Real-world complexities of nearshore integrated multi-trophic aquaculture in crowded coastal environments

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Abstract

The expansion of coastal aquaculture is challenged by a lack of coastal space and competition from maritime activities. In the Mediterranean Sea, commercial aquaculture in a multiple-use port in Marsaxlokk Bay, Malta, encounters complex natural and anthropogenic interactions. Intermittent water currents observed near the fish cages were not explained by atmospheric forcing but corresponded to vessel movements within the vicinity. At farm-level, cage management practices such as cohort dynamics and cage movements also influenced waste deposition around fish cages. The farm-scale model, Cage Aquaculture Particulate Output and Transport (CAPOT) was used to estimate waste distribution from the fish farm between October 2018 and July 2019 from the production of multiple species from different cohorts and cage arrangements. CAPOT then informed the placement of the sea cucumbers *Holothuria (Roweothuria) poli*, in an integrated multi-trophic aquaculture (IMTA) system. Between October 2018 and September 2019, *H. poli* was cultured directly below a fish cage at 0 m (E0), at 10 m (E10) and 25 m (E25) distances from a commercial fish cage, and at two reference sites (R1 and R2) over 800 m from the fish farm. Sea cucumbers grew better near the fish cages but mass mortalities were recorded at E0 within the first month of the study and poor survival rates were recorded at all sites. Still, their ability to recycle aquaculture organic wastes was validated using carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios and fatty acids in February, May and September of 2019. Isotopic analysis indicated that sea cucumbers at IMTA sites primarily relied on fish farm organic waste as their primary dietary source. This was complemented by a higher abundance of individual fatty acids, oleic (18:1*n*-9), linoleic (18:2*n*-6) and eicosenoic (20:1*n*-9) acids at E10 and E25, presumably linked to the terrestrial plant oil content of fish feeds, and marine-based fatty acids dominant in sea cucumber tissue at the reference sites. The full complexity of bay-wide processes and farm-level practices should be represented for effective management of wastes and maximised use of space in multiple-use environments. Detailed input data and finer scale modelling approaches are required to represent changes in waste deposition and food resources for sea cucumbers in IMTA. Additionally, the impact of temporal variation in food availability and quality in sediment, and the bioaccumulation of substances like mercury and arsenic that bioaccumulated in sea cucumbers under fish cages should be considered. Overall, a comprehensive representation of these factors is essential for effective waste management and sustainable aquaculture practices in multiple-use coastal areas.

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Building upon the investigation of the effects of wind and maritime traffic on inshore currents around a fish farm in a crowded coastal space, described in Chapter 3, this research was published as a scientific paper to present important implications of anthropogenic activities in port areas for the development of sustainable marine aquaculture in these highly contested areas.

Cutajar, K., Gauci, A., Falconer, L., Massa-Gallucci, A., Cox, R.E., Beltri, M.E., Bardócz, T., Deidun, A. and Telfer, T.C., 2023. Wind and shipping influences on sea currents around an inshore fish farm in a heavily contested Mediterranean embayment. *Regional Studies in Marine Science*, *62*, p.102855.

Based on Chapter 5, this publication analyses the potential of a Mediterranean sea cucumber species for open-water integrated multi-trophic aquaculture when cultured in a commercial fish farm at lower latitudes in Europe to drive the push for research in IMTA where the local environment and fish farming conditions present familiar challenges for the growth of sustainable aquaculture.

Cutajar, K., Falconer, L., Massa-Gallucci, A., Cox, R.E., Schenke, L., Bardócz, T., Sharman, A., Deguara, S. and Telfer, T.C., 2022. Culturing the sea cucumber *Holothuria poli* in open-water integrated multi-trophic aquaculture at a coastal Mediterranean fish farm. *Aquaculture*, *550*, p.737881.

The research presented in Chapter 6 is presented in this publication and leverages the foundational understanding of fish-sea cucumber IMTA obtained from Chapter 5. This paper investigates the trophic connectivity between deposit-feeding extractive organisms and fed fish in cages in an open-water environment to accentuate the significance of fish-sea cucumber IMTA within the broader context of sustainable aquaculture in the marine environment.

Cutajar, K., Falconer, L., Massa-Gallucci, A., Cox, R.E., Schenke, L., Bardócz, T., Andolina, C., Signa, G., Vizzini, S., Sprague, M. and Telfer, T.C., 2022. Stable isotope and fatty acid analysis reveal the ability of sea cucumbers to use fish farm waste in integrated multi-trophic aquaculture. *Journal of Environmental Management*, *318*, p.115511.

Chapter 1. Introduction

1.1 IMTA, a strategy for sustainable aquaculture in crowded coastal spaces

As the global demand for food continues to rise, marine aquaculture has emerged as a crucial solution to meet the increasing need for seafood. Aquaculture is recognized as an essential source of protein and food security; however, the expansion of this industry is hindered by the limited availability of suitable coastal locations (Sanchez-Jerez et al., 2016; Cavallo et al., 2020; Galparsoro et al., 2020). Coastal regions are highly valuable and extensively vyed for areas with competing activities and increasing pressure from various human activities that limit the available space for aquaculture operations. Consequently, there is a need to consider how space is utilized to enable the sustainable growth of aquaculture with minimal impacts on other coastal users and the environment. To grow in crowded coastal areas, natural and anthropogenic interactions in these spaces and their implications for planning and management of aquaculture need to be understood first. It is essential to explore innovative approaches that can maximise the use of space and resources for sustainable aquaculture to expand, particularly in crowded coastal areas.

Malta, at the centre of the Mediterranean, has spatial restrictions and challenges for the expansion of marine aquaculture. In Malta, and elsewhere in the world, nearshore areas are used as sheltered coastal spaces that provide suitable environments for marine aquaculture. In the Mediterranean alone, marine cage facilities and shellfish farms in multiple-use environments are common e.g., Port d'Andratx in Mallorca, Port of Cagliari and Port of Olbia in Sardinia, Port of Ashdod in Israel, Port of Villagarcia de Arosa in Galicia and the Andalusia region in Spain (Cavallo et al., 2020). Multiple-use coastal areas where inshore aquaculture exists pose real-world complexities and unique challenges for the management and mitigation of open-water aquaculture impacts. Resolving some of the complexities, helps address knowledge gaps that can otherwise impede the development of marine aquaculture in these areas.

In crowded coastal spaces where horizontal space can be limited, integrated multi-trophic aquaculture (IMTA)is considered a potential strategy that maximizes the use of vertical space below open-water fish farms (Mazzola and Sarà, 2001; Cheshuk et al., 2003; Handå et al., 2012; Irisarri et al., 2013, 2014; Jiang et al., 2013; Giangrande et al., 2020). IMTA has been described as a strategy for the uptake and conversion of aquaculture waste into marketable biomass of extractive organisms co-cultured with fed species (Troell et al., 2009; Chopin et al., 2012). IMTA is often recommended as a system for environmental mitigation of intensive monoculture that can help maximise production (Troell et al., 2009; Chopin et

al., 2012). Nonetheless, studies have reported contradictory findings and demonstrate challenges associated with IMTA, particularly in the open-water environment (Mazzola and Sarà, 2001; Cheshuk et al., 2003; Handå et al., 2012; Irisarri et al., 2013, 2014; Jiang et al., 2013; Giangrande et al., 2020). Specifically, evidence for the effectiveness of IMTA in land-based systems has been well documented, whereas the commercial viability of integrated aquaculture in open-water environments requires that the performance of extractive organisms within the system be substantiated in different settings. Open-water IMTA studies have revealed the utilisation of aquaculture-derived organic matter (Mazzola and Sarà, 2001; Handå et al., 2012; Irisarri et al., 2014; Jiang et al., 2013; Giangrande et al., 2020) but others contribute the lack of improved growth performance in extractive organisms and reduced organic enrichment closer to fish cages to limitations in food availability and quality (Cheshuk et al., 2003; Irisarri et al., 2013). In view of this, it still unclear whether IMTA can be the solution for better use of space and resources where these are a limitation for the development of sustainable aquaculture in the open environment.

1.2 Sea cucumbers in IMTA

Among the proposed candidates for IMTA, sea cucumbers have been widely recommended for their ability to take up and reduce organic matter in wastes (uneaten food and fish faeces) that accumulate under fish cages (Slater and Carton, 2009; MacDonald et al., 2013; Cubillo et al., 2016; Zamora et al., 2016; Grosso et al., 2021). Deposit-feeding sea cucumbers ingest detritus, organic matter, and planktonic particles from the substrate or the water column. They are important ecosystem engineers that can collect and process organic material to expel clean sediment, contributing to the recycling of nutrients and having an essential role in sediment dynamics (Lee et al., 2018). This helps the decomposition of organic matter on the seabed and promotes nutrient turnover. Beyond their ecological significance, sea cucumbers have considerable economic value (Toral-Granda et al., 2008; Purcell, 2015). They are a highly prized food delicacy and recognised for their purported health benefits in traditional medicine, particularly in Asian countries. This has led to overharvesting and concerns for the sustainability of wild populations have driven moratoria on sea cucumber fishing and an increase in aquaculture efforts (Rakaj et al., 2019).

While hatchery production of sea cucumbers is relatively new to the aquaculture industry in the Mediterranean region, this has been increasingly developing to meet the ever-growing demands of the Asian markets for this delicacy as bêche-de-mer. In the Mediterranean region, active fisheries exist for commercially important sea cucumber species with

Holothuria poli and *Holothuria tubulosa* among the increasingly popular target species (González-Wangüemert et al., 2018) and aquaculture candidates (Rakaj et al., 2019). Mediterranean countries have increasingly exploited these holothurians (González-Wangüemert et al., 2014, 2018). There is little data available in literature about the growth performance of these species within their natural environment and their nutrient and organic matter uptake capacities; however, holothurians like *H. poli* can effectively select and assimilate organic-rich particles (Mezali and Soualili, 2013; Belbachir et al., 2014) from the organically poor sediments they inhabit. This presents potential opportunities for these sea cucumbers to extract organic matter from aquaculture-derived wastes near fish cages. Moreover, the artificial breeding and juvenile production in hatchery cultures of *H. poli* not only presents a potential strategic response to unregulated harvesting in this region, but the availability of hatchery-produced sea cucumber juveniles supports the development of fish-sea cucumber IMTA in the Mediterranean. While the potential of *H. tubulosa* to use organic matter in fish farm biodeposits near fish cages has been subject of pilot research (Tolon et al., 2017a, Neofitou, 2019), studies on the potential of *H. poli* to reduce and grow organic matter in fish farm wastes near fish cages are lacking.

Sea cucumbers show promise for waste bioremediation in aquaculture, with experimental land-based systems confirming their capacity to filter and process organic matter in wastes (Nelson et al., 2012; MacDonald et al., 2013). Pilot studies further substantiate the potential of sea cucumbers to survive and grow in open-water IMTA setups (Nelson et al., 2012; Yu et al., 2012; Hannah et al., 2013; Yokoyama, 2013; Yu et al., 2014a, b). Although attractive and widely piloted, there are additional considerations for fish-sea cucumber IMTA to transition from pilot to commercial systems (Zamora et al., 2018). Scaling IMTA to commercial production is still limited by knowledge gaps that are associated with practicalities, operational challenges and technological limitations that exist at this level of production (Troell et al., 2009; Alexander et al., 2015; Kleitou et al., 2018). For instance, the ability of sea cucumbers to assimilate and grow on organic matter from aquaculture-derived wastes under commercial fish cages has yet to be validated. In an urban port area where environmental and anthropogenic processes can obscure the distinction between organic sources of dietary contributions for sea cucumbers in IMTA, it is important to provide substantial evidence to validate the capacity of sea cucumbers to uptake aquaculture-derived organic matter in waste.

In an open-water environment, conditions are more variable with less control over the availability and quality of uneaten food and faeces that deposit to the seafloor throughout the production of fish under commercial setups. This can affect the transfer of organic

material in fish-sea cucumber IMTA and influence the ecological and economic performance of sea cucumbers that have been presented in previous land-based studies. Therefore, inconsistent survival and growth performances of sea cucumbers in open-water IMTA research are not surprising (Yokoyama, 2013; Yu et al., 2014a, b; Günay et al., 2015; Tolon et al., 2017b; Neofitou et al., 2019; Grosso et al., 2021). This highlights the need to understand the real-world implications of commercial open-water aquaculture conditions for IMTA development. Understanding the natural and anthropogenic dynamics that surround open-water IMTA is essential for evidence-based planning and management of fish-sea cucumber IMTA.

Despite the wealth of data, commercial open-water IMTA systems are still underdeveloped. The complexities of coastal and open-water environments pose challenges for IMTA (Falconer et al., 2023) and there are clear uncertainties about the feasibility and effectiveness of open-water IMTA (Reid et al., 2020; Falconer et al., 2023). This requires research to build on lessons learnt from land-based studies and small-scale experiments to investigate the practical application of commercial-scale IMTA. Site-specific environmental conditions and farm-level processes can influence the performance of open-water IMTA (Falconer et al., 2023) and need to be assessed to inform the transition from experimental systems to more real complex scenarios.

1.3 Practical considerations for IMTA development in multiple-use environments

1.3.1 Natural and anthropogenic dynamics

In the Mediterranean (Magill et al., 2006), in Asia (Ferreira et al., 2008), and elsewhere in the world, intensive aquaculture is complicated by natural and anthropogenic dynamics. Existing fish farm facilities in complex multiple-use areas like ports and harbour areas are subject to additional pressures from anthropogenic activities (Soomere, 2007; Rapaglia et al., 2011; Parnell et al., 2016; Scarpa et al., 2019; Mao et al., 2020). These can affect water movement near fish cages (Grifoll et al., 2009; De Marchis et al., 2014; Grifoll et al., 2014; Ward et al., 2023) and while the hydrodynamic effects on aquaculture have been well documented, the interactions between these activities and water movement near fish cages need further consideration (Grifoll et al., 2009; De Marchis et al., 2014; Grifoll et al., 2014; Ward et al., 2023). Unsurprisingly, water movement is an important element of decision-making in aquaculture planning, licensing and regulation (Montaño-Ley et al., 2007).

Environmental regulation relies on decision-support tools to provide representative impact assessments for production to be within the ecological carrying capacity of the system (Ferreira et al., 2012; Ross et al., 2013). However, multiple-use environments are complex areas where site-specific natural and anthropogenic complexities around existing aquaculture facilities may not be truly represented by short-term hydrography and simplified scenarios used in planning and licensing processes (Grifoll et al., 2009; De Marchis et al., 2014; Grifoll et al., 2014). If so, it is necessary to understand the natural and anthropogenic interactions with water movement near fish cages for decision-support tools to represent the distribution of wastes so benthic impacts can be managed effectively through IMTA.

The development of IMTA in multiple-use areas requires additional consideration for bay-level processes that can influence the performance of extractive species in IMTA. For instance, terrestrial effluents and anthropogenic activities in urban ports (Andral et al., 2004; Lafabrie et al., 2008; Benali et al., 2015) can be sources of contaminants that can influence the behaviour and physiology of extractive species in IMTA. In these industrial environments, there can be a myriad of anthropogenic inputs and contaminants that can affect fish behaviour and health (Gravato and Guilhermino, 2009; Almeida et al., 2010; Guardiola et al., 2012; Sfakianakis et al., 2015). These can influence key ecological services provided by extractive organisms in IMTA that have yet to be understood. There is limited information about the effects of sedimentary contaminants on the performance of sea cucumbers despite their affinity for metal bioaccumulation (Aydın et al., 2017; Parra-Luna et al., 2020; Montero et al., 2021). In areas where coastal and maritime activities are prominent and anthropogenic inputs and pollutants are expected (Andral et al. 2004; Benali et al. 2015; Lafabrie et al. 2008), assessing the bioavailability and bioaccumulation of metals associated with these activities (Sutherland et al., 2007; Basaran et al., 2010) can inform the viability of placing sea cucumbers under commercial fish cages. The development of commercial-scale IMTA may need the full complexity of bay dynamics to be represented where this may be subject to anthropogenic activities.

1.3.2 Farm-level complexities

In heavily contested multiple-use environments, the performance of fish-sea cucumber IMTA can also be complicated by complexities of real-world cage production. The challenges for IMTA development can be exacerbated by farm-level practices where cage production involves multiple species from different cohorts and complex cage arrangements. In different regions of the world, marine fish farms are not consistent in terms of size, intensity, cultured species and combinations of, cage arrangements and husbandry

and management practices (Magill et al., 2006; Ferreira et al., 2008). These farm-level practices pose a challenge in determining the optimal placement of sea cucumbers within IMTA setups. In addition, these practices present complications for models that are widely used to support planning and licensing in aquaculture by simulating the distribution of wastes around fish cages (Cromey et al., 2002; Corner et al., 2006) and predicting benthic impact in aquaculture (Cromey et al., 2002; Stigebrandt et al., 2004; Chamberlain and Stucchi, 2007; Jusup et al., 2009; Cromey et al., 2012). Mathematical models have been used in fish-sea cucumber integrated production scenarios to examine the uptake of nutrients and organic material by extractive organisms (Ren et al., 2012; Watanabe et al., 2015; Zhang and Kitazawa, 2016; Chary et al. 2020). Model simulations of waste dispersion can be used to inform the placement of sea cucumbers in open-water IMTA; however, studies have rarely relied on waste dispersion models to plan and manage open-water integrated systems (Baltadakis et al., 2020; Telfer et al., 2022). Effective mitigation of wastes depends on suitable siting of extractive species relative to the distribution of waste streams to allow synchronous waste deposition and recycling (Reid et al. 2020; Chary et al., 2019). Farm-scale models can provide estimates of particulate waste deposition and show promise as decision-support tools to help set up IMTA system, hence the need to simulate waste dispersion for different species combinations and fish farming conditions. Where aquaculture is complicated by farm-level practices, there could be ramifications for waste deposition and food transfer in IMTA that can disrupt the balance between the waste discharge and uptake in fish-sea cucumber integrated system. It is necessary to address knowledge gaps regarding the effects of cage management practices on waste deposition around fish cages where aquaculture is complicated by farm-level processes if waste dispersion simulations to have further scope in setting up IMTA systems in complex environments.

The aim of this thesis is to assess real-world complexities of nearshore aquaculture in crowded coastal environments and evaluate the ability of a fish-sea cucumber IMTA system to extract organic resources in aquaculture-derived wastes and make better use of space under commercial conditions in these areas. This research explores real-world aquaculture practices where fish farming takes place in a multiple-use coastal environment that is limited in space and complicated by anthropogenic activities near the fish farm. It considers challenges that have still to be addressed for the development of IMTA under complex commercial conditions in these environments. These considerations include an understanding of complications for waste distribution near fish cages, the applicability of farm-scale models for appropriate placement of sea cucumbers in open-water IMTA, and

the ability of sea cucumbers to take up and grow on organic matter in aquaculture-derived wastes near fish cages in a fish-sea cucumber integrated system.

The primary objectives of this study involve:

1. Investigating the relationship between wind forcing and water currents and identify potential correlations between ship traffic and water movement near the fish farm. This study assesses some of the complexities associated with anthropogenic activities that can add to natural forcing to influence water movement through inshore fish farms where these exist in busy multiple use bays and port areas.

2. Describing complexities of real-world cage management practices at the farm level and their influence on waste deposition. To achieve this, a flexible farm-scale model is adapted to incorporate detailed input data to predict the distribution of sediment carbon around the fish cages at the fish farm where multiple species from different cohorts were farmed at the same time.

