern was observed with volumetric loading of DIP. In combination with the long residence times of Northwater this suggests that this area would be more likely to be impacted by the dissolved nutrient released, but that there is also potential for mitigation through additional bivalve culture. This is confirmed from studies in Mulroy Bay from Moreno et al. (2011a,b) suggesting high stratification in both these two sub-regions, while Telfer and Robinson (2003) documented externalities in terms of nutrient and chlorophyll-a levels in Broadwater. Calculation of mass balance equations and constants used within the model from

Wang et al. 2012 relied on a yearly averaged feed supply based on the FCR in terms of annual biomass production. It is more likely that feeding regimes withing a year will vary based on each farms feeding regime and consequently dissolved nutrient release might also vary. However, when looking at a number of salmon farm sites at a bigger regional scale in combination with the data scarcity of production biomass, FCR was considered to be a reasonable approach.

Mussels and oysters cumulatively incorporated and removed through harvesting 14.6 tonnes of DIN and 1.5 tonnes of DIP across Mulroy Bay within a year. Nutrient removal by mussels was solely coming from Broadwater, where the mussel production was based, whereas oysters were located both in the Narrows and Northwater. In terms of nutrient removal efficiency mussels removed 0.11 t N ha^{-1} yr^{-1} and 0.006 t P ha^{-1} yr^{-1}, the results come in agreement with other studies such as Rose et al. (2015) suggesting a net modelled removal of 0.11 t N ha^{-1} yr^{-1} or with mussels deployed as buffer zones in wetlands suggesting a net removal of 0.1 t N ha^{-1} yr^{-1} (Anderson et al. 2012). Oysters removed 0.14 t N ha^{-1} yr^{-1} and 0.02 11 t P ha^{-1} yr^{-1}, which also comes in agreement with Rose et al. 2015 suggested a net P removal of 0.02-0.14 11 t N ha^{-1} yr^{-1} and Silva et al. 2011 indicating a net N removal of 00.7-1.2 11 t N ha^{-1} yr^{-1} Oysters removed higher amount of DIN and DIP due to the higher harvestable DW, especially the shells. Another route of uptake that has not be examined in this study is the denitrification process which until this point there have been variable results and conservation still continues regarding the potential for long time burial. Bio deposition under shellfish farms can result in N removal from the system, consequently the role of denitrification may be as equally important as of harvesting (Clements et al. 2019), with Humphries et al. (2016) suggesting that denitrification by shellfish farms is very likely to happen. However, denitrification is driven by parameters such hydrodynamics and specific farm scale parameters (e.g depth, farm density, residence times, water velocity) which were not readily available and fall outside of the scope of this research chapter. Furthermore, modelling the annual denitrification rates has been proven to be rather inconsistent for mussels due to temporal variations between seasons, (Stephenson et al. 2010; Kellogg et al. 2013; Humphries et al. 2016).

The calculation of the nutrient budget of the Mulroy Bay RIMTA system suggest that bivalves removed 6.6% of the total DIN excreted from the salmon farms and slightly higher levels of DIP with 10.2% of the total removed. This, in terms of a static system is acceptable, however flushing rates and residence times of the water body had to be also taken in account. The CT/RT ratio indicated that based on the culture biomass of the bivalves, there was not considerable regulation of the phytoplankton levels of the water system in the Narrows sub-region with CT/RT>1. That meant water within each sub-region would be replenished faster than filtered from the bivalves and therefore potential increase in bivalve production in terms of regulating services would be unnecessary. However, locations such as Northwater or Broadwater, CT/RT ratio was near to 1 which meant that potential increase of production of mussels would lead to regulation of the system through their production. An increase in mussel production in Broadwater would be desirable, particularly due to space availability. On the contrary, Northwater would pose limitation in terms of carrying capacity, due to small available area for cultivation. Carrying capacity of each-subregion would be very important taking in account the local physical conditions where the culture will take place (McKinney 2012).