3. Establishing an open-water integrated system and assess the potential viability of *Holothuria poli*, a Mediterranean sea cucumber species, within a fish-sea cucumber IMTA context. This is based on farm-scale predictions of waste deposition to inform the proper setting up of a fish-sea cucumber IMTA system under complex farming conditions.

4. Evaluating trophic connectivity within an open-water fish-sea cucumber IMTA system through a dual tracer method that combines stable isotope techniques and fatty acid profiling. This research contributes insight into biological aspects of fish-sea cucumber IMTA and operational and environmental challenges inherent in open-water IMTA under commercial conditions, factors that currently constrain the broader adoption of this strategy, especially at lower latitudes in Europe where this approach remains underdeveloped.

5. Furthermore, this thesis evaluates the bioaccumulation of metal contaminants in sea cucumbers cultured near fish cages and assesses their potential implications for the extractive role played by sea cucumbers in open-water IMTA.

   This thesis elucidates important conclusions about the complexities of open-water IMTA under commercial conditions and the implications for scalability of fish-sea cucumber integrated systems in these environments.

Chapter 2. Essential environmental data for sustainable aquaculture in crowded coastal areas: insights from Marsaxlokk Bay, Malta

2.1 Introduction

For decades, sheltered inshore areas have provided suitable environmental conditions for marine aquaculture. However, lack of space and competition from other coastal activities challenge the development of marine aquaculture (Sanchez-Jerez et al., 2016; Cavallo et al., 2020; Galparsoro et al., 2020) especially where it exists in multiple-use coastal zones. Knowledge gaps about the effects of coastal anthropogenic activities on aquaculture in heavily contested zones still exist, creating challenges for aquaculture planning and licensing (Falconer et al., 2023). For aquaculture to grow in these coastal areas, there needs to be a better understanding of the user-environment interactions and the implications of anthropogenic influences for aquaculture co-existing in the same space. Otherwise, uncertainties will continue to be barriers that hinder effective marine spatial planning and development, as has been the case in Malta (Deidun et al., 2011).

With limited coastal space for expansion, Malta echoes concerns that competition for space is a serious limitation for marine aquaculture (Sanchez-Jerez et al., 2016; Cavallo et al., 2020; Galparsoro et al., 2020). However, as the global human population grows to a projected 9.7 billion in 2050 (United Nations Department of Economic and Social Affairs, 2022), marine aquaculture will inevitably need to expand, as will other maritime activities, to continue to secure the provision of goods and services and to support livelihoods. Port areas are archetypal of multiple user coastal environments where marine fish and shellfish aquaculture, berthing, cargo transhipment, bunkering operations, and other maritime activities, co-exist in the different parts of the world. Across the Mediterranean, fish cages have been sited near other maritime industries in urban port areas (e.g., Yucel-Gier et al., 2013; Israel et al., 2019), but at times these situations have led to conflicts (Cavallo et al., 2020). In these marine environments, elevated levels of nutrients and contaminants have been attributed to anthropogenic sources ubiquitous in these coastal areas (Andral et al. 2004; Sutherland et al., 2007; Lafabrie et al. 2008; Basaran et al., 2010; Benali et al. 2015). For instance, the accumulation of heavy metals in sediments has been ascribed to the extensive use of antifouling in ships and fishing vessels in the maritime industry (Sutherland et al., 2007; Basaran et al., 2010) and constituents in fish feed and faeces in aquaculture (Kalantzi et al., 2013). Meanwhile, fuel oil leaks and discharges from shipping activities can introduce a range of pollutants, including heavy metals, to the water and sediment environment (Trottet et al., 2021). Rural and urban sources can release nutrients, organic matter and chemicals into the sea while activities like marine traffic and dredging can mobilise substances (e.g. nutrients and suspended solids and heavy metals) that deposit and accumulate in sediments. These activities can alter water quality by increasing turbidity,

producing biological oxygen demand, reducing oxygen levels, and potentially increasing stress in fish and susceptibility to diseases. Exposure to pollutants from these anthropogenic sources can alter the behaviour (e.g. swimming and feeding) and physiology (e.g. blood composition and immune functions) of fish (Gravato and Guilhermino, 2009; Almeida et al., 2010; Guardiola et al., 2012; Sfakianakis et al., 2015; Lester et al., 2018; Brooks and Conkle, 2019). These stressors can reduce growth rates and disrupt production trends in aquaculture and the bioaccumulation of these pollutants in seafood can have health implications for consumers.

Where aquaculture exists in heavily contested coastal areas and competes with existing industries, coastal management and expansion of aquaculture is complicated by the multitude of anthropogenic processes and the interactions these have with the marine environment where they occur. These activities can have different effects on the quality of water and sediment and consequently, data becomes indispensable for the sustainable management of this area. For instance, adequate water and sediment quality are important criteria in aquaculture planning and development (Pérez et al., 2003; Karakassis et al., 2005; Pérez et al., 2005; Ross et al., 2009; Borg et al., 2011; Ross et al., 2013; Price et al., 2015) and therefore, the availability of environmental data that represents the complex dynamics of crowded coastal spaces is a pressing requirement for aquaculture in these areas (Pérez et al., 2005; Falconer et al., 2013, 2020; Stelzenmüller et al., 2017). The complex processes and interactions that exist in multi-use coastal areas require profound comprehension, however gaps in environmental data and the conspicuous absence of information still exist. Bridging these data deficits becomes imperative if we are to untangle the intricate dynamics of multi-use spaces for long-term comprehensive and integrated approaches to sustainable management and development.

This chapter provides an overview of the diverse range of human activities taking place within an urban port area situated at the heart of the Mediterranean where marine aquaculture has existed for over 30 years. These activities have the potential to exert significant influence on the quality of water and sediment, not only in the immediate vicinity but also over varying spatial and temporal scales. Then, the study underscores the crucial importance of identifying the data required to understand the potential effects of these activities and characterise the site. To achieve this, it emphasizes the necessity of collecting and monitoring specific types of environmental data. This data is instrumental in assessing the implications that increased coastal activities may have on the marine aquaculture operations within the bay.

2.2 Study site

Marsaxlokk Bay, at 35°49'32.23" N in longitude and 14°32'35.46" E in latitude, is located in the southeast of the Maltese Islands, at the centre of the Mediterranean (Fig. 2.1 A). The bay indents the east coast of Malta between Delimara Point, and Bengħajsa Point that is about 1.6 km southwest (Fig. 2.1 B). Marsaxlokk bay is partly sheltered by a breakwater at the mouth of the bay with an additional stretch of 850 m to Delimara Point. The coastline of Marsaxlokk Bay extends through Marsaxlokk, a traditional fishing village at the head of the bay, and the town of Birzebbugia on the western side. The coastline is around 15 km long and the bay is approximately 3.78 km^2.

The bay shows spatial variation in grain size with heterogeneous soft sediments (very fine to medium grain size) and muddy sediments at the centre of the bay, near the navigation channels (Adi Associates Environmental Consultancy Ltd., 2007). The benthic environment throughout the bay is characterised by biocoenosis of infralittoral algae, biocoenosis of infralittoral stones and pebbles, biocoenosis of superficially muddy sands in sheltered waters, and biocoenosis of *Posidonia oceanica* meadows.

At the centre of the bay, an open-water fish farm is below a headland at the north of the bay and lies 130 m northwest of navigation channels close to transhipment terminals that are just over 500 m to the south (Fig. 2.1 B). The fish farm is a nursery and juvenile facility for grow-out production that has been in operation since the early 1990s and is run by Malta Fish Farming Ltd. In 2019, the fish farm produced 719 t of gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) in 19 fish cages (Fig. 2.1 B). At the start of this study in 2018, one fish cage was used to hold greater amberjack (*Seriola dumerili*). The aquaculture site is in shallow waters between 8 m and 12 m and surrounded by a patchy distribution of *P. oceanica*.

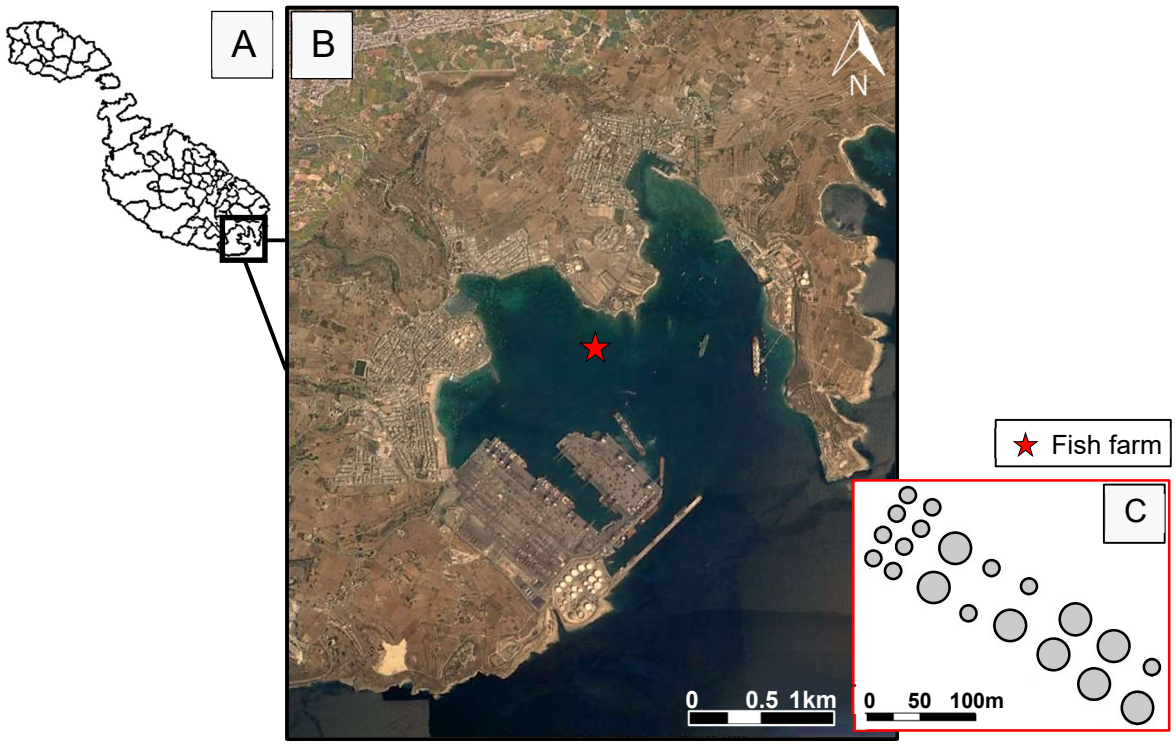


Figure 2.1. A. Location of study site, Marsaxlokk Bay in the southeast of Malta shown in B. as a zoomed Google Earth image. C. Arrangement of fish cages at the existing aquaculture facility at the site at the time of the experiment.

Surrounding the fish farm, the bay hosts an array of land-based and sea-based anthropogenic activities and is archetypal of a multiple user coastal environment to make it one of the busiest coastal locations of the island. The bay serves as a port for international traffic and as the main fishing port of the islands. As a major container terminal in the Mediterranean, the port accommodates maritime activities in this bay associated with this major port in the Mediterranean, including towage, pilotage, and cargo transhipment. The bay hosts industrial fuel storage facilities, an electricity generation plant, a commercial marine fish farm, a land-based aquaculture facility, and a residential and urban coastline. In addition, berthing facilities inside the bay accommodate bunkering activities and fishing vessels that comprise 70% of the Maltese fishing fleet. These land and sea-based activities characterise Marsaxlokk Bay and can be sources of point and non-point discharges into these coastal waters that can affect the environment and users that co-exist in the bay.

Wind data for a 44-year sequence reveals strong and frequent winds that prevail from the north-west and with increasing frequencies of north easterly winds during autumn and south-easterly wind components during spring (AIS Environment, 2016). Data showed dominant wind direction between 285° (WNW) and 315° (NW), and wind speeds greater than 5.7 m s^{-1} for 35.3% of the time and that rarely exceed 17.5 m s^{-1} (Adi Associates

Environmental Consultants Ltd., 2007). This 44-year sequence revealed that the wave climate in Marsaxlokk Bay is dominated by wind-generated waves between 30° N and 210° N rather than being related to tides (Svašek Hydraulics, 2007). Data for surface currents inside the bay is not available although these are reportedly never expected to become larger than 0.4 m s^{-1} since wind speed should not exceed 15 m s^{-1} (Svašek Hydraulics, 2007). Data on precipitation was not available, however episodes of heavy rainfall are expected to cause the accumulation of rainwater in valleys that empty into different parts of Marsaxlokk Bay via two run-off routes and four major valleys (Paris, 2010). This could explain observations of elevated nutrients, organic matter and contaminants from point source discharges or run-off from agricultural activity or other anthropogenic land uses.

2.3 Environmental data

The spatial identification and mapping of coastal activities were established through an extensive review and compilation of records of anthropogenic uses in the bay that were derived from data sources used for environmental data in this study. These were substantiated by land surveys of human activities along the coastline of Marsaxlokk Bay conducted between January and February 2023. The assessment of water and sediment conditions within the bay was informed by environmental data sourced from both unpublished and published research that was originally collected during prior monitoring surveys and impact assessments conducted within the study area.

The physical and chemical status of the water column was based on measurements of nutrients (mg L^{-1}) that include concentrations of total nitrogen (TN), total nitrates, phosphorus (TP), total phosphates, total chlorophyll *a* (µg L^{-1}) and total suspended solids (TSS, mg L^{-1}). In this analysis, measurements obtained from sub-surface water depths of 1 to 5 m were considered primarily, as detailed in Table 2.1. Water quality data was extracted from dataset sources available for different parts of the bay between 2008 and 2021 (Fig. 2.2 – 2.7). Data was normalised to a uniform unit of measurement.

Table 2.1. Sources of data for water quality parameters between 2008 and 2021 in Marsaxlokk Bay.

Parameter	Description	Methodology	Detection limit	Sampling period		Reference
Nitrate	*In situ* measurements from local unpublished study	Spectrophotometric determination according to Parsons et al. (1984)	0.01 µg L^{-1}	November 2008	May 2009	Paris, 2009
Phosphate			0.005 µg L^{-1}			
Chlorophyll a		Submersible spectrofluorimeter	n.d.			
Nitrate	*In situ* measurements from local unpublished study	Spectrophotometric determination according to Strickland and Parsons (1972)	0.05 µmol L^{-1}	December 2009	October 2010	Pisani, 2011
Phosphate			0.03 µmol L^{-1}			
Total Phosphorus			0.15 µmol L^{-1}			
Chlorophyll a		Submersible spectrofluorimeter	n.d.			
Nitrate	Average data (*n* = 8) from the Water Framework Directive (WFD) monitoring surveillance of coastal waters	Ion chromatography, standard method EN ISO 10304-1:2009	0.03 mg L^{-1}	June 2012	March 2013	MEPA, 2013
Nitrite			0.01 mg L^{-1}			
Phosphate			0.1 mg L^{-1}			
Total Nitrogen		Spectrophotometric determination APAT CNR-IRSA 4060 Man 29 2003	0.06 mg L^{-1}			
Total Phosphorus		Inductively coupled plasma atomic emission spectroscopy according to method 200.7 (USEPA, 1994)	0.05 mg L^{-1}			
Nitrate	Average data (n = 2) from environmental impact assessment	Spectrophotometric determination APAT CNR-IRSA 4060 Man 29 2003	0.01 mg L^{-1}	June 2013		Axiak, 2013
Phosphate			0.01 mg L^{-1}			
Total suspended solids		Gravimetric determination APAT CNR-IRSA 2090 B Man 29 2003	n.d.			
Chlorophyll *a*		Spectrophotometry according to Strickland and Parsons (1972)	n.d.			
Nitrate	*In situ* measurements at 1 m depth as part of environmental impact assessment	Spectrophotometric determination using methods in APAT CNR-IRSA (2003)	5 mg L^{-1}	May 2015		AIS Environment, 2016
Nitrite			0.2 mg L^{-1}			
Phosphate			0.1 mg L^{-1}			
Nitrate	Average data (*n* = 7) on the assessment of status of Maltese waters as part of the EU Marine Strategy Framework Directive (MSFD)	Spectrophotometric determination using methods in APAT CNR-IRSA (2003)	0.14 µg L^{-1}	2017	2019	ERA, 2020
Phosphate			0.15 µg L^{-1}			
Total Nitrogen			1.4 µg L^{-1}			
Total Phosphorus			0.3 µg L^{-1}			
Chlorophyll a			n.d.			
Nitrate	Measurements for the Water Information System for Europe (WISE-SoE) for the coastal waters	Methods utilised in the WFD monitoring surveillance of coastal waters (MEPA, 2013)	0.14 µg L^{-1}	2017	2019	EEA, 2021
Phosphate			0.16 µg L^{-1}			
Total Nitrogen			1.40 g L^{-1}			
Total Phosphorus			0.31 µg L^{-1}			
Chlorophyll *a*		Submersible spectrofluorimeter	n.d.			
Total Nitrogen	Average data (*n* = 2) at 1 m water depth as part of environmental monitoring near the fish farm	Spectrophotometric determination using methods in APAT CNR-IRSA (2003)	1.4 µg L^{-1}	December 2018	July 2021	Ecoserv Ltd., 2018, 2019b, 2020a, b, 2021a, b
Total Phosphorus			0.3 µg L^{-1}			
Total suspended solids			n.d.			
Chlorophyll a			n.d.			

n.d. indicates that data was not retrieved

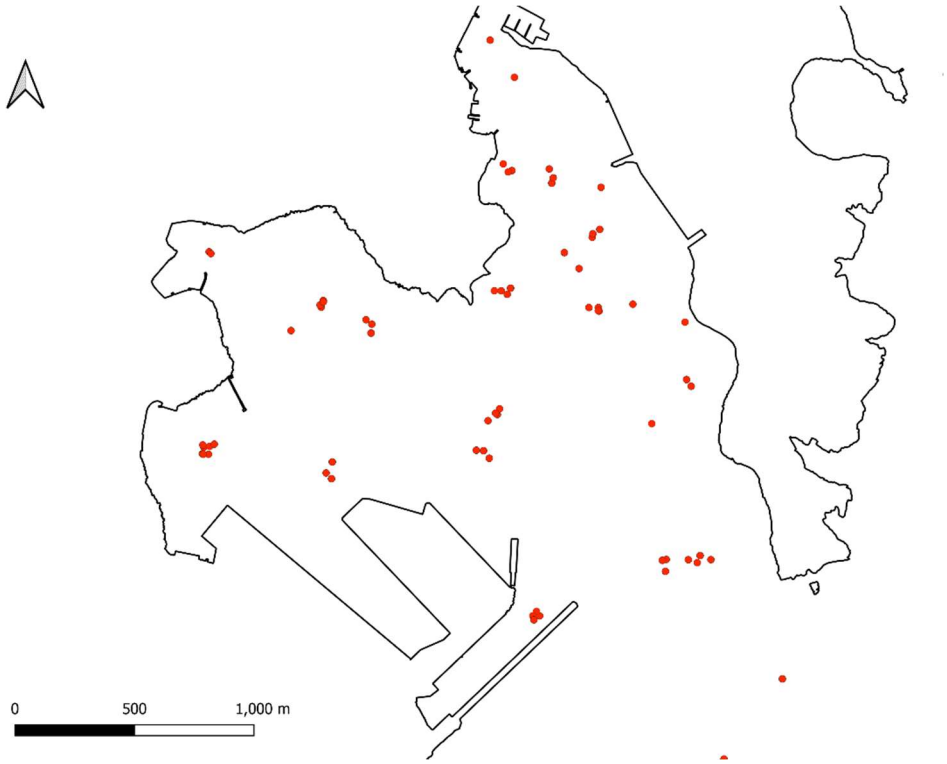


Figure 2.2. Positions of all sampling stations in Marsaxlokk Bay for the assessment of nitrates in water from dataset sources considered in this study. Available datasets for water quality parameters share some positions of sampling stations.

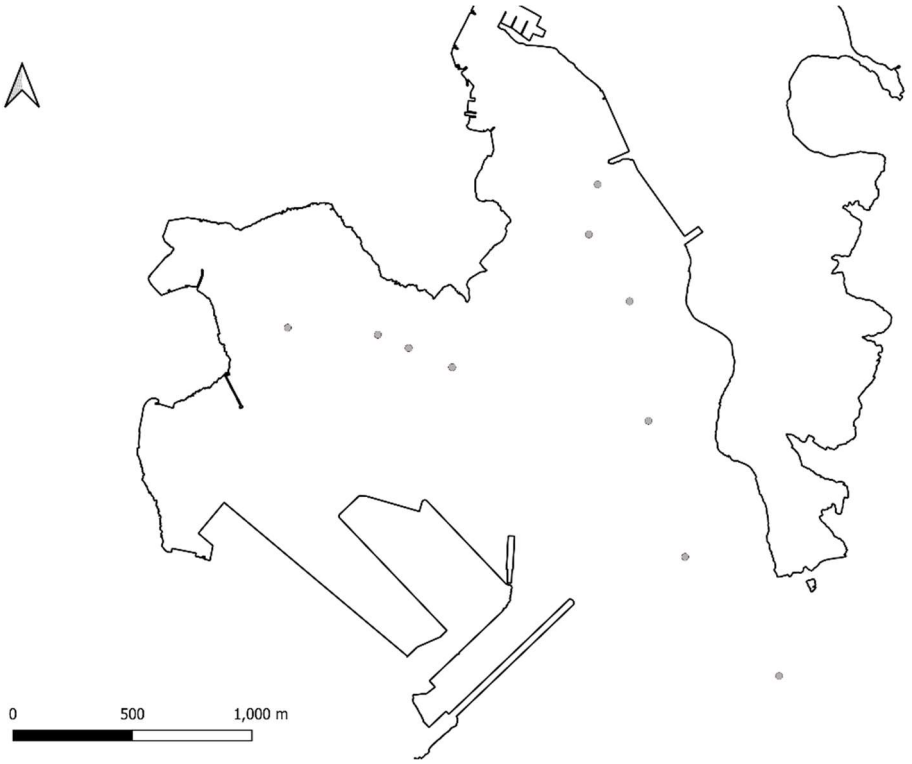


Figure 2.3. Positions of all sampling stations in Marsaxlokk Bay for the assessment of total nitrogen in water from dataset sources considered in this study. Available datasets for water quality parameters share some positions of sampling stations.

Figure 2.4. Positions of all sampling stations in Marsaxlokk Bay for the assessment of phosphates in water from dataset sources considered in this study. Available datasets for water quality parameters share some positions of sampling stations.

Figure 2.5. Positions of all sampling stations in Marsaxlokk Bay for the assessment of total phosphorus in water from dataset sources considered in this study. Available datasets for water quality parameters share some positions of sampling stations.

Figure 2.6. Positions of all sampling stations in Marsaxlokk Bay for the assessment of total suspended solids in water from dataset sources considered in this study. Available datasets for water quality parameters share some positions of sampling stations.

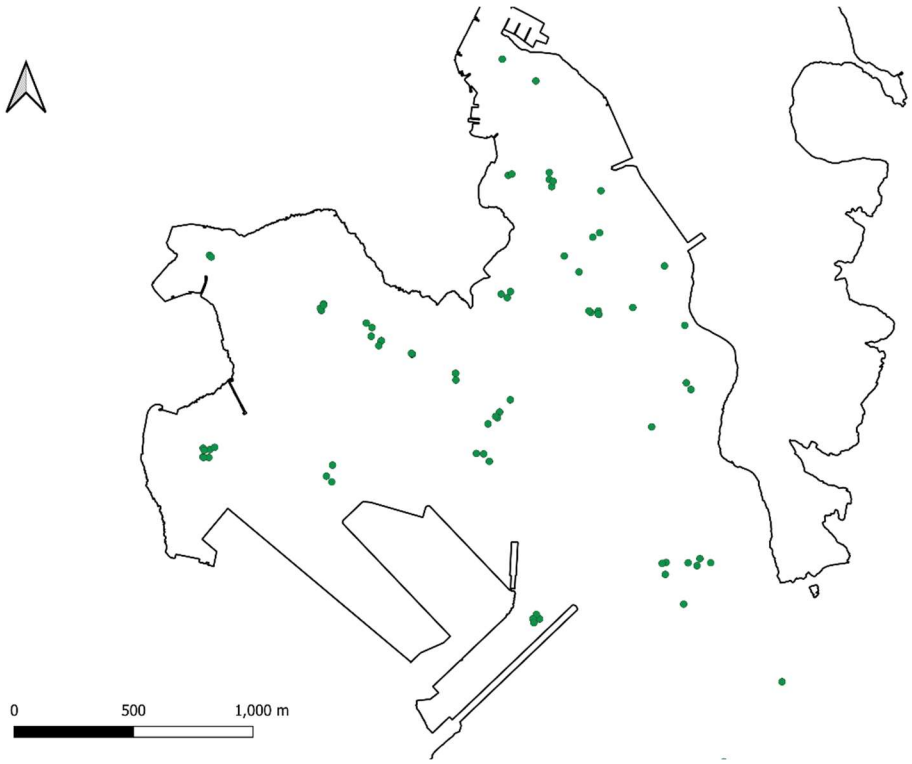


Figure 2.7. Positions of all sampling stations in Marsaxlokk Bay for the assessment of chlorophyll a in water from dataset sources considered in this study. Available datasets for water quality parameters share some positions of sampling stations.

Measurements of total organic carbon (%) in surface sediments were extracted from sources of data recorded in different parts of the bay between 2013 and 2019 (Table 2.2).

Table 2.2. Sources of data for total organic carbon measurements between 2008 and 2019 in Marsaxlokk Bay.

Description	Methodology	Sampling period	Reference
Average data (n = 2) from environmental impact assessment	Standard methodologies in APAT/IRSA-CNR (2003)	June 2013	Axiak, 2013
Average data (n = 2) from environmental impact assessment	Standard methodology UNI EN 13137:2002	October 2018	Adi Associates Environmental Consultants Ltd., 2018
Data collected as part of environmental monitoring near the fish farm	Standard methodology UNI EN 13137:2002	July 2019	Ecoserv Ltd., 2019a

Figure 2.8. Positions of all sampling stations in Marsaxlokk Bay for the assessment of total organic content in seafloor sediment from dataset sources considered in this study. Available datasets may share positions of sampling stations.

2.4 Site characterisation

Open-water aquaculture installations need suitable environmental conditions. However, anthropogenic activities in crowded coastal spaces can challenge this integral requirement to site selection processes in aquaculture (Ross et al., 2013). Therefore, optimising the use of space where marine aquaculture already exists in these coastal areas means that anthropogenic inputs and potential impacts on the environment and co-existing users need to be identified and represented. The variety and multitude of land and sea-based anthropogenic activities identified across different locations along the coastline of Marsaxlokk Bay (Fig. 2.9) reveal multiple sources of potential discharges and inputs into the marine environment. For aquaculture to grow within the carrying capacity of the system, understanding the anthropogenic dynamics and the impacts on the environment around marine aquaculture facilities can resolve challenges that may impede further expansion (Ross et al., 2013; Sanchez-Jerez et al., 2016; Cavallo et al., 2020; Galparsoro et al., 2020).

As part of the data analysis, human uses were classified according to the type of facility or activity carried out at the site and visualised in the geographic information system QGIS (v.3.30.0). Anthropogenic activities were interpreted in terms of the inputs and waste outputs associated with operation processes at the facilities and their potential contributions and impacts on the marine environment. Environmental data from each different time point and source were visualised as separate layers and interpreted for each parameter individually.

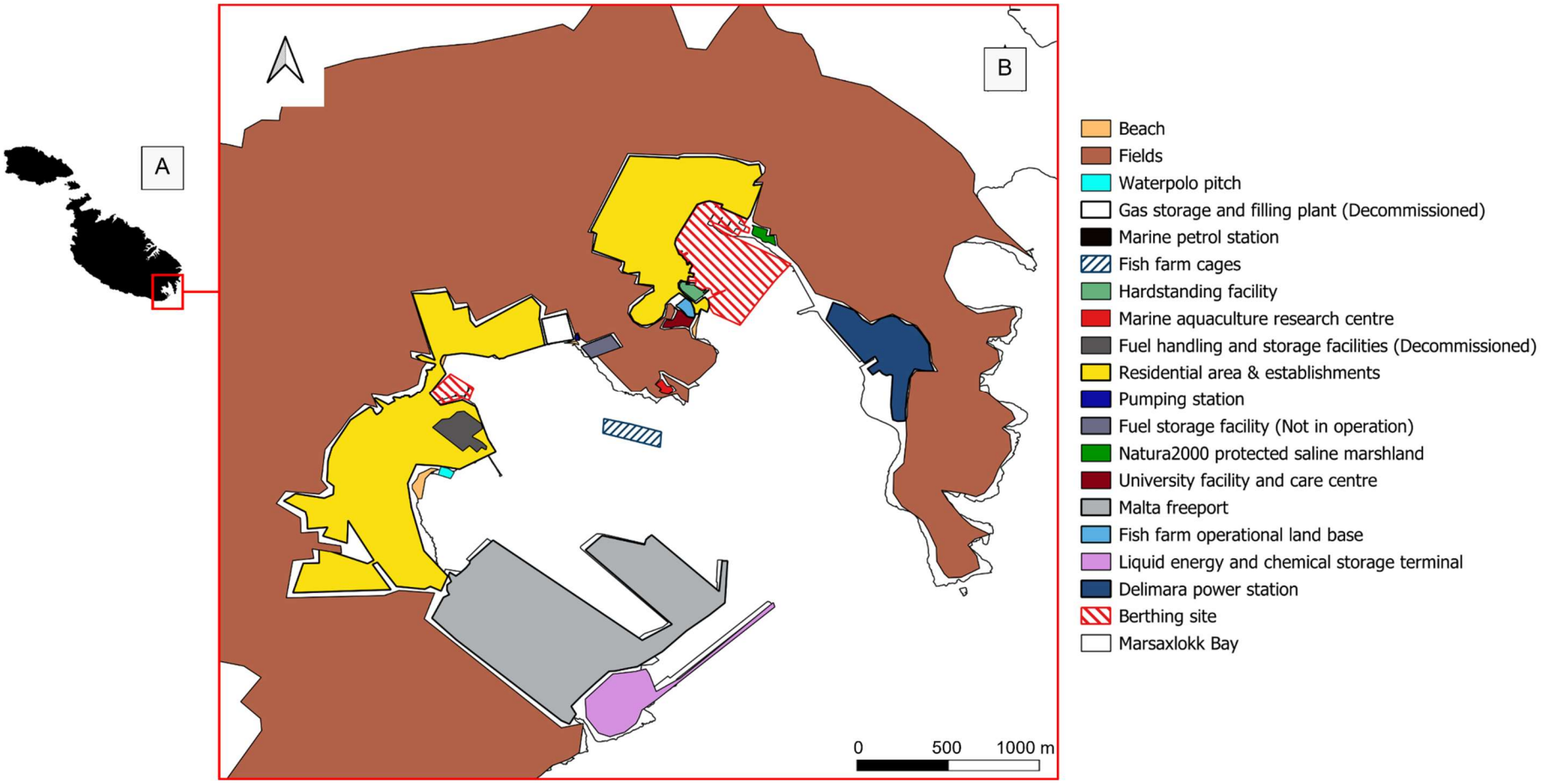


Figure 2.9. Map showing the area of study in the southeast of Malta with B. natural features and anthropogenic activities and facilities mapped for the coastal area of Marsaxlokk Bay.

2.4.1 Coastal uses

Historically, ports and shipping areas are considered more susceptible to the threat of pollution particularly associated with oil and fuel discharges (Andral et al. 2004; Benali et al. 2015; Lafabrie et al. 2008). Between 2012 and 2018, up to 25% of pollution events associated with the release of oil and hazardous and noxious substances in Malta occurred in Marsaxlokk Bay (ERA, 2020). This area accommodates a commercial fish farm facility, situated at the centre of the bay and lies a mere kilometre northwest from a liquid energy and chemical storage terminal that has a total capacity of 568,399 cubic meters (Malta Maritime Forum, 2022) for the storage of heavy fuels (e.g. marine diesel oil and bunker oil) and light petroleum products (e.g. gasoline and methyl tert-butyl ether). Additionally, the bay accommodated industrial fuel handling and storage facilities (e.g. petrol, unleaded, gasoline, kerosene, aviation fuel, and diesel oil) (Axiak, 2003; Paris, 2010) that were decommissioned by 2021. These facilities have been reported to release petroleum hydrocarbons into ground water (Axiak, 2003; Paris, 2010), and to sea through run-off and storm water drainage (Axiak and Delia, 2000; Paris, 2010). Within 450 m of the fish farm, the transhipment terminal at Malta Freeport Ltd. occupies 0.77 km^2 to serve container and cargo transhipment and storage. A hard standing facility of about 6000 m^2 elsewhere in the bay is another source of oils and lubricants (Pisani, 2011). Heavy fuel oils are sources of heavy metals that can contaminate waters and accumulate in sediments (Trottet et al., 2021). Given the broad environmental implications, any infrastructural growth within the port necessitates a thorough representation of potential inputs. This is particularly crucial in anticipation of possible intermittent anthropogenic leaks, which may go undetected during routine monitoring sessions.

As populations grow, coastal areas are inevitably increasingly urbanised. Sustainable aquaculture in these areas can have an important role in the provision of food where the ever-growing demand needs to be met. However, where densely populated, production can be limited by increased nutrient discharges and pollutants in the environment. As an urban port area, the large stretch of residential area and establishments that characterise the shoreline of the innermost parts of the bay attracts crowds that can be a source of organic waste discharges and effluents. Effluents from a land-based aquaculture facility on top of the headland at the centre of the bay can introduce chemicals (e.g. sodium hypochlorite, phenoxyethanol, and oxytetracyclin) and elevate nutrient concentrations where discharged. The entire periphery of the Marsaxlokk coastline boasts agricultural activity that is a source of point and non-point discharges (e.g. fertilizers and pesticides) (Axiak and Delia, 2000; Paris, 2010) which can expose marine organisms to toxic contaminants. Among these

contaminants, heavy metals can accumulate in sediment and other substrates, and bioconcentrate in the tissues of fish and other aquatic organisms (Bado-Nilles et al., 2009; Gravato and Guilhermino, 2009; Almeida et al., 2010; Danion et al., 2011; Guardiola et al., 2012; Sfakianakis et al., 2015; Lester et al., 2018; Brooks and Conkle, 2019).

In Marsaxlokk Bay, present day electricity generation plants on the eastern side of the bay run on a natural gas and gasoil-fired systems however, these facilities were operated using heavy fuel oils until 2017. This power station has been a source of several contaminated wastewater streams containing antifouling chlorine agent and metal compounds from cooling and demineralisation processes (Axiak, 2013). The upgraded facilities still release an estimated 18,200 m^3 annual discharge of wastewater streams, floor washings and rain run-off with possible traces of oils directly into Marsaxlokk Bay (Axiak, 2003; Paris, 2010). Sea-based activities associated with transhipment terminals and the multiple designated berthing sites in the innermost parts of the bay can release contaminants directly into seawater (Andral et al. 2004; Sutherland et al., 2007; Lafabrie et al. 2008; Basaran et al., 2010; Benali et al. 2015). Marine traffic, dredging operations and industrial processes (e.g. de-ballasting) in the bay (Paris, 2010) can mobilise sediments into the water column to release suspended solids, nutrients, metals, and organic contaminants that can deteriorate the local water quality conditions near marine aquaculture facilities. Considering the multitude and variety of anthropogenic activities in the bay, it is critical to understand the possible causes and effects of contamination in marine environments that host fish cage installations and to develop effective strategies to mitigate potential impacts on aquaculture production.

2.4.2 Water and sediment quality

The techniques for analysis applied in the dataset sources used to evaluate environmental parameters in this study varied. The datasets comprise measurements taken intermittently over a 13-year period and at different times of year. Detection limits for various parameters differ between datasets depending on the sensitivities of the analytical methods used in each dataset source. Measurements below these limits were still represented by markers at the georeferenced sampling positions as an indication of ‘below detection limit’. Long-term regular monitoring data is not available over the entire scale of the bay and this work attempts to consolidate information that is accessible while recognising the limitations of the data.

During the observation period, changes in nutrient levels were assessed as part of isolated investigations (Paris, 2009; Pisani, 2011), impact assessments (Axiak, 2013; AIS Environment, 2016) and environmental monitoring initiatives (MEPA, 2013; ERA, 2020; EEA, 2021). Impact assessments and monitoring programmes typically evaluated conditions within the specific area of interest or from a single monitoring station (e.g., EEA, 2021) at intermittent sampling intervals. Consistent monitoring is related to the assessment of water and sediment quality near fish cages from 2018 to 2021, that still represent the local conditions around the aquaculture installation rather than bay-wide characteristics (Ecoserv Ltd., 2018, 2019b, 2020a, b, 2021a, b). Nonetheless, nutrient and chlorophyll levels in Marsaxlokk Bay provide an indication of water quality near anthropogenic activities from the innermost inlets of the bay extending to the mouth of the bay at the time of sampling.

Nitrate concentrations were below 0.5 mg L^{-1} across different parts of the bay (Fig. 2.10 A). At different times of the year, nitrate levels were generally higher close to the headland at the centre of the bay and towards the fishing harbour in the innermost inlet at the north of the bay. The available datasets for TN measurements share some sampling stations, and typically show TN concentrations below 0.01 mg L^{-1}. However, data revealed a range of values between 2.18 mg L^{-1} and 2.71 mg L^{-1} in different parts of the bay (MEPA, 2013). These elevated concentrations indicate bay-level changes at the time of sampling or possibly different analytical sensitivities. Nitrate levels (Fig. 2.10 B, C) at various locations within the bay over a two-year period reveal temporal changes with increased concentrations at specific sampling periods and locations but not at others. Like TN concentrations, phosphate levels remained low and rarely exceeded detection limits of 0.005 mg L^{-1} (Fig. 2.11), while TP levels reached 0.02 mg L^{-1} in different parts of the bay (Fig. 2.12). Exceedingly low nutrient concentrations were not represented (e.g. EEA, 2021); still, distinctly elevated concentrations reveal differences in phosphorus levels across datasets that may suggest changing conditions within different parts of the bay (Fig. 2.11 B, C; Fig. 2.12 B, C). These warrant further consideration for changes in anthropogenic inputs over a longer timescale.

Chlorophyll levels ranged between 0.1 µg L^{1} and 0.4 µg L^{1} within 5 m waters in the bay however, surveys closer to the fish cages revealed a peak of 1.54 µg L^{1} in surface waters (Fig. 2.13). Similar to nutrient levels, chlorophyll remained within the range of concentrations recorded in other oligotrophic areas in the Mediterranean that accommodate anthropogenic activities (Pitta et al. 1999; Puigserver et al., 2002; Kontas et al. 2004; Yucel-Gier et al. 2007; Neofitou and Klaoudatos, 2008; Kaymaz and Özdemir, 2019; Kucuksezgin et al.,

2021; Morsy et al., 2022). Considering the diverse range of activities occurring in different parts of the bay and the observed variability in chlorophyll levels during similar periods across different datasets (Fig. 2.13 B - E), routine monitoring efforts, like that of EEA (2021), and that can truly represent chlorophyll conditions across the bay continue to be encouraged.