RIMTA is a newly emergent term which attempts to consider IMTA at a regional level and take into account the larger scale connections in nutrient transfer between organisms at different trophic levels. RIMTA attempts to redefine mitigation of dissolved nutrients through independent allocation of different culture systems within the same body of water. As showing in this study large water bodies will incorporate several farming activities with different species cultured and stocking biomass, whilst sub-regions will be characterised by different physical conditions (e.g volume) and hydrodynamic conditions such as residence times. The role of bivalves as a measure to provide regulating services has been promoted over the years for their regulating services, affecting ecosystem interactions such as primary productivity (Newell et al. 2004). Considered as a potential tool for nutrient extraction through farming of bivalves (Bricker et al. 2014; Grizzle et al. 2017). Bivalve farming irrespective of salmon farming has already been suggested as measure to remove a substantial amount of nutrient from Bays in Canada as a measure to manage the nutrient budgets in the area (Clements et al. 2019). Mussel culture has also been incorporating into a modelling study in Canada, suggested to act as a measure to manage mussel biomass production for the regulation of the environment (Filgueira and Grant 2009) The same regulating service have been suggested for seaweeds as well, for mitigation of potential eutrophic events by (Holdt and Edwards 2014; Kim et al. 2015; Hasselstrom et al. 2018). The relationship of these two source and sinks within a system have been initially suggested from Wang et al. 2012 where he followed a nutrient mass balance approach looking at wastes entering the system for the whole of Norway, whilst mentioning the potential for IMTA considerations at broader scale. This study attempted to merge the studies from Wang et al. 2012 and Clements et al. 2019 by calculating the nutrients budgets under the newly emerged concept of RIMTA suggested by (Sanz-Lazaro and Sanzez-Perez (2020). RIMTA as shown in this study could potential serve as a planning and management concept of waterbodies, suggesting extractive organisms potential locations where biomass increases could offer regulating services into the system by looking at particularly dissolved nutrients.

5.5 Conclusion

This chapter investigated the concept of RIMTA by looking at Mulroy Bay as study site location offering a variety of production systems and relatively rich in background information. For the better management of RIMTA it is important to assess initially the production biomass of each culture system and consequently map the different hydrographic conditions of the system. As this chapter suggested there will be areas where salmon production would not constitute a huge threat in terms of environmental impacts due to high flushing rates of the specific sub-region and that secondary organisms such as bivalves would not play a big role on regulating potential impacts. Conversely, there were sub-regions associated with high residence times and a history of increased background nutrient values, prone to eutrophic events. In these regions bivalve farming could play a significant role on regulating the food web, mitigation potential impacts. By treating a whole region as a RIMTA system and compartmentalising sub-regions, information could be given to regulators, and local stakeholders in relation to best management practises of the relevant culture systems. That could be further investigated by looking at human induced stresses originating from other activities, such as cattle farming, wastewater treatments and include those sources into the nutrient budgets of the RIMTA system as well. RIMTA demonstrates than even though organisms are not closely connected, there are still considerations that can be made in terms of dissolved inorganic waste management at a waterbody scale.

Chapter 6 Discussion

6.1 General Introduction

The growing demand for fish protein as populations rises and stagnation of fisheries landings has led to the need for aquaculture production to grow in order to meet the projected global demands in high quality protein (FAO 2018). However, presently growth of aquaculture in Europe is slow, with growth rate of aquaculture fish production since 2001 being the lowest among the other continents and even lower than the world average (FAO 2020). One of the reasons for this is the environmental concerns particularly for open water cage farming where majority of aquaculture takes place (FAO 2020), leading to societal scepticism regarding the sustainability of the sector. One of the solutions for the sustainable growth of the sector suggested is IMTA (Neori et al. 2004; Troell et al. 2003). Initially after this concept received positive feedback, this led to a series of in situ pilot studies over in the last decade. It is generally perceived as a promising way to alleviate potential environmental impacts originating from fin-fish farming, whilst offering an extra source of income for the farmers, diversifying production and ultimately increase public perception and social acceptability of aquaculture. In addition, IMTA has been presented as a challenge to the scientific community due to its complexity in addressing both the circular economy and its multidisciplinary nature. Even though there is a considerable body of literature published, IMTA has few examples of full commercialization in Europe yet, that can most likely be attributed to lack of understanding and support from policy and regulatory bodies to allow incentives for the adoption of IMTA (Hughes and Black 2016; Alexander and Hughes 2017). One of the reasons planning and management of IMTA is limited is the knowledge gap regarding the spatial management and allocation of the species, and at different spatial scales under examination. The same applies for fish farming in general, different standards and issues occur when looking at suitable sites for salmon farming at a national based level and at farm scale level and are considered differently in legislation and regulation. The same should apply for IMTA to create a holistic view regarding the challenges and opportunities presented in each spatial scale, allowing better information for planning and setting up an IMTA site. However due to the multidisciplinary nature of IMTA, combining different types of systems, rearing designs and species has been difficult. To add to the complexity, the variable nature of the natural environment conditions along with the site-specific characteristics of each farm site in terms of production creates additional difficulties.

The premise of this PhD research was to investigate IMTA by assessing its limitations, considerations and opportunities presented at different spatial scales when planning to establish a system.