TSS levels inside port and harbour areas, like Marsaxlokk Bay, are expected to vary as a consequence of anthropogenic activities, such as marine traffic and dredging. TSS measurements remained below 1.6 mg L^{-1} inside the bay (Fig. 2.14 A), and never beyond maximum values reported for other port and harbour areas in the Mediterranean (Aydin-Onen et al., 2012; Kucuksezgin et al., 2021). Maximum concentrations of suspended solids near fish cages in Marsaxlokk Bay were lower than values reported in proximity of intensive aquaculture facilities that have continued to persevere in multiple-use areas in the Mediterranean (Kucuksezgin et al., 2021). TSS levels were not conducive to poor water conditions at the time of sampling; however, the propensity for water quality to vary with changes in port activities, demands more frequent monitoring beyond existing efforts (Fig. 2.14 B).

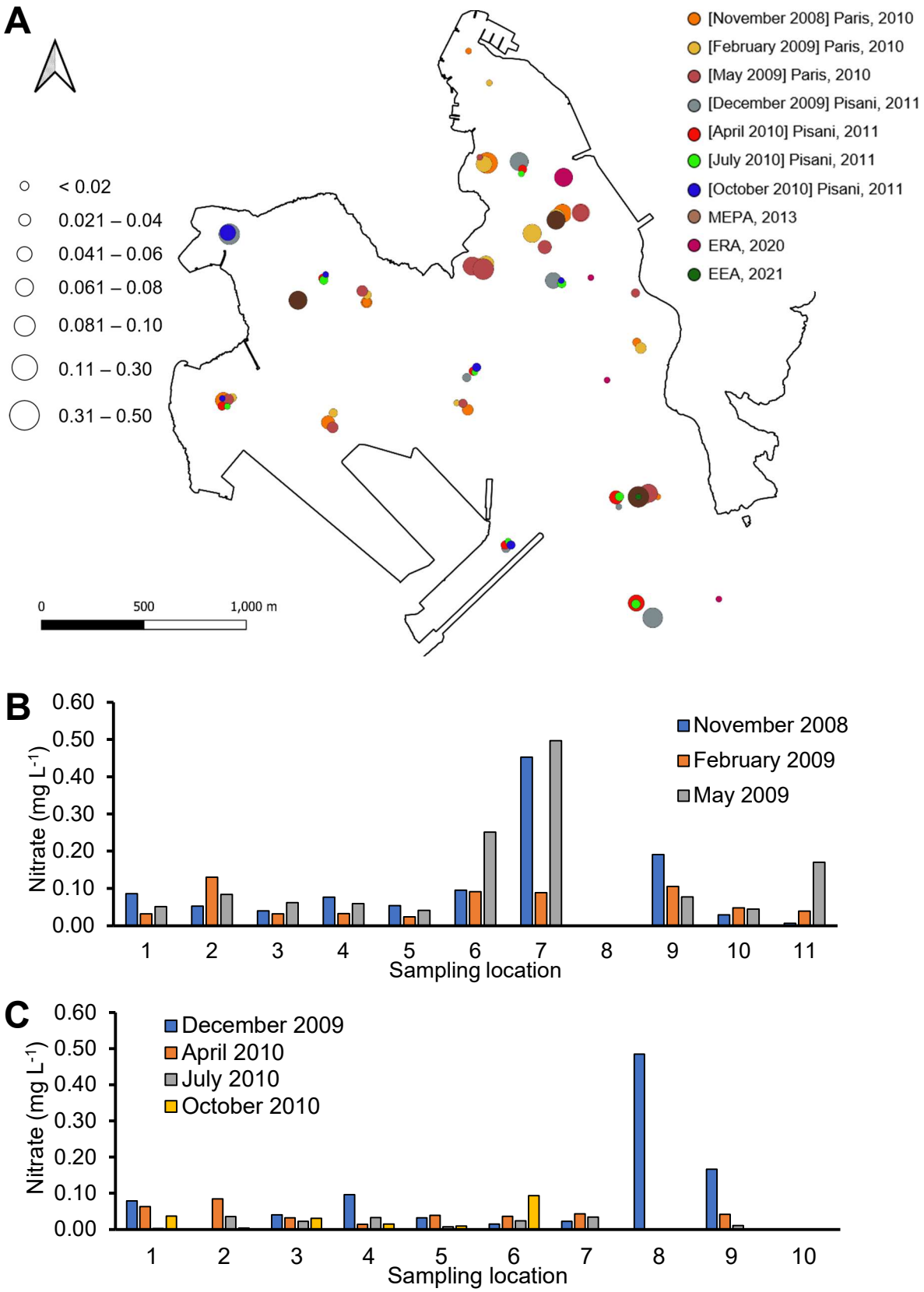


Figure 2.10. A. Concentrations of nitrates in sub-surface waters in Marsaxlokk Bay mapped in GIS from various datasets between 2008 and 2021. Nitrate levels across the bay at different sampling periods in B. Paris (2009) and C. Pisani (2011). Missing data indicates concentrations below detection limits.

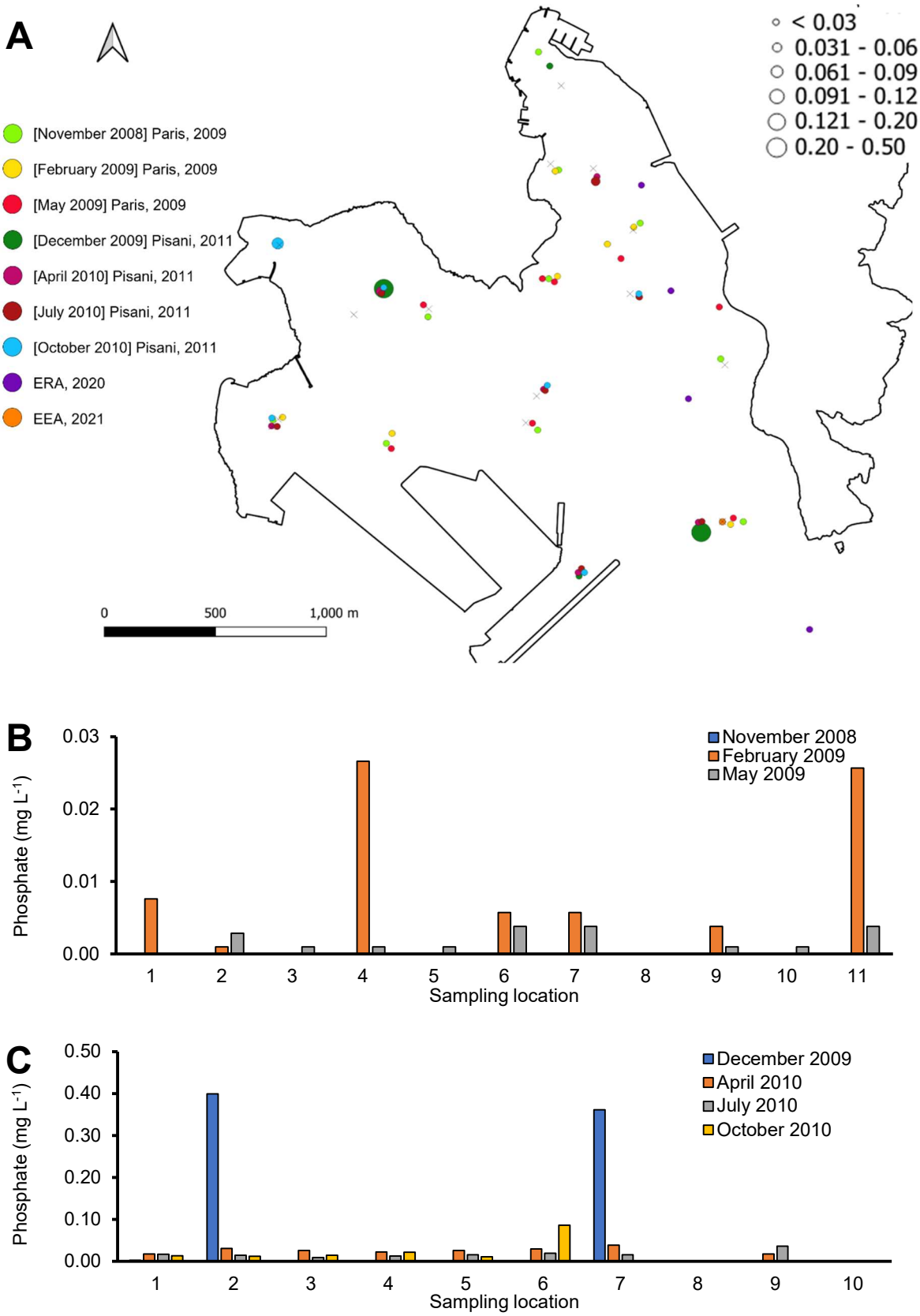


Figure 2.11. A. Concentrations of phosphates in sub-surface waters in Marsaxlokk Bay mapped in GIS from various datasets between 2008 and 2021. Nutrient levels that fell below detection limits were indicated by a cross marker. Phosphate levels across the bay at different sampling periods in B. Paris (2009) and C. Pisani (2011). Missing data indicates concentrations below detection limits.

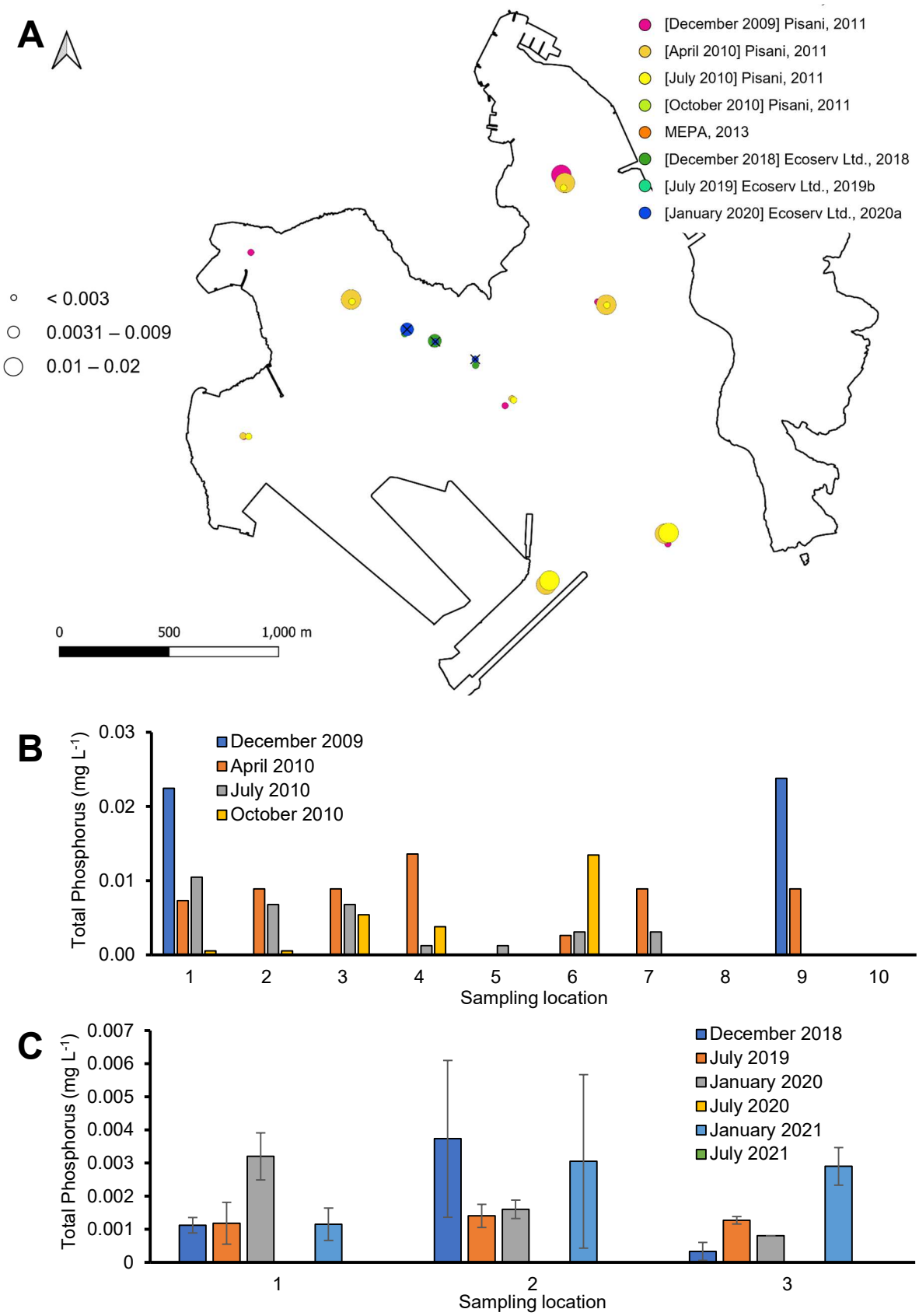
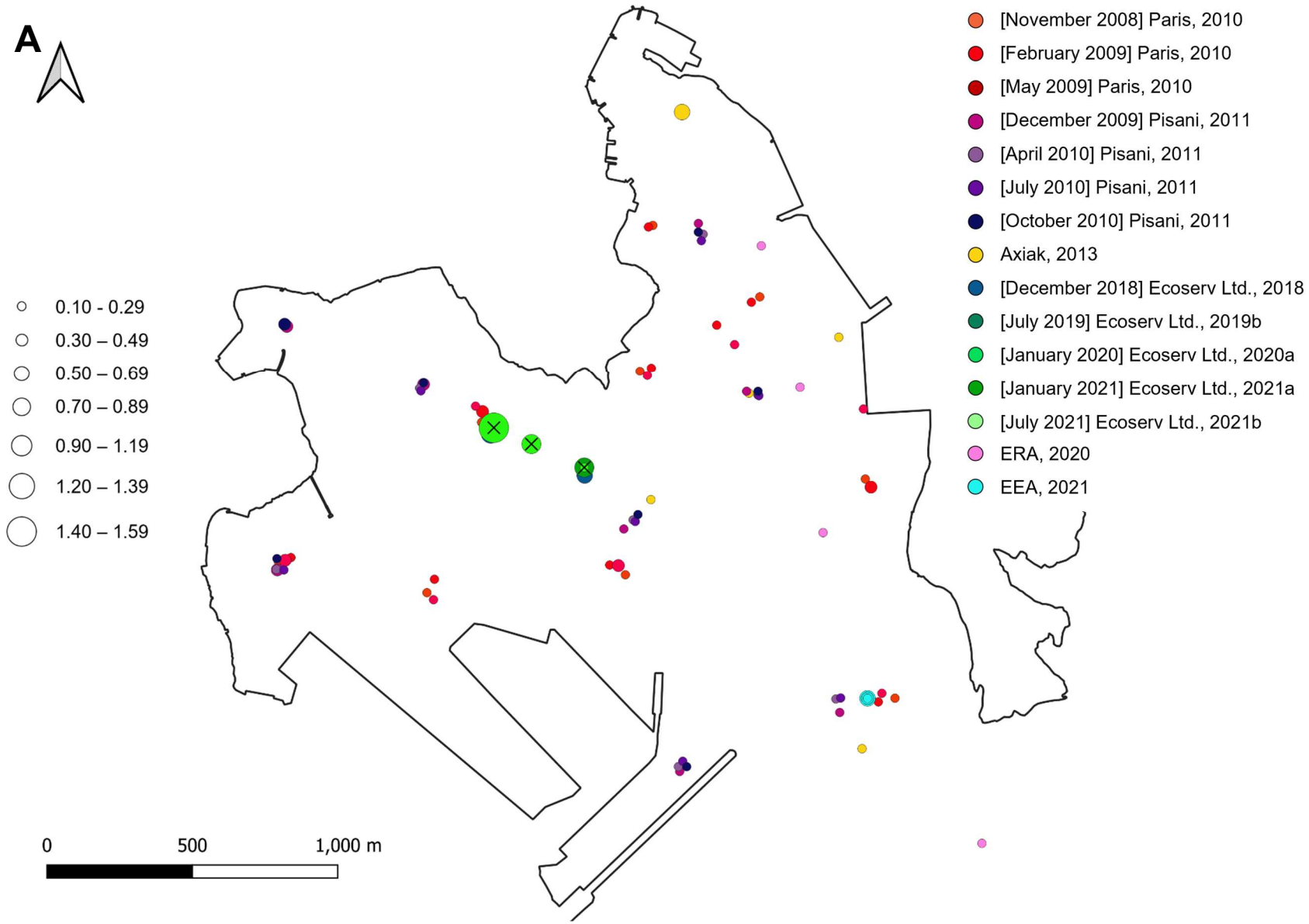


Figure 2.12. The concentrations of total phosphorus in sub-surface waters in Marsaxlokk Bay mapped in GIS from various datasets between 2009 and 2021. Nutrient levels that fell below detection limits were indicated by a cross marker. Total phosphorus levels across the bay at different sampling periods in B. Pisani (2011) and C. Ecoserv Ltd. (2018, 2019b, 2020a, b, 2021a, b). Missing data indicates concentrations below detection limits.


A
0.10 - 0.29
0.30 – 0.49
0.50 – 0.69
0.70 – 0.89
0.90 – 1.19
1.20 – 1.39
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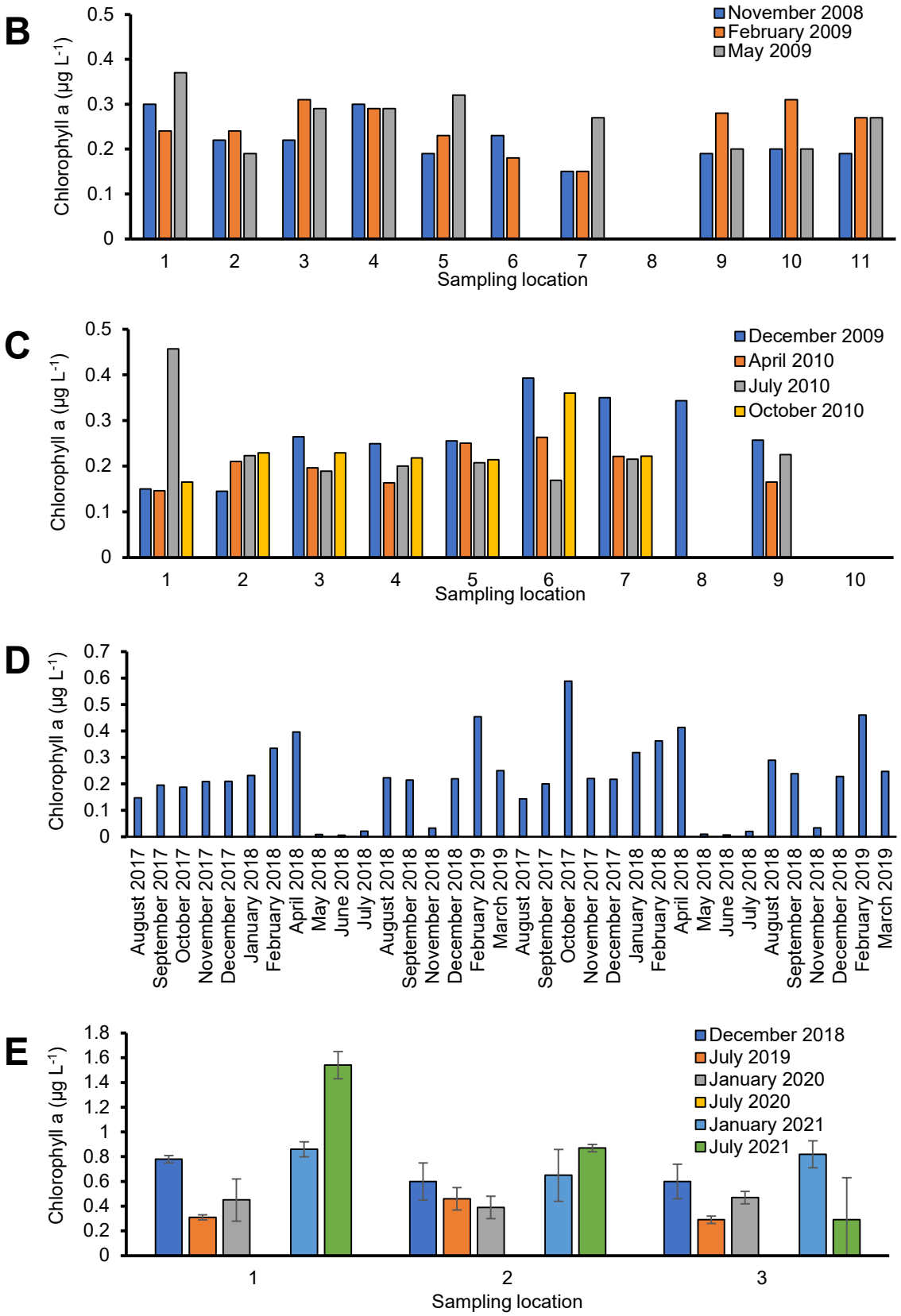

Figure 2.13. Concentrations of chlorophyll a in sub-surface waters in Marsaxlokk Bay mapped from various datasets between 2008 and 2021 in GIS. Chlorophyll levels across the bay at different sampling periods in B. Paris (2009), C. Pisani (2011), D. EEA (2021) and E. Ecoserv Ltd. (2018, 2019b, 2020a, b, 2021a, b). Missing data indicates concentrations below detection limits.

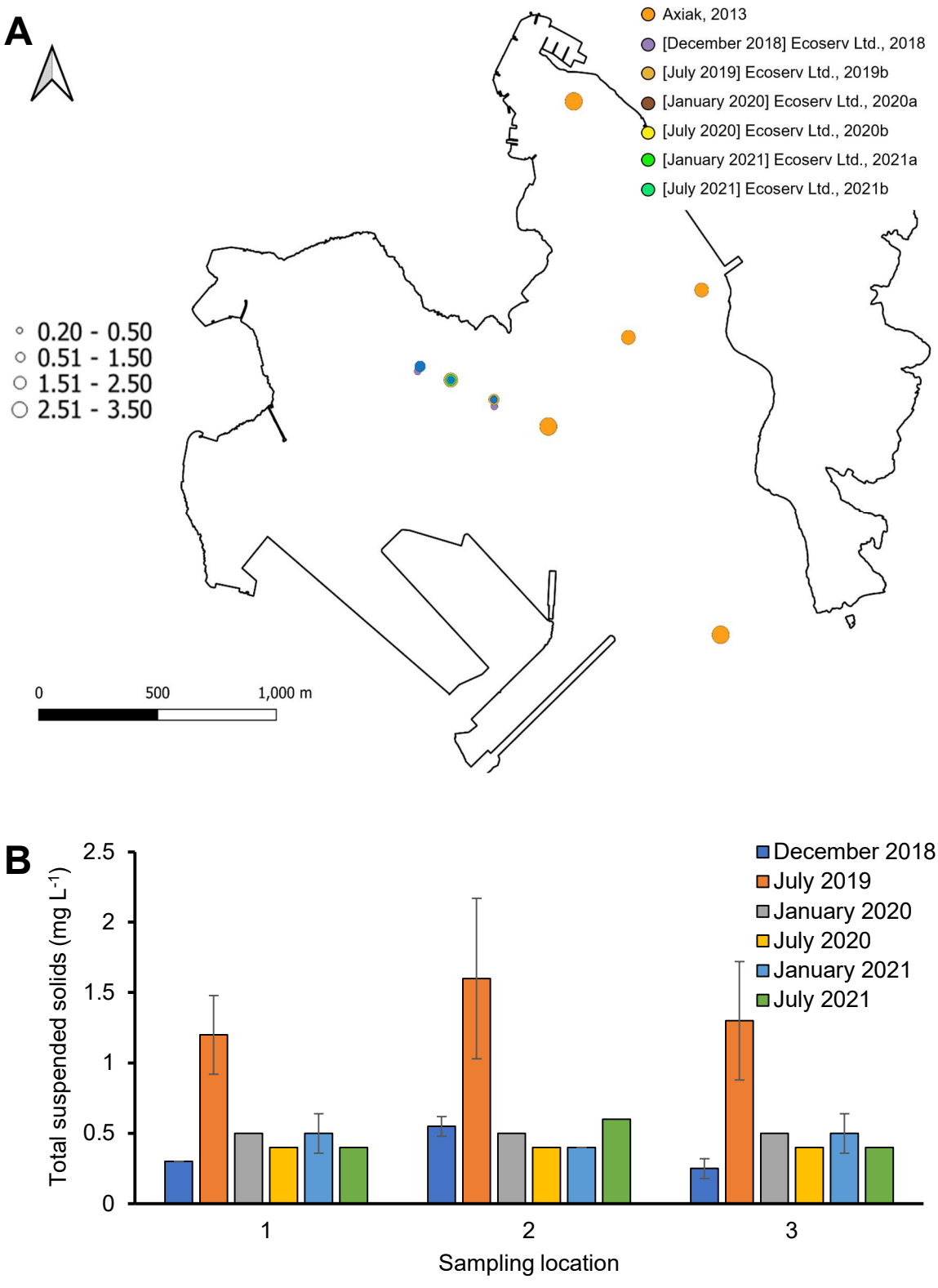

Figure 2.14. Total suspended solids in sub-surface waters in Marsaxlokk Bay mapped in GIS from various datasets between 2013 and 2021. Mean concentration of total suspended solids at stations near the fish farm at different sampling periods in B. Ecoserv Ltd. (2018, 2019b, 2020a, b, 2021a, b). Missing data indicates concentrations below detection limits.

Anthropogenic activities have caused nutrient enrichment in the Mediterranean region (La Rosa et al., 2002; Belias et al., 2003; Holmer et al., 2008; Basaran et al., 2010; Holmer, 2010; Simboura et al., 2016). However, these inputs and impacts can be influenced by short-term temporal and seasonal variations (Pitta et al., 1999; Culha et al., 2020; Neofitou and Klaoudatos, 2008; Price et al., 2015). For instance, in marine aquaculture, the release of solute wastes from uneaten feed and fish wastes increases during feeding activities (Pitta et al., 1999). Traditional static monitoring approaches fail to capture the complexity of the dynamic marine environment and the activities that it supports. While a water residence time of 27 days in the bay may help flush the area (Axiak, 2003), management strategies must focus on minimizing nutrient contributions (Stickney, 2002; Braaten, 2007; Pittenger et al. 2007; Belle and Nash 2008; Olsen et al. 2008; Bureau and Hua 2010) and reducing adverse impact (Pitta et al., 1999; Price et al., 2015) despite the challenges of attributing discharges to a single source in multiple-use coastal areas (Price et al., 2015). Given that the expected upsurge in port activities could lead to additional nutrient contributions that can be a concern for aquaculture production (Culha et al., 2022; Morsy et al., 2022), the focus on real-time, adaptive management supported by continuous monitoring is not just a strategy but also an imperative for sustainable development.

The impact of anthropogenic inputs on sedimentation and the accumulation of particulate organic wastes is more noticeable than that of solutes in water (Karakassis et al., 2001; Stagličić et al., 2017). Organic carbon levels in Marsaxlokk ranged from 0.3% to 9.5% bay (Fig. 2.15). In different parts of the bay, organic carbon concentrations were higher than levels recorded below fish cages (0.4% to 3.4%). The concentrations of organic carbon beneath fish cages were consistent with those near other fish farms in the Mediterranean (Karakassis et al., 2000; Porello et al., 2005). However, accumulation rates vary between fish farms due to different local environmental conditions, production capacity and feed inputs. Moreover, the concentrations of organic carbon near fish cages may be amplified by the settling of excess food and fish waste close to the cages due to feeding in intensive farming (Sarà et al., 2004; Kalantzi et al., 2006; Holmer et al., 2007). This accumulation has the potential to boost bacterial activity and deplete oxygen levels (Brooks and Mahnken, 2003; Vita et al., 2004), as well as increase the bioavailability of toxic contaminants in seafloor sediments.

The distribution pattern of organic carbon in these sediments serves as an important indicator of human activities and impacts within the bay area. The absence of comprehensive environmental data in the bay despite well-documented impacts of various human activities on sediment organic carbon enrichment raises significant concerns. As sediment serves as a historical record of environmental changes, its organic carbon content

reflects past anthropogenic influences. Without up-to-date data, it becomes challenging to track and comprehend the evolving consequences of human actions on sedimentation patterns and organic carbon accumulation. Given the dynamic nature of both anthropogenic processes and environmental conditions, a lack of data impedes our ability to assess, adapt to, and mitigate the effects of these changes, hindering effective environmental management and sustainable decision-making. Considering the dynamic nature of these activities, spatiotemporal monitoring of organic enrichment in sediments is fundamental to decision-making in management and spatial development. In light of evolving human activities, such as modifications to feed used in aquaculture (Yabanli and Egemen, 2009; Weitzman et al., 2019) and the growth of port infrastructure, it becomes particularly significant to represent fluctuations in sediment quality and the complex environment interactions that influence benthic conditions. This plays a crucial role in planning effective strategies to recycle organic matter in aquaculture-derived wastes in seafloor sediment.

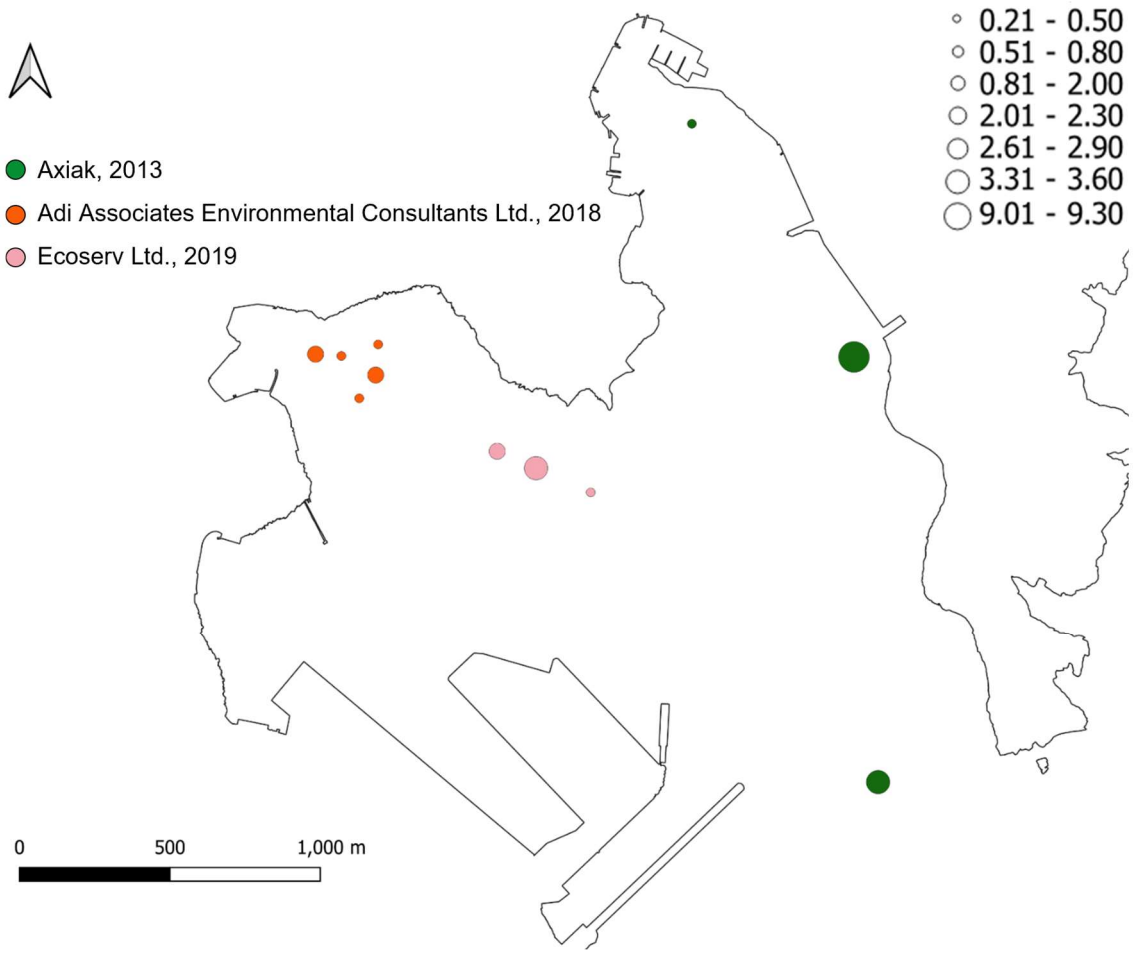

Figure 2.15. Levels of total organic carbon (mg L^{-1}) in sediments in Marsaxlokk Bay mapped in GIS from various datasets between 2013 and 2021.

2.5 Implications for aquaculture

Spatial data derived from real-world observations of environmental conditions is essential for informing marine spatial plans and management (Pérez et al, 2003; Falconer et al., 2018; Falconer et al., 2020). Maps provide valuable insight into coastal usage (Collie et al., 20 13; Falconer et al., 2023), however, the anthropogenic activities in coastal spaces like Marsaxlokk Bay are dynamic and their potential impacts on the environment and co-existing users vary over time. Among the marine industries, aquaculture is expected to continue growing but as coastal cities become increasingly urbanized, inshore areas will remain important sheltered coastal spaces for aquaculture and competition for these areas continues to intensify. In these areas, the dilution of anthropogenic waste may no longer suffice and effective management will rely on representative monitoring that accounts for spatiotemporal changes in the potential inputs of anthropogenic activities in these environments.

Model advancements and improved farm management practices have contributed to better waste management. Models help predict anthropogenic impacts on the environment to inform planning and provide effective strategies for waste management (Kapetsky et al. 2013; Lovatelli et al. 2013, Ross et al. 2013b). However, these multiple-use coastal areas are complex and simplified scenarios used in planning processes are not representative enough of the real-world conditions at these sites. Untangling some of these complications requires long-term data to represent the full complexity of these crowded coastal spaces. Given the diversity of anthropogenic activities in coastal bays, it is important to identify which inputs are of greater concern and then to monitor certain activities regularly and take timely management decisions, particularly in anticipation of their expansion or that of aquaculture. Then, research is needed to validate the anthropogenic contributions to specific activities through techniques that exploit distinct environmental signatures as stable isotopes.

While offshore aquaculture offers promising prospects (Gentry et al., 2016), enhancing the efficiency of existing coastal aquaculture by optimizing space and resource utilization can also be a viable avenue for expansion. Increasingly, innovative approaches to optimizing our coastal spaces are being explored (Klinger and Naylor, 2012; Falconer et al., 2023). Integrated multi-trophic aquaculture (IMTA) systems, in particular, present a potential solution that can maximize production while minimizing environmental impact. These systems achieve this balance by offsetting waste discharge through the assimilation of nutrients and organic matter found in anthropogenic waste inputs. However, the successful implementation of IMTA systems in multi-use coastal areas is complicated by bay-scale and farm-level dynamics. A thorough understanding of these dynamics, informed by high-

resolution long-term data, is a prerequisite for adequate planning and optimal resource utilization. This understanding should be bolstered by advanced monitoring programs that offer a dynamic approach to spatial planning, allowing for adaptive responses to changes, future trend predictions, and the refinement of management strategies. IMTA systems, in particular, hinge on the transfer and availability of resources in an open-water environment and depend on how these change within the system over time. Monitoring becomes critical to comprehend the flow and interaction of these elements that includes nutrients, organic matter and contaminants, thus providing the necessary insight to manage IMTA systems effectively. The advent of advanced decision-support tools and methods for environmental monitoring is a fundamental necessity. However, their effectiveness relies on the availability of representative environmental data that can allow for dynamic spatial planning and the development of adaptable integrated systems, capable of responding to the constant flux in the marine environment.

2.6 Conclusion

Multi-use environments can be suitable coastal areas that provide adequate environmental conditions for marine aquaculture to co-exist with other industries and possibly grow sustainably. However, these remain complex and dynamic systems where the concern of potential individual and cumulative anthropogenic effects on marine aquaculture, where this exists in these areas, comes from the multitude and variety of sources of inputs in these areas. The elucidation of these inputs and effects is necessary to gain a comprehensive understanding of bay-scale dynamics, which is essential for effective planning and development of marine aquaculture. The resolution of interactions between anthropogenic activities and the environment can lead to improved management and utilization of space, especially in the context of expanding industries.

Given the diverse range of anthropogenic activities in coastal bays, it is crucial to identify and monitor inputs that are of greater concern regularly. A more representative comparison of certain water and sediment quality parameters would require additional sampling at other locations in the bay. This approach enables timely management decisions in anticipation of changes in these activities or those of aquaculture. To promote the growth of aquaculture sustainably, innovative approaches such as IMTA could be means to optimize the use of space and resources. However, this requires detailed data and a high-resolution understanding of system dynamics at bay and farm levels in complex multi-use environments.

Chapter 3. Investigating the effects of wind and maritime traffic on inshore currents around a fish farm in Marsaxlokk Bay, a busy multi-user coastal space, at the centre of the Mediterranean

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Abstract

Marine aquaculture expansion will continue to be challenged by a lack of space in areas of the marine domain that can support aquaculture, due to competition from other maritime activities vying for the same spaces. This research attempted to characterise those natural and anthropogenic forces that influence and drive sea currents measured over a 16-month period around a nearshore fish farm located within a busy multiple-use bay in the central Mediterranean Sea. Evidence from a concomitant two-year-long meteorological dataset revealed the occurrence of variable winds that result in a dominant and perpetual forcing on near-surface current magnitude and direction. The correlation coefficient between wind and sea currents decreased with increasing depth and hourly time lag. Moreover, the observed water level variations were more related to meteorological forcing factors than to tidal influences recorded at the mouth of the bay. However, intermittently observed water current values could not be exclusively explained by atmospheric forcing variables when the relationship between *in-situ* measurements and sea current values predicted by the hydrodynamic-wave model (Marine Forecasting System) was analysed. Consequently, this lack of correlation spurred further analysis, which revealed that relevant water current disturbances, particularly in near-surface sea currents, corresponded to 131 different Automatic Identification System (AIS) records of vessels. These vessels included bunkering barges, pilot boats and dredging vessels operating and navigating within a 650 m radius from the fish farm and during a 10-min window. This study thus provided evidence for natural and anthropogenically-derived influences on local fish farm-scale hydrodynamics that have important implications for the effective and sustainable development of aquaculture within a marine spatial context, especially in congested, multi-use environments.