6.2 National scale

By looking at IMTA from a national scale perspective in this study, the research highlighted that not all established commercial farm sites and regions across Scotland will be suitable for IMTA, and that this suitability will vary depending the type of the extractive species chosen. In particular, the results suggested that the regions of Argyll, Firth of Clyde and West Highlands should be prioritized when looking on setting up an IMTA system. Variability of site suitability scores was evident within seal loch systems as well, where the majority of aquaculture salmon farming takes places. Loch Linnhe located in Argyll hosts the majority of salmon farming activities and locations which were found to be suitable only for the lobsters. Comparatively, in the Firth of Clyde marine region, Loch Fyne it was observed to have suitable areas for only lobster in the northern section and suitable locations for all three species in the southern part of the loch. The evident variability between suitability scores for the species originates from the difference between their biological and rearing design requirements. Suspended designs for the production of lobsters were highly appropriate for more areas than sediment benthic ones used for sea cucumber and urchin production. This originates from the greater flexibility of suspended culture methods and without its dependence on sediment type and depth. Another component which was crucial and required attention is the seasonal variability, suggesting that there were some locations with high temperature ranges that were not favourable for culturing any of the extractive organisms. The method for site suitability was subject to data gaps as much of the required information was dependent on published literature, such as the specific biological and rearing design tolerances for the lobsters was often limited. For sea cucumbers and sea urchins, primarily due to their popularity due to proven mitigation potential and economic significance, finding literature related to the biological tolerances was easier. A suggested reason for the slow growth of IMTA is the lack of regulatory tools and legislation for planning and management of the combined systems, and their lack of inclusion into marine spatial plans (European Commission 2020). The purpose of the regional models created in this study was to lay down the foundations of the main challenges and show the variability of the natural environment along with the biological and rearing design requirement of each species, influencing the suitability of IMTA.

6.3 Farm scale

Once a location has been indicated as suitable for the whole IMTA system at a national scale, production considerations have to be made at the farm scale level regarding where to farm the extractive species and the bioremediation performance of the system. This study demonstrated for benthic environments that the variability of the natural environment and particularly the site-specific characteristics of each farm site will influence the positioning of the extractive species, the amount of the extractive species and consequently the bioremediation potential of an IMTA system. I was demonstrated that across all six farm locations modelled that 15 to 34 % of the total area cover of a farms' particulate waste footprint would be available for the deployment of the extractive species. As a consequence, 22 to 47 % of the total particulate organic waste released from the farm sites would be available for consumption. This approach, focusing on particulate wastes, demonstrated that majority of the wastes would be settled in areas where extractive benthic organisms such as sea cucumbers would be limited by their biological tolerance to anoxic conditions or limited by food supply. This approach can be extended further by modelling for other benthic detritivores, such as sea urchins and annelids, where there is also growing interest in their use for IMTA. In addition, it was shown that the proposed equipment design, stocking densities and biological tolerances of other species would be different, influencing their potential for waste extraction and bioremediation. The approach taken in this study, attempted to enhance the knowledge around farm scale spatial management when planning a site. The optimal areas for production near the cages along with the amount of additional carbon source available are significantly correlated to the current flow and production biomass at the farm sites, respectively. This relationship could be extended for any potential site by using the equations developed as a first estimator of economic and bioremediation potential for the extractive benthic species. However, this relationship would have been stronger if more sites were evaluated to have a greater sample size. The total waste removal from the two scenarios used of 200 and 500 crates in relation to the total amount of waste deposited ranged between 0.21 and 0.26 % and 0.53 and 1.27 % respectively, indicating that the potential for a significant amount of waste removal from this type of system based for these conditions is minimal. A considerable amount of waste could be reduced if the whole suitable area was filled with sea cucumbers through sea ranching ranging from 5% to 16 %, but this was shown to be impractical. The same conclusion was made by Reid et al. (2013), who suggested for a considerable amount of wate reduction, a very large biomass of mussels and seaweeds would be needed, exceeding any space available. Sea ranching, by not containing the extractive species, would create further implications such as genetic contamination of naturally wild populations (Uthicke and Purcell 2004), overlap with everyday farm activities (Alexander and Hughes et al. 2017) and high levels of mortalities and escape events (Han et al. 2016). By looking at remediation cost efficiency of the system it was suggested that the range of values for the six salmon farm locations modelled would be £80 for the removal of 1 kg of carbon waste for 200 or 500 crates. Suggesting the cost efficiency of the system is irrespective of the mitigation provided, with expenses being proportional to mitigation.