3.1 Introduction

Around the world, coastal bays and inlets are multiple use areas that cater for different activities. For decades, sheltered inshore areas have provided suitable environmental conditions for marine aquaculture, particularly for juvenile production. However, lack of space and competition from other coastal activities challenges the development of marine aquaculture (Sanchez-Jerez et al., 2016; Cavallo et al., 2020; Galparsoro et al., 2020) especially where it exists in multi-used bays or port and harbour areas. Recently, aquaculture and other coastal activities are increasingly undertaken within the wider context of marine spatial planning (Sanchez-Jerez et al., 2016). Indeed, Deidun et al. (2011) advocated for scientific guidance to address knowledge gaps that could otherwise be a barrier for effective planning. For this reason, spatial development and management of coastal activities should be based on evidence-based decision-making (Pınarbaşı et al., 2017). The development of marine aquaculture, especially where space is limited and competition is significant, requires a thorough understanding of the natural and anthropogenic factors that affect it.

There is increasing reliance on hydrodynamic models in planning and management of marine space. Planning measures and policies for spatial development and management in coastal waters rely on sound understanding of the hydrodynamics and the forces that drive them in these coastal areas (Montaño-Ley et al., 2007). Still, research on the forcing factors and the processes that influence and drive water movement in many of these coastal environments is limited. In the Mediterranean region, model simulations have revealed wind-dominant influences on water movement over tidal effects in sheltered coastal areas (Ferrarin and Umgiesser, 2005; De Marchis et al., 2014; Grifoll et al., 2014; Balsells et al., 2020). However, the hydrodynamics of complex coastal areas may not necessarily be simulated accurately through simplistic or idealised scenarios (Grifoll et al., 2009; De Marchis et al., 2014; Grifoll et al., 2014). For instance, port and harbour hydrodynamics can also be influenced by event-specific factors like anthropogenic forcing. Specifically, hydrodynamics in ports and harbour areas are also influenced by human-induced activities. When these are not accounted for, simulations fail to represent the true water current conditions around fish farm cages. In these situations, decision-support tools may have limited applicability. For marine aquaculture, a detailed description of hydrography can aid decision-support tools in providing a more accurate assessment of waste dispersion, farm production, hydrodynamic effects of fish farm infrastructure and environmental impacts in these coastal areas.

Anthropogenic activity adds distinct disturbances to natural processes and may have different effects and implications for these coastal systems (Soomere, 2007; Scarpa et al., 2019). Notably, ship-generated water movement has different characteristics to wind-induced hydrodynamics with sediment resuspension altered by vessels by as much as one order of magnitude greater within seconds (Soomere, 2007; Rapaglia et al., 2011). These abrupt ship-related hydrodynamic disturbances can occur more frequently and have greater effect on the surrounding aquatic environment, more so when combined with wind effects (Gabel et al., 2017). Numerous studies have observed ship-induced waves and currents from single ship passages in different water body systems (Rapaglia et al., 2011; Parnell et al., 2016; Scarpa et al., 2019; Mao and Chen, 2020; Mao et al., 2020). However, considering the challenges and pressures that the rapidly increasing maritime traffic will continue to put on the environment (Rapaglia et al., 2015; Fleit et al., 2016; Gabel et al., 2017) and coastal users like marine aquaculture (Pearson et al., 2016; Gabel et al., 2017), further research attention should be devoted to understanding the effects and implications for coastal management and development.

Research has described different challenges in human-dominated seascapes with the rapid development of maritime activity across the world (Fernández et al., 2016; Pearson et al., 2016). For instance, intermittent dredging not only has hydrological impact but can also cause resuspension and dispersion of contaminants from sediment (Airoldi et al., 2016). Water movement can facilitate the resuspension and transport of sediments and affect fish behaviour and physiology, especially if exposed to contaminated sediments associated with industrial coastal areas, ports and shipping. Flow around fish cages disperses waste that is generated from marine aquaculture. Moreover, the supply of oxygenated waters affects fish welfare and production in marine aquaculture (Klebert et al., 2013). Similarly, shellfish production is strongly influenced by water movement for the supply of food and oxygen (Dame and Kenneth, 2011; Campbell and Hall, 2018). Therefore, water movement is key in marine aquaculture and requires thorough understanding in planning, managing and developing the sector. In the present study, the forcing that influence and drive currents allude to the hydrodynamic effects on marine aquaculture and the implications for the sustainable development of the sector.

In the Mediterranean region, multi-use bays and port areas are locations were marine fish farms sited have been sited. This observational study describes water current variability around an inshore fish farm situated in a busy multiple user bay and port area in the Mediterranean Sea. This research investigates the influence of the natural and anthropogenic forces, wind and ship traffic, on water currents, and then describes the

implications for other coastal activities, particularly marine aquaculture. In more detail, *in-situ* hydrodynamic data is used to establish statistical relationship with forecast wind data and to describe whether sea currents surrounding this nearshore fish farm in this multiple user marine space are influenced or driven by wind forcing. Then, the link between ship traffic and water movement near the fish farm is investigated by corresponding ship entries with any observational current data points that deviate from modelled hydrodynamic data. This work aims to contribute towards new solutions for the sustainable development of aquaculture, especially where it is challenged by coastal space and co-existing maritime industries.

3.2 Materials and methods

3.2.1 Study site

This study was carried out in Marsaxlokk Bay, in southeast Malta (Fig. 3.1 A), described in Chapter 2. The eastern and westernmost parts of the bay have a mean water depth of 10 m (Axiak, 2013) but at the centre of the bay, navigation channels associated with the transhipment terminals are dredged to a designated depth below 17 m (Adi Associates Environmental Consultants Ltd., 2007) while the water depth at the breakwater leading to the open sea is 26 m. Fig. 3.1 B shows a gridded bathymetric map of the area referenced to the mean sea level and with a resolution of 10 m that was rendered using bathymetric LIDAR data (Hili, 2014).

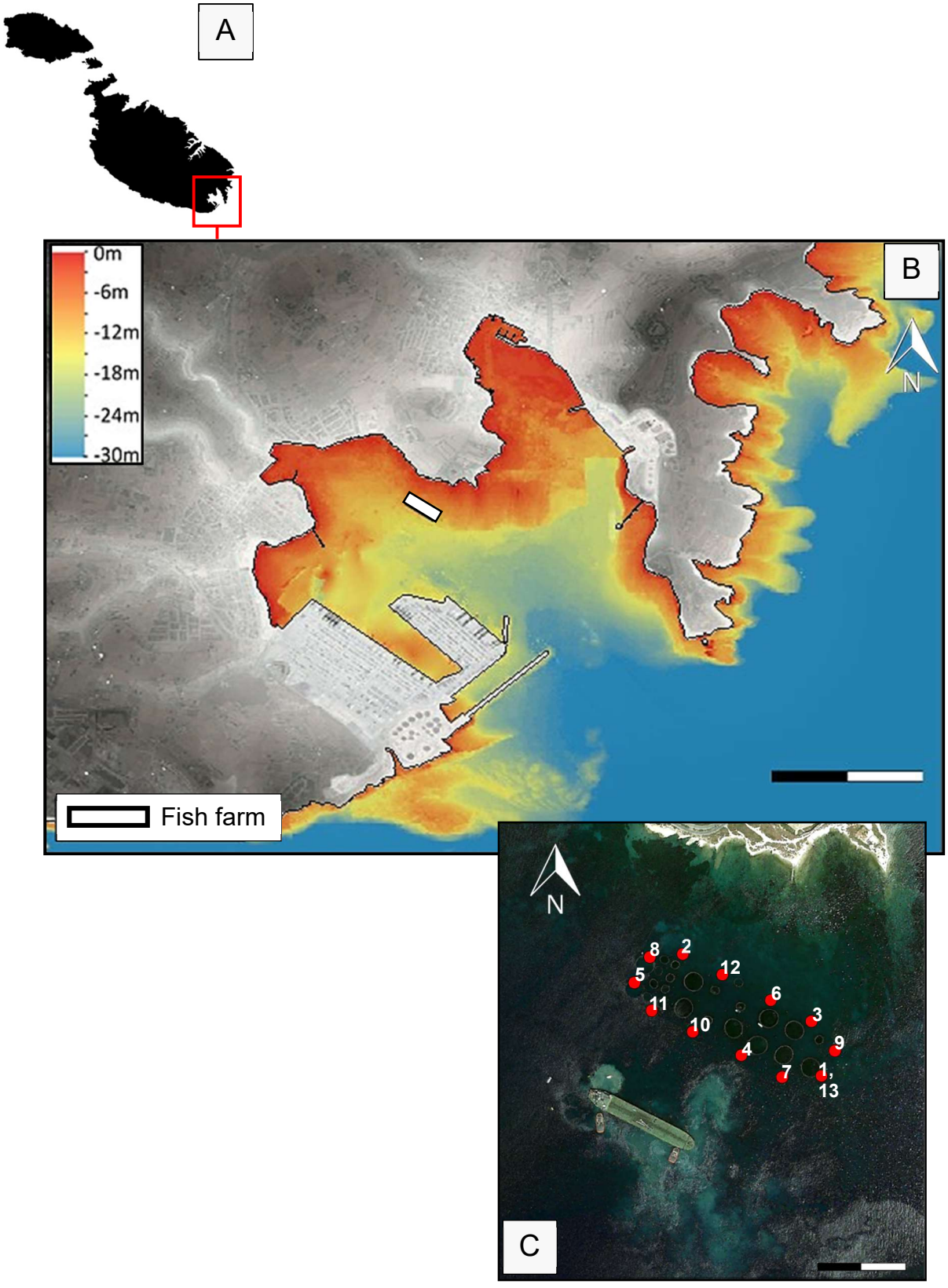

Figure 3.1. A. Test site location within Marsaxlokk Port, southeast Malta, and B. bathymetric map of the area rendered from LIDAR data (Hili, 2014) showing the location of the fish farm (Scale bar 1 km). C. Deployment positions of the acoustic Doppler current profiler in consecutive order of placement around the fish farm in Google Earth (Scale bar 200m).

Marsaxlokk Bay has various berthing facilities that include a major maritime transhipment terminal on the western side of the outer bay and that lie just 500 m southwest of the fish farm. These are operated continuously and have a capacity for 3.8 million TEU (twenty-foot equivalent units)[1] in its deep-water quays (total operational area of 2,463 m) (Malta Freeport, 2021). In 2020, these terminals registered 1553 calls and 2.44 million TEU (Malta Freeport, 2021). Different types of cargo are handled inside the port area at the centre of the bay, whereas various maritime uses from leisure crafts, traditional vessels and trawlers are associated with the traditional fishing village in the harbour at the northern end of the bay.

3.2.2 Data collection

3.2.2.1 Hydrographic and meteorological data

Hydrographic data was collected at the study site using an acoustic Doppler Current Profiler (ADCP), Aquadopp Profiler 400 kHz (Nortek, Norway), between May 2018 and August 2019 (Table 3.1). The ADCP was deployed on the seabed at different sites and at different water depths next to fish cages around the fish farm (Fig. 3.1 C). The current meter was secured in a tripod stainless steel frame (140cm x 66cm (*l* x *h*)) that was affixed to three 40 kg concrete blocks (34 cm x 20 cm (*d* x *h*)). A marker buoy was attached to the frame with ample rope length. Current speed, direction and pressure were measured at 1m interval depths every 20 minutes. The water depth was recorded from the surface using a hand-held echo sounder at each deployment site. Prior to each monthly redeployment, the battery was replaced and data was extracted.

[1] 1 TEU is 6.1 m x 2.4 m x 2.6 m (length *x* width x height) and a maximum load of 24 tons

Table 3.1 Sites and periods of deployment of the current profiler around the fish farm in the Marsaxlokk Bay as indicated in Fig. 3.1 C.

Site Number	Deployment date	Retrieval date	Deployment period (days)	Latitude (º N)	Longitude (º E)	Water depth (m)
1	07/05/2018 08:20	31/05/2018 08:20	24	35.826378	14.543042	11.9
2	31/05/2018 08:40	02/07/2018 10:45	32	35.82859	14.54089	7.5
3	02/07/2018 11:00	02/08/2018 13:40	31	35.827111	14.54362	8.4
4	02/08/2018 13:59	07/09/2018 10:30	36	35.82698	14.54213	12.5
5	07/09/2018 11:35	11/10/2018 11:35	34	35.8279	14.54008	11.4
6	11/10/2018 12:00	05/11/2018 11:50	25	35.827983	14.542425	8.0
7	05/11/2018 12:16	05/12/2018 12:45	30	35.82675	14.54258	13.3
8	12/12/2018 13:00	17/01/2019 12:00	36	35.82852	14.54079	9.8
9	17/01/2019 13:00	07/02/2019 12:40	21	35.82747	14.54342	8.5
10	07/02/2019 14:00	06/03/2019 09:40	27	35.82718	14.54136	13.0
11	06/03/2019 10:20	10/04/2019 07:40	35	35.8277	14.54067	10.5
12	10/04/2019 07:45	28/05/2019 10:30	48	35.82805	14.54196	10.2
13	28/05/2019 10:50	20/08/2019 09:01	84	35.82647	14.5432	12.8

The ADCP was set an upward-direction configuration on the seabed to obtain current measurements at each one-metre water depth intervals to record hydrography through the fish farm, across a varied bathymetric profile. This allowed for accurate velocity measurements albeit with limitations in measuring the surface layer (10% of water column) due to side lobe interferences, and the near-bottom currents within the blanking distance and the height of the current meter bottom mount. Data was recorded with a temporal

resolution of 20 minutes. The values represent an average of the collected measurements at 60s intervals over the sampling duration.

A WaveGuide sea level monitoring sensor (Radac B. V., Netherlands), installed at tip of the breakwater at the mouth of Marsaxlokk Bay in March 2021, was used to measure sea level, wave height, and wave period. This radar sea level gauge measures heave with a resolution of 3 mm at a frequency of 10 Hz and wave height with an accuracy of 1 cm at one-minute intervals. The sea level sensor recorded *in-situ* range of free surface elevation as tides entered the Marsaxlokk Bay between the 1st and 7th November 2021. This sensor is operated and maintained by the Physical Oceanography Research Group (PO-Res Group) of the University of Malta, as part of the SIMIT-THARSY project (Physical Oceanography Research Group, 2021).

Concomitant two-year meteorological data between 2018 and 2019 was extracted from the validated 'MARIA/Eta' high-resolution atmospheric forecasting system for the Central Mediterranean and the Maltese Islands that is run and maintained by the PO-Res Group. The model runs daily starting from 12.00 h GMT of the current day and produces a 48 h forecast that has 3 h outputs (Physical Oceanography Unit, 2006). *In-situ* measurements from the meteorological station located on the breakwater at the mouth of the bay could not be used for this present study. Instead, the modelled wind forecast at 10 m above sea level at the cell centred at 35.8333° N of latitude and 14.5417° E of longitude was used.

3.2.2.2 Vessel data

In the present study, data from a local Automatic Identification System (AIS) receiver (AIS100, Digital Yacht, UK) that was set up by the PO-Res Group was used to track the ship activity inside the Marsaxlokk Port and around the inshore fish farm. As of 2004, the AIS was mandatory for ships as per the Vessel Traffic Monitoring and Reporting Requirements Regulations (S.L.499.23) of Malta and in accordance with standards of Chapter V/19 of the SOLAS convention.

Position and speed data transmitted by these vessels were collected every 30 minutes to extract records between 12th April 2018 and 28th May 2019 in the Marsaxlokk Port area between 35.80° N and 35.85° N latitude, and 14.52° E and 14.57° E longitude. The AIS data provided static information on the vessel such as the ship name, the Maritime Mobile Service Identity (MMSI) code, as well as the dynamic and voyage-related details that include vessel position and navigation status.

3.2.3 Data analysis

This study assessed the relationship between *in-situ* current observations and modelled wind data. The *in-situ* measurements of water current magnitude and direction that were captured at different depths by the ADCP were normalised to represent depths at every 1 m. The speed and direction of measured current data at different depths and modelled wind data were standardised to 1 h temporal resolutions and correlated (Pearson Product Moment Correlation using SPSS *v*1.0.0.1327). This was carried out for every 1 m water depth downward from the uppermost near-surface layer until no statistical relationship was established. Correlations were calculated in Matlab® (v2001b) with no time delays and with hourly lags to a maximum lag of 12 h between wind and near-surface sea currents, in terms of magnitude and direction, over a whole time series to determine the effect of time delays on sea current response. Data was tested for normality using the D'Agostino-Pearson's K^2 to meet assumptions of Pearson's correlation.

The relationship between observed current speeds and values predicted by the hydrodynamic-wave model, Marine Forecasting System (MFS) (Clementi et al., 2019), was assessed at the same water depths through regression in Matlab®. MFS is a high-resolution open-water hydrodynamic-wave forecast available on Copernicus that runs over the entire Mediterranean region at a spatial resolution of 0.042 degrees and provides 141 unevenly spaced depth levels. The forecast from MFS was taken as at 35.8125^{o} N of longitude and 14.58333^{o} E of latitude. This grid cell is at the land-sea boundary, specifically at the mouth of the Marsaxlokk Bay, and the closest to the area of study.

The schematic illustration in Fig. 3.2 conveys the main elements of the present study, specifically the atmospheric (MARIA/Eta) and hydrodynamic-wave (MFS) models, and simplifies how they have been linked to investigate the effects of wind and ship traffic on water current variability in Marsaxlokk Bay.

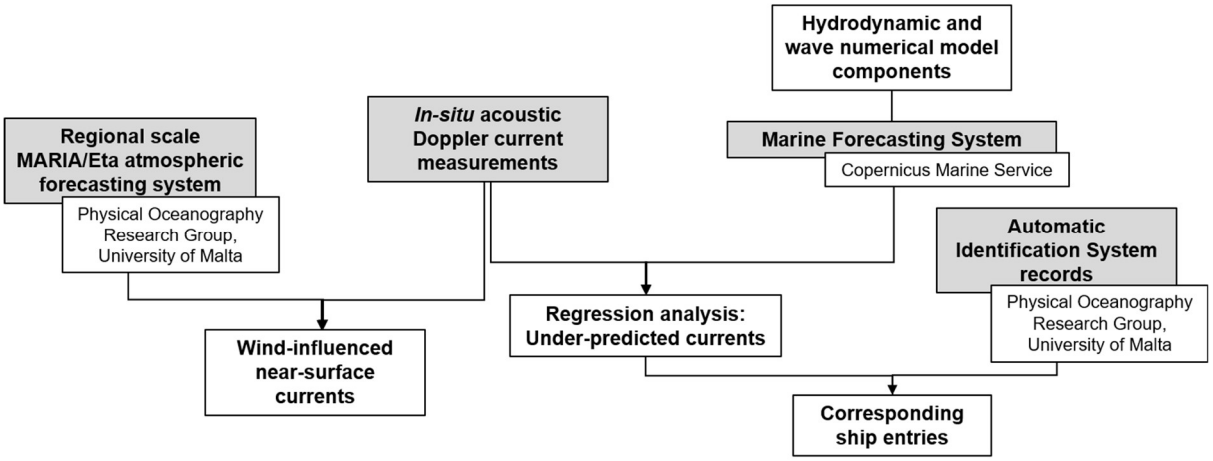

Figure 3.2. Schematic illustration interrelating the atmospheric (MARIA/Eta) and the hydrodynamic-wave (Marine Forecasting System) with the observed water currents from the acoustic Doppler current profiler and ship entries from the Automatic Identification System.

MFS data was only available from October 2018 and therefore, comparisons with the ADCP current values were only possible from this date onwards until the end of the study in August 2019, covering the deployment site numbers 5 to 13 (Table 3.1). A fixed threshold value (0.2 m s^{-1}) was set *a posteriori* as maximum residual value between 'observed' and 'predicted' values for current magnitude to identify data under-predicted by the hydrodynamic-wave model and therefore not explained by the atmospheric forcing variables that are assimilated into the model. This data could be explained by external forces that the hydrodynamic and wave components of the model did not account for, possibly including human-induced disturbances such as marine traffic near the ADCP deployed around the fish farm. To explain this data, the AIS records for ship activity inside the port and near the fish cages were traversed to identify any corresponding ship entries underway within a 650 m radius and within a 10-minute window from the under-predicted data points. Furthermore, the frequency and the type of vessels that corresponded with the extracted under-predicted current data were identified.

3.3 Results

3.3.1 Water currents

The time series data provides *in-situ* measurements that recorded the local currents conditions near inshore fish cages that are situated in this busy Mediterranean bay and port

area, over a 16-month period. This dataset is available through the EMODNET repository at: [https://www.emodnet-ingestion.eu/submissions/submissions_details.php?menu=39&tpd=550&step_more=9]. Measurements of water current magnitudes taken at different sites and depths and time series plots of currents near the fish farm between May 2018 and August 2019 are presented in Appendices 3.1 and 3.2.

The current magnitude fluctuated between peaks and intervals throughout the observed periods. The highest recorded current magnitude was 1.389 m s^{-1} at site 3 in July 2018 whereas low current magnitudes (< 0.001 m s^{-1}) were observed in different water depth layers repeatedly throughout the study (Appendix 3.1). Intermittent data points reveal high current magnitudes in the dataset recorded by the ADCP that were only limited to and did not extend beyond single measurements. During specific periods, that include May and December 2018, higher average speeds were apparent in the near-surface layers (Appendix 3.1) at the different sites around the fish farm (Table 3.1).

The hydrographic dataset shows that the direction of currents changed over time at the different deployment sites of the ADCP (Appendix 3.2). Moreover, the data revealed variation in the direction of currents between different water depth layers, particularly between near-surface and near-bottom currents, in specific periods of observation. This temporal and spatial variation in water currents, specifically between different depth layers in the vertical water profile, provide empirical grounds for the possible influences of different and dynamic forcing factors, such as wind and ship traffic, on water currents around the static infrastructure of the fish farm.

The *in-situ* observations reveal a small constant diurnal cycle with a tidal period of 12 h in the bay (Fig. 3.3). Tidal fluctuations are superimposed by smaller fluctuations presumably due to swell waves, internal resonance of the particular basin and other factors. The tidal range is generally less than 0.4 m and therefore small tidal currents are expected, a trend that follows the general Mediterranean tidal fluctuation. The weak tidal influences that are expected at the mouth of the Marsaxlokk Bay reveal that water level variations seem more related to meteorological influences. This analysis of forcing factor influence on the hydrodynamic variability in this bay provides an account of the negligible tidal component and consequently, tidal effects on sea currents were not considered in the present study.

Real-time water surface elevation data from the sensor is available at: [http://ioi.research.um.edu.mt/porto-stations/index.php/welcome/open/MRXB/marine/0].

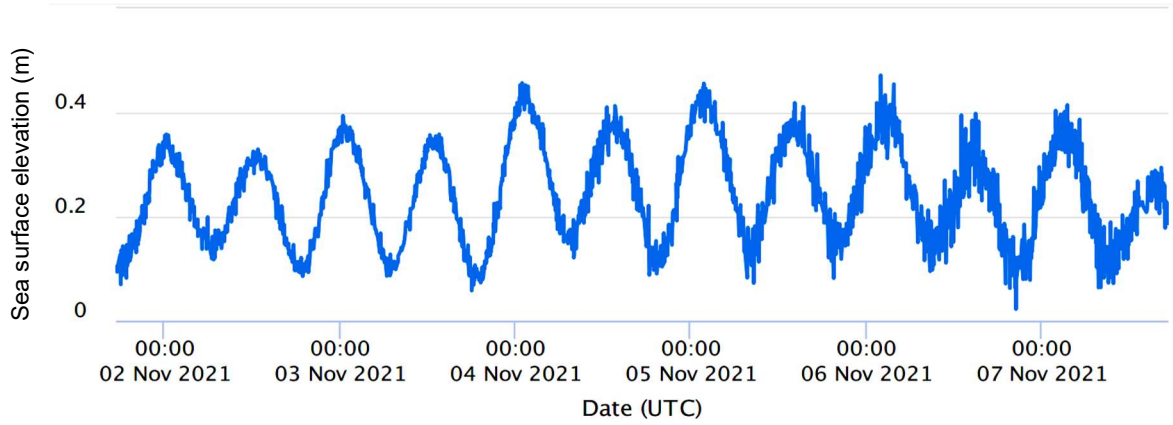

Figure 3.3. Sea level variations recorded at the oceanographic station in Marsaxlokk Bay between 1st and 7th November 2021.

3.3.2 Wind direction and speed

The easterly component winds from 90.0° to 112.5° were the strongest and most frequent accounting for 15% of the total predicted wind direction (Fig. 3.4). The wind direction was variable throughout the observation period. The corresponding time series shows stable periods and intervals of transformation between the stable stages (Fig. 3.5 A). The wind speed predicted by the MARIA/Eta model ranged between 0.02 m s^{-1} and 17.97 m s^{-1} and averaged 3.62 m s^{-1} (Fig. 3.5 B). Throughout the study, moderate winds between 5.5 m s^{-1} and 7.9 m s^{-1}, and winds travelling faster than 8 m s^{-1}, were predominantly recorded between November and May, in 2018 and 2019.

Daily atmospheric regional scale forecast for central Mediterranean is available at: [http://www.capemalta.net/maria/regional/results.html].

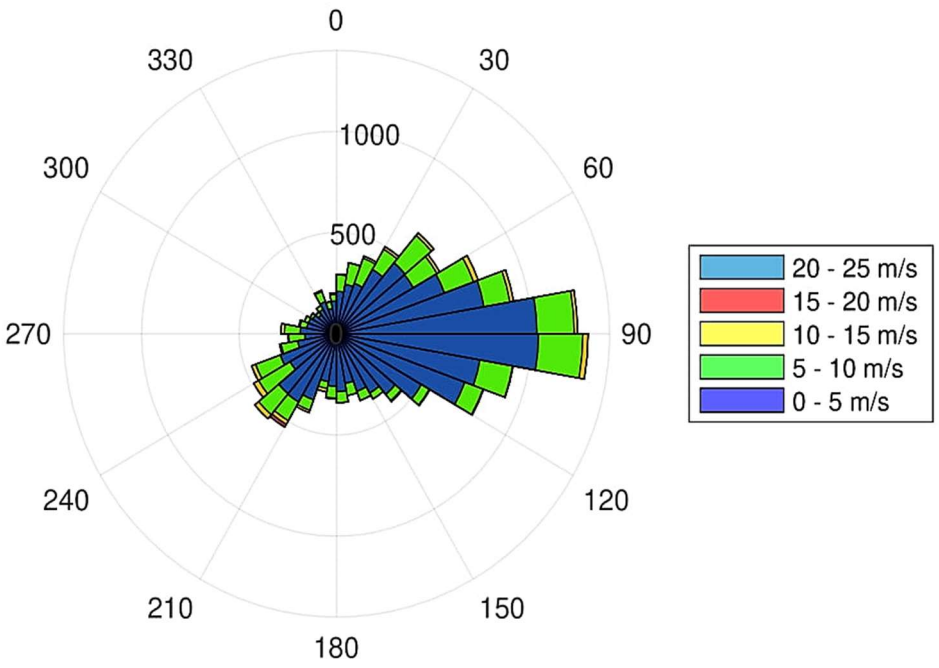

Figure 3.4. Wind rose plot from the numeric high-resolution atmospheric forecasting system for the Central Mediterranean and the Maltese Islands (MARIA/Eta) at 10 m above sea level for 35.8333° N and14.5417° E, between 2018 and 2019.

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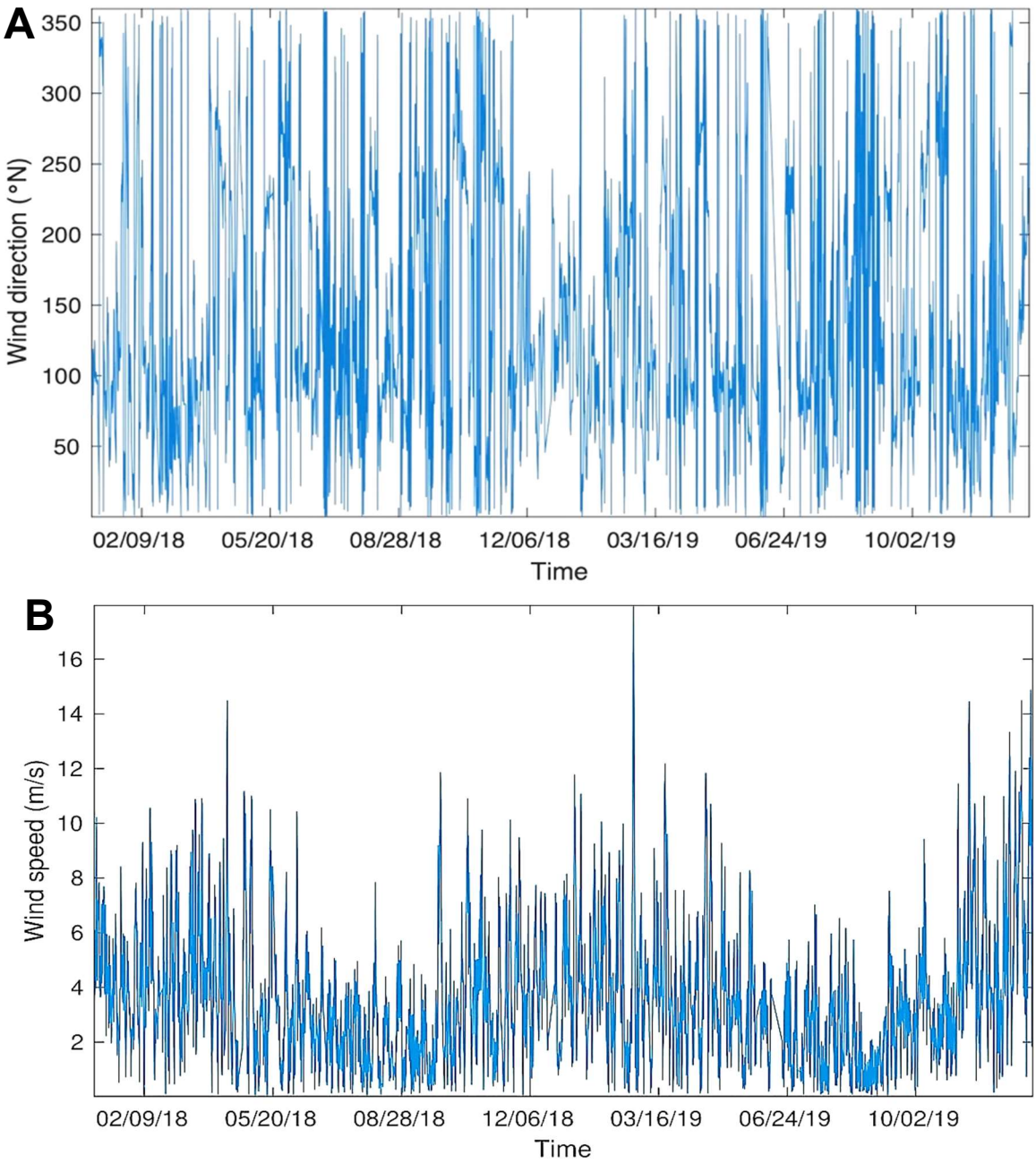


Figure 3.5. Time series of A. wind direction and B. wind speed from the numeric high-resolution atmospheric forecasting system for the Central Mediterranean and the Maltese Islands (MARIA/Eta), at 10 m above sea level for 35.8333° N and14.5417 ° E, between 2018 and 2019.

3.3.3 Wind and currents' relationship

The magnitude and direction of the sea currents at different water depth levels near the fish farm were correlated with the outputs of the MARIA/Eta wind model. A significant

relationship was frequently established between wind flow and near-surface hydrography, particularly at the greater distances from the seabed (Table 3.2). Time series plots for the uppermost near-surface water layer reflect a consistent relationship established between currents and wind flow direction for 20-minute and 3 h temporal resolutions respectively and without any time lags (Appendix 3.3). Results show the correlation coefficient was almost always highest for correlations with zero time lag that decayed from their maximum correlation value with increased hourly time shifts. This reveals the momentum transfer through wind stress that generates immediate response in near-surface currents, between the first and second water depth layers measured at one-metre intervals. Crossed-correlations reveal weaker relationships between wind and near-surface current series in lagged correlations compared to those that have the same mode (zero time lag). At this point, while lagged correlations were calculated in terms of direction and magnitude, and not shown, only zero lag correlations between wind and sea currents are presented in this study.

The magnitude of the water currents was positively correlated with predicted wind velocity (MARIA/Eta), particularly at the uppermost-recorded near-surface layer (Table 3.2). Despite the correlation between wind and current speed for the uppermost layer across all observed periods, the strength of this relationship decreased with increasing depth. A significant relationship was observed between observed ADCP currents and forecast wind (MARIA/Eta) in the uppermost near-surface layers and therefore only the statistical outcomes for the near-surface water layers are presented in Table 3.2.

Table 3.2. Correlation between wind predictions from the numeric forecast model 'Malta Atmospheric and Wave Forecasting System' (MARIA) and current data, for magnitude and direction for the upper water layers at each site.

Current profiler deployment period	Distance from seabed (m)	Wind and current magnitude (m s^{-1})			Wind and current direction (degrees)		
		r	r^2	*p*-value	*r*	r^2	*p*-value
07/05/2018 - 31/05/2018	9	0.093	0.009	0.038**	0.041	0.002	0.360
	10	0.164	0.027	<0.001**	0.083	0.007	0.063
	11	0.373	0.139	<0.001**	0.166*	0.028	<0.001**
	12	0.583	0.340	<0.001**	0.403*	0.162	<0.001**
31/05/2018 - 02/07/2018	4	0.034	0.001	0.376	0.027	0.001	0.469
	5	0.036	0.001	0.340	0.060	0.004	0.113
	6	0.094	0.009	0.013**	0.063	0.004	0.098
	7	0.278	0.077	<0.001**	0.112*	0.012	0.003**
02/07/2018 - 02/08/2018	5	0.007	0.000	0.848	0.035	0.001	0.347
	6	0.045	0.002	0.222	0.066	0.004	0.077
	7	0.032	0.001	0.393	0.008	0.000	0.823
	8	0.232	0.054	<0.001**	0.020	0.000	0.592
02/08/2018 - 07/09/2018	9	0.024	0.001	0.499	0.054	0.003	0.133
	10	0.020	0.000	0.583	0.020	0.000	0.576
	11	0.076	0.006	0.035**	0.030	0.001	0.404
	12	0.430	0.185	<0.001**	0.188	0.035	<0.001**
07/09/2018 - 11/10/2018	8	0.124*	0.015	0.000**	0.025	0.001	0.481
	9	0.004	0.000	0.911	0.046	0.002	0.190
	10	0.320	0.102	<0.001**	0.015	0.000	0.677
	11	0.422	0.178	<0.001**	0.037	0.001	0.285

11/10/2018 - 05/11/2018	4	0.015	0.000	0.712	0.065	0.004	0.114
	5	0.052	0.003	0.202	0.134*	0.018	0.001**
	6	0.000	0.000	0.994	0.119*	0.014	0.004**
	7	0.106	0.011	0.009**	0.048	0.002	0.244
05/11/2018 - 05/12/2018	10	0.031	0.001	0.411	0.034	0.001	0.375
	11	0.010	0.000	0.797	0.016	0.000	0.677
	12	0.124	0.015	0.001**	0.103*	0.011	0.006**
	13	0.573	0.329	<0.001**	0.358*	0.128	<0.001**
12/12/2018 - 17/01/2019	6	0.039	0.002	0.288	0.056	0.003	0.128
	7	0.006	0.000	0.861	0.003	0.000	0.941
	8	0.057	0.003	0.121	0.074	0.005	0.043**
	9	0.414	0.171	<0.001**	0.194*	0.038	<0.001**
17/01/2019 - 07/02/2019	5	0.025	0.001	0.585	0.018	0.000	0.694
	6	0.064	0.004	0.160	0.011	0.000	0.808
	7	0.046	0.002	0.313	0.037	0.001	0.421
	8	0.272	0.074	<0.001**	0.466	0.217	<0.001**
07/02/2019 - 06/03/2019	9	0.073	0.005	0.063	0.020	0.000	0.605
	10	0.080	0.006	0.041**	0.001	0.000	0.973
	11	0.025	0.001	0.521	0.042	0.002	0.293
	12	0.264	0.070	<0.001**	0.131*	0.017	0.001**
06/03/2019 - 10/04/2019	7	0.085*	0.007	0.014**	0.044	0.002	0.204
	8	0.061	0.004	0.081	0.034	0.001	0.326
	9	0.013	0.000	0.708	0.063	0.004	0.071
	10	0.181	0.033	<0.001**	0.263*	0.069	<0.001**
	6	0.060*	0.004	0.046**	0.105	0.011	<0.001**

10/04/2019 - 28/05/2019	7	0.021	0.000	0.480	0.026	0.001	0.380
	8	0.135	0.018	<0.001**	0.075	0.006	0.013**
	9	0.132	0.017	<0.001**	0.169	0.028	<0.001**
28/05/2019 - 20/08/2019	8	0.041	0.002	0.091	0.010	0.000	0.684
	9	0.012	0.000	0.628	0.026	0.001	0.296
	10	0.023	0.001	0.352	0.030	0.001	0.219
	11	0.255	0.065	<0.001**	0.144*	0.021	<0.001**

r represents the Pearson coefficient of correlation and r^2 signifies the coefficient of determination. * shows negative Pearson correlation; ** correlation is statistically significant at $p< 0.05$ level (2-tailed)

Wind and current direction were significantly correlated at the uppermost near-surface water layer, except in July 2018 and between September and October 2018. Where there is a statistically significant relationship, the correlation between the direction of wind and currents was negative, except in August and September 2018. Generally, the relationship between wind flow and current direction decreased rapidly with increasing water depth so that current direction was less affected by wind, or not at all, below near-surface waters. These observations show the extent of the effect exerted by wind forcing on the current conditions at different depths and attests to wind influences on the near-surface water currents in the Marsaxlokk bay. Where no correlation was established or the relationship strength was weak (low r^2 value) (Table 3.2), current conditions around the fish farm may not be explained by meteorological effects but could be influenced and driven by other forces or processes.

3.3.4 Current velocity anomalies and ship traffic

The Automatic Identification System (AIS) data for the period between April 2018 and May 2019 showed that 2,371 passages of vessels were recorded in the study area. The density map, produced over a regular grid with a resolution of 0.0001 degrees, illustrates the frequency of ship and boat passages in the area to highlight inbound and outbound trajectories of vessels and to describe their sailing line (Fig. 3.6). Ship activity was higher around the transhipment terminals and in the various inlets of the Marsaxlokk Port, notably the Marsaxlokk fishing harbour at the head of the bay. A higher frequency of vessel passages was recorded near the fish farm especially to the southwest of the aquaculture site, the location of a mooring site used for bunkering. Different types of vessels frequent the transhipment terminals, the fishing harbour and the other berthing facilities in the area. These navigate the coastal waters of Marsaxlokk Bay to carry out different activities, such as dredging, towage and bunkering, in different designated locations in the area.