6.4 Individual scale

The present study also incorporated a pilot scale experimental site in Ireland to investigate IMTA at an individual scale and the potential trophic relationships between salmon and a novel secondary trophic organism. The outcomes suggest that environmental conditions at the location is a very important component of

IMTA and particularly the position of the extractive organisms in relation to the prevalent waste stream. Once potential for consumption of wastes has been established the resulting potential for trophic connectivity was explored with the use of biological tracers such as stable isotopes and fatty acids. The study showed fatty acids have been shown to be a robust method to access trophic connectivity particularly for the lobsters showing a clear difference of the fatty acids profiles of the lobsters located on the farm station in relation to the lobsters in the control station. Accumulation of specific fatty acids markers such as 18:1n-19, 18:2n-6, 18:3n-3, 20:1n-9 and 22:1n-11 originating from terrestrial sources and consequently in the salmon feed were in higher concentration on the farm site. On the other hand, stable isotopes analysis did not indicate any significant differences of $\delta^{13}C$ and $\delta^{15}N$ between the cage and control sites for lobsters. However, the contribution of nitrogen in kelp tissues of salmon farm origin were relatively higher at the farm site with 92.9% and control site with 91.6%, suggesting that soluble nitrogen released from the farm site was reaching the control station. The results suggested that when looking for a trophic connection between two components of an IMTA system the method used depends on the feeding regime of the extractive organism and type of waste used for this connection. For the case of particulate organic matter fatty acids was a more robust method of tracing, indicating that the impact of the particulate organic waste stretches only few meters away from the farm site. Comparatively, stable isotopes were more successful in detecting the trophic connectivity for soluble nutrients, which occurred near to and at considerable distances from the farm. In a more general context this re-enforces the understanding that in their majority particulate organic wastes tend to be dispersed only within few meters from the farm site in large quantities. Conversely, dissolved wastes tend to be dispersed over greater distances, meaning that extractive organisms that exploit these wastes have the potential to be places considerable distances from the farm. This in a way changes the meaning of IMTA which treats the two compounds of the system as one, being spatially closely connected.

6.5 Waterbody scale (RIMTA)

RIMTA is where the whole water body containing multiple aquaculture activities are treated as a single system, even if not being closely interconnected, and looking at an ecosystem-based level. The findings of the investigation suggest that within a waterbody scale, sub-regions will have different production characteristics in terms of biomass and species cultured. Each sub-region would have areas where fin-fish farming would act as a nutrient source and bivalves, in our case, as nutrient sinks. However, the hydrodynamics of each subregion and particularly the residence times and water volume exchange will vary. Therefore, careful consideration is needed when assessing a RIMTA system from a nutrient budget point of view. In the present investigation, there were areas such as the fast flowing Narrows where dissolved nutrient addition through salmon would not constitute a potential threat for the system due to the low residence times of the water, and consequently nutrient extraction by bivalve culture would make little difference. However, in sub-regions such as Broadwater, with slow flowing condition and poor water exchange, addition of nutrients would not be quickly flushed away, potential allocation of bivalves would help balance the dissolved nutrients within the system through bivalve harvesting.

6.6 Future considerations

Holistically, when planning for IMTA in coastal waters initially there needs to be the selection of appropriate sites for the deployment of the species at national level to narrow down suitable locations that should further be explored in greater detail. The next step would be to assess the conditions both physical such as current speeds and direction along with the depth of the surrounding environment at these locations together with the production characteristics of the site. Based on these conditions, an area suitable for the deployment of the species should be designated based on the biological tolerances of the extractive organisms along with the proposed design. Once this area is predefined, careful selection of the areas suitable for the deployment of the species should be made based on the prevailing stream of waste in order to ensure connectivity. In the case of dissolved waste, a different spatial consideration should be taken in account when considering larger scale integrated systems like RIMTA. However, careful consideration of the flushing and residence rates of each subregion along with the stocking biomass of each aquaculture activity must be made in order to decide which sub-region is more likely to be affected by potential impacts. The RIMTA concept could potentially incorporate IMTA as well. RIMTA should be designated for dissolved wastes at water body scale, whilst IMTA withing the same waterbody could be used to assess farm scale particulate organic matter considerations taking in account the suggestions made in Chapter 2, 3 and 4.

6.7 Concluding remarks

The outcomes and finding from this study are important, because firstly they demonstrate that to look at IMTA from different spatial scales, different methodologies and disciplines are required. Each spatial scale faces its own challenges highlighted in this thesis which offers a holistic view of issues related to planning and selection of farm sites suitable for IMTA. The study demonstrates, that site suitability, model performance of a system, trophic connectivity between the different parts of the system and finally the nutrients budgets of a water body system are affected by the spatial variability of the natural environment. In addition, these are influenced by the technical design of each system and the subsequent biological tolerances of the selected extractive organisms. The study suggests that the purpose of IMTA needs rethinking as bioremediation (as the main ecosystem service of IMTA) may not be as efficient as presumed. However, there might be other potential routes of ecosystem services that need further exploration.

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