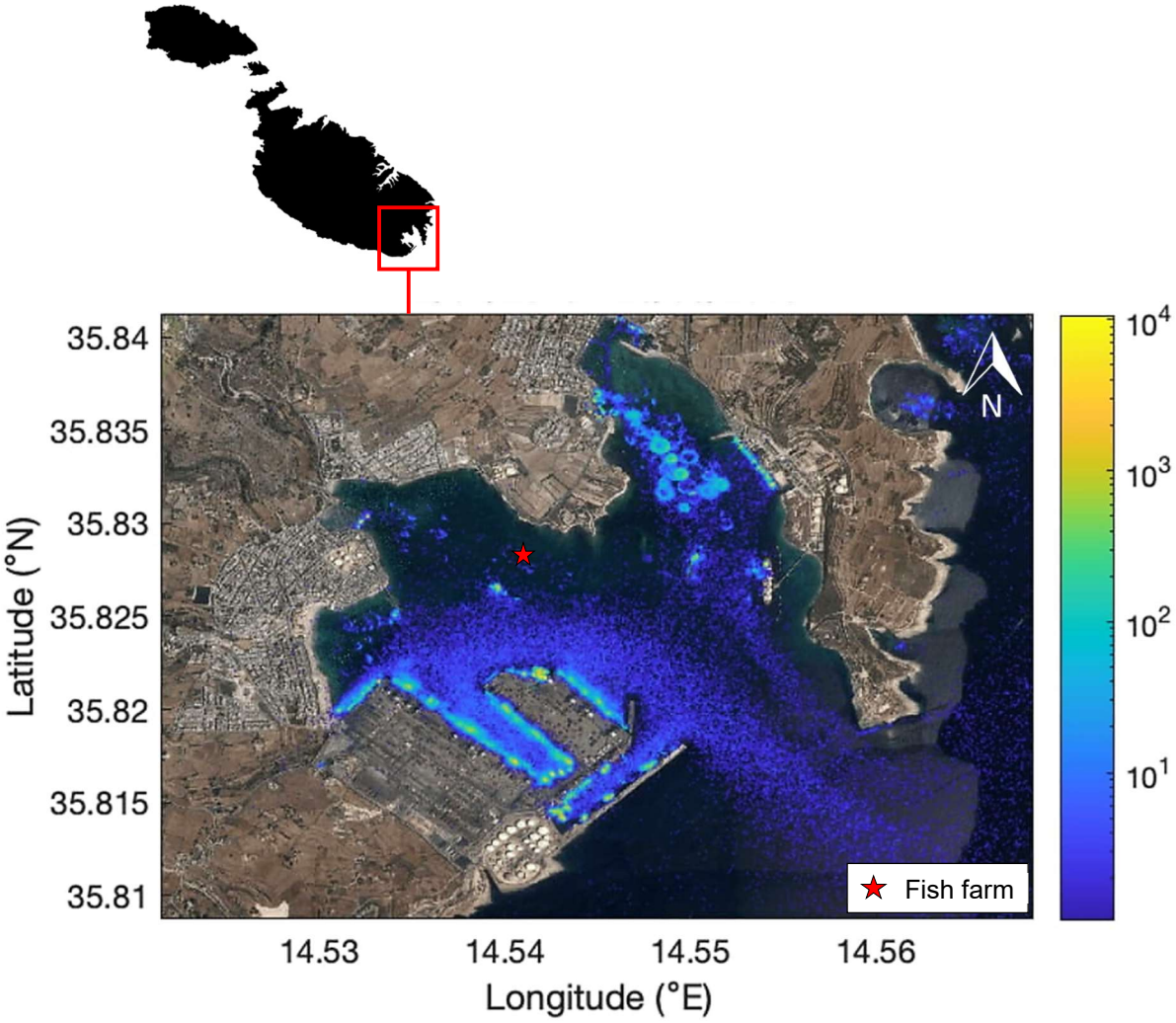
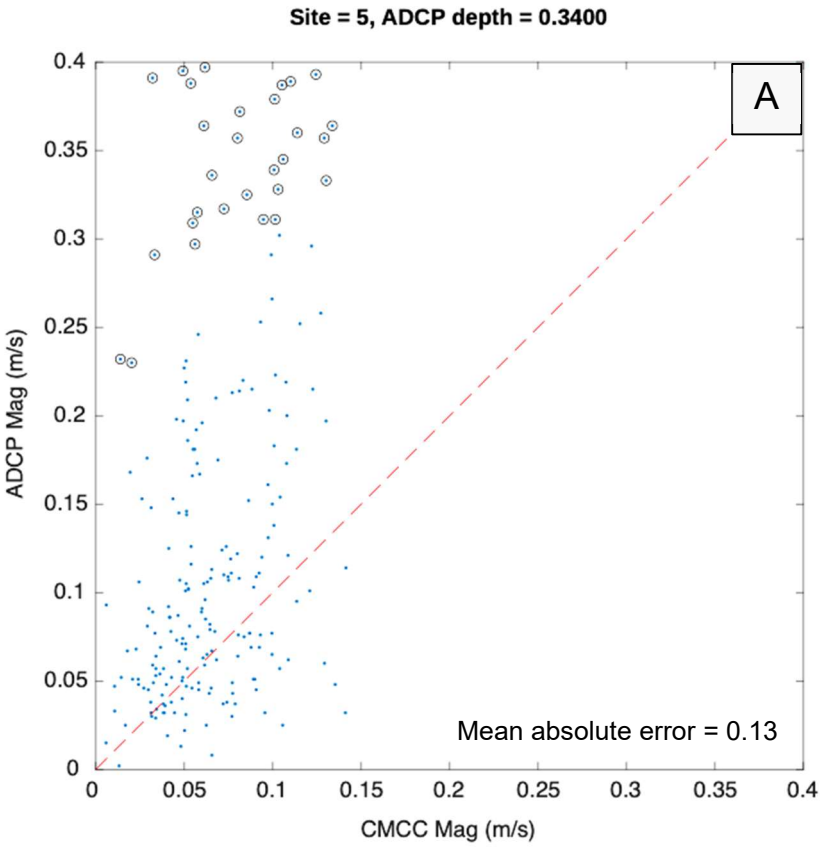
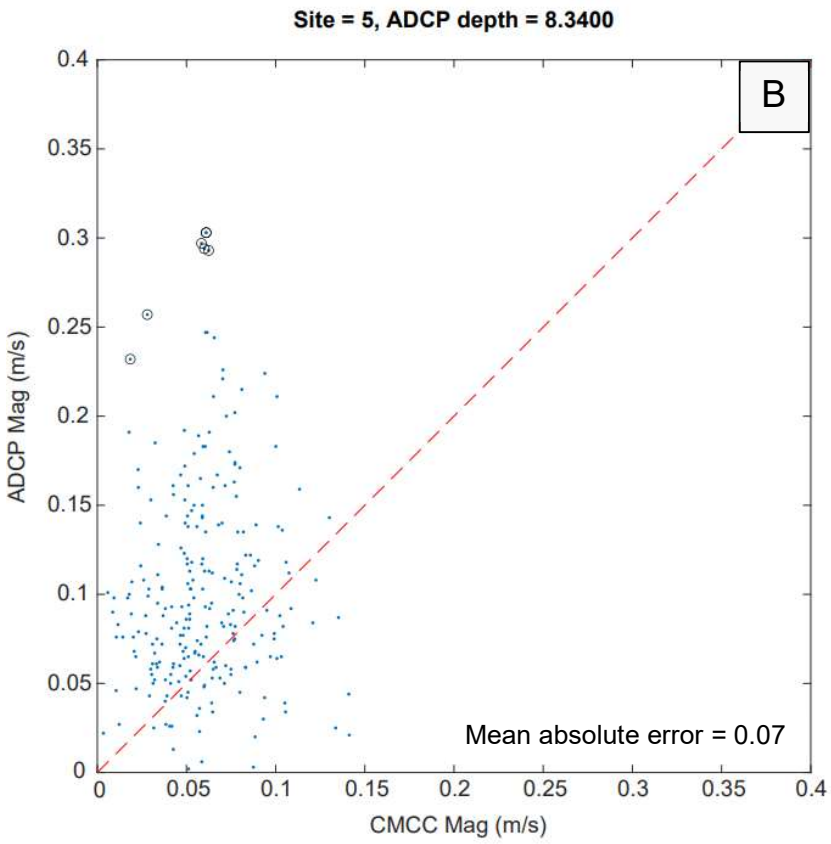
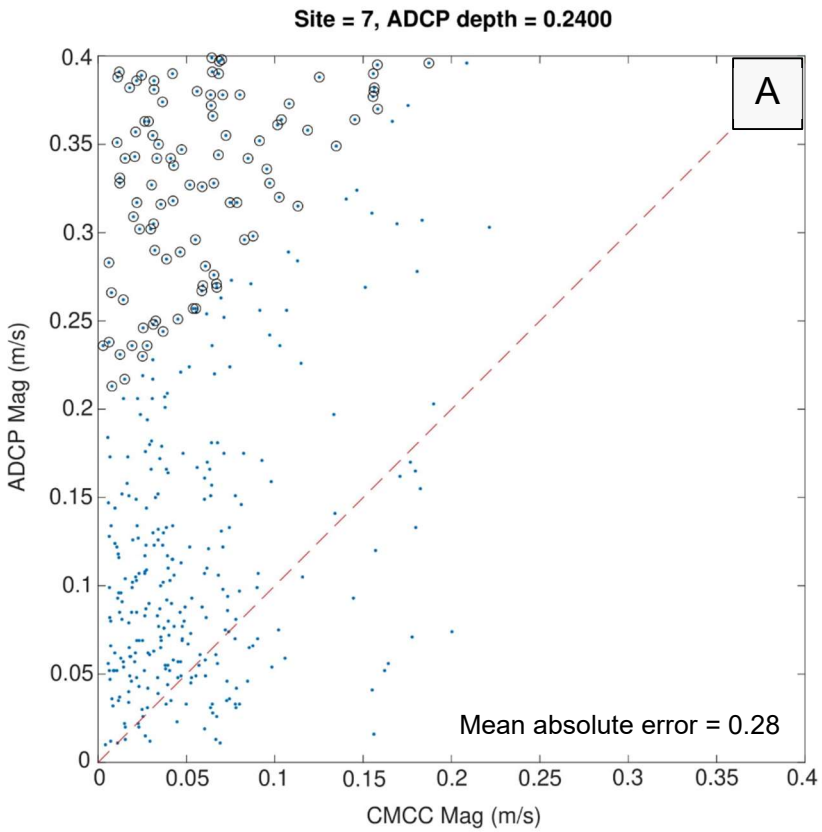


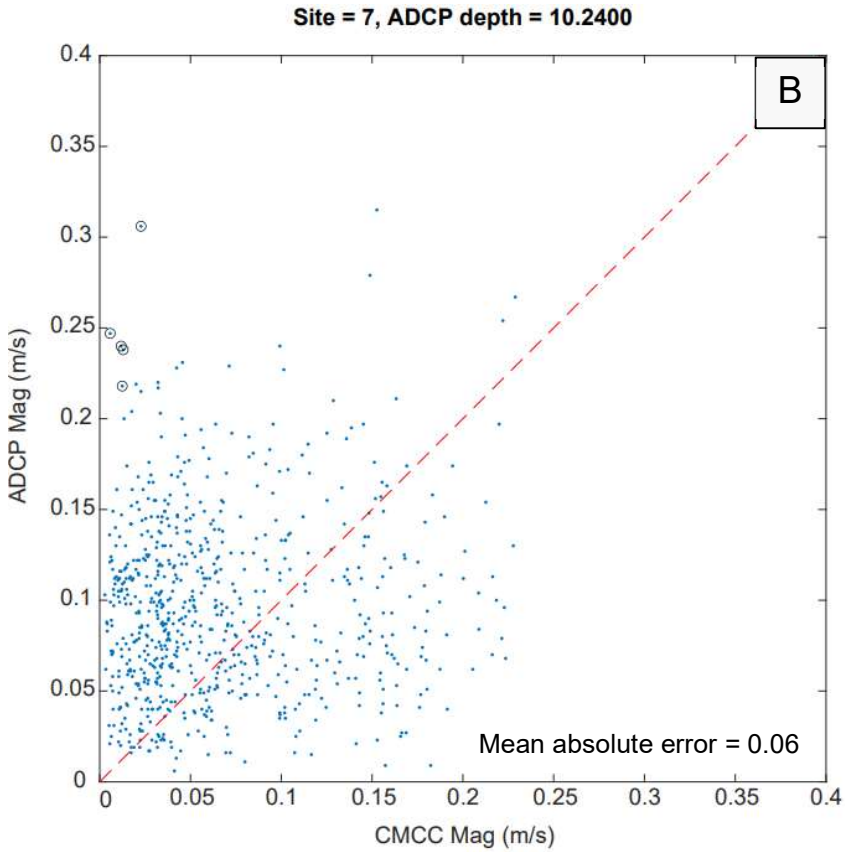
Figure 3.6. Density map of ship records from the Automatic Identification System in Marsaxlokk Bay, between 12th April 2018 and 28th May 2019, produced over a regular grid with a resolution of 0.0001 degrees.

The relationship between the current magnitude values predicted by the model (MFS) and those measured *in-situ* by the ADCP identified disturbances in the current state that were not explained by the Copernicus hydrodynamic-wave forecast (Fig. 3.7). Regression analysis between predicted and observed current magnitude values, at similar water depth layers revealed that the mean absolute error decreases with increasing water depth between the near-surface and the near-bottom water currents.


Site = 5, ADCP depth = 0.3400
A
Mean absolute error = 0.13
ADCP Mag (m/s)
CMCC Mag (m/s)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4


Site = 5, ADCP depth = 8.3400
B
Mean absolute error = 0.07
ADCP Mag (m/s)
CMCC Mag (m/s)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4


Site = 7, ADCP depth = 0.2400
A
Mean absolute error = 0.28
ADCP Mag (m/s)
CMCC Mag (m/s)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4


Site = 7, ADCP depth = 10.2400
B
Mean absolute error = 0.06
ADCP Mag (m/s)
CMCC Mag (m/s)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4

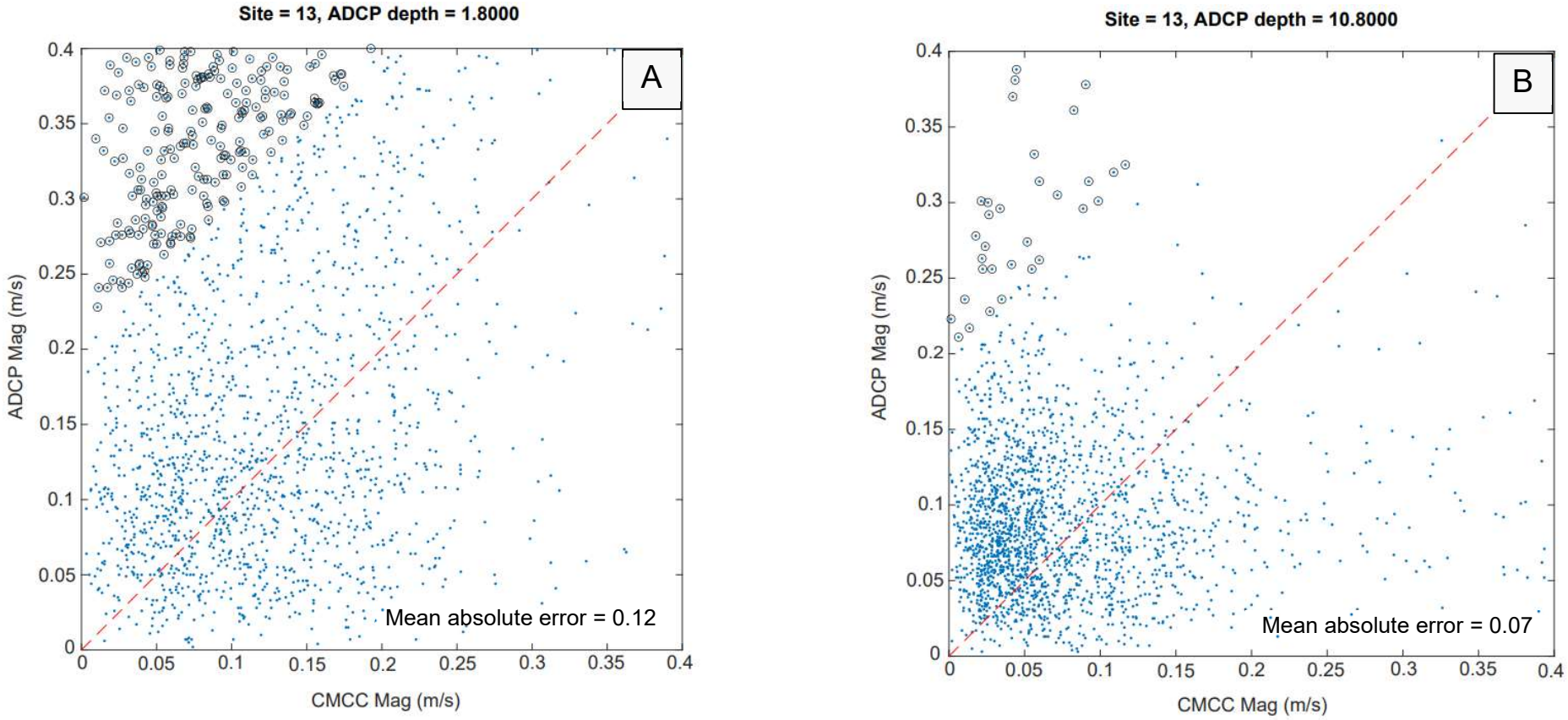


Figure 3.7. Relationship between *in-situ* observations of seawater current magnitudes and predicted values from the hydrodynamic-wave forecast model, 'Marine Forecasting System' (MFS), for a subset of sites (5, 7 and 13) and water depth levels (near-surface (A) and near-bottom (B)). Marked data points were identified as under-predicted current magnitude values.

There were 131 records of AIS equipped vessels within a 650 m radius of the ADCP and a 10-minute window of instances when data for predicted current magnitude was in poor agreement with the *in-situ* measurements. These vessel records corresponded with under-predicted current magnitude values when the ADCP was deployed at site numbers 9 to 13, excluding site number 12. Most of these records (87%) were registered by the ADCP at site 13 between 8th June and 19th July 2019. The vessel typology varied from a bunkering barge of 97 m LOA to a pilot boat, 11 m LOA (Table 3.3). The maximum draught of these vessels ranged between 3 m and 6.2 m, not including the missing information for the pilot boat, BRAVO I.

Table 3.3. Counts of unique vessels associated with under-predicted current magnitude measurements by the hydrodynamic-wave forecast model, 'Marine Forecasting System'.

Ship type	MMSI Code	Ship name	Length (m)	Maximum draught (m)	Count
Bunkering barge	248230000	SANTA ELENA	81	5.0	61
Tug	256607000	ST. ELMO	30	5.8	7
Tanker (HAZ-A	248047000	SPIRO F	55	4.5	7
Pilot boat	249000473	BRAVO I	11	n.d.	5
Cargo ship (HAZ-A)	256269000	SAULUS	58	3.0	3
Dredging or UW ops	215351000	AVE CAESAR	55	3.5	46
Bunkering barge	215953000	ELAURA	97	6.2	2
Tanker	248138000	BAWA I	30	5.8	1

n.d. indicates that data is not available.

The bunkering barge, 'SANTA ELENA' (n = 61), had the highest frequency of records, which was followed by the dredging vessel 'AVE CAEASAR' (n = 46). An apparent higher frequency of vessels corresponded with under-predicted *in-situ* measurements at the near-surface water layers (Fig. 3.8). The under-predicted near-bottom currents between 8 m and 11 m at the ADCP deployment site 13 were linked with the bunkering barge, 'SANTA ELENA', the dredger, and the tanker 'SPIRO F'.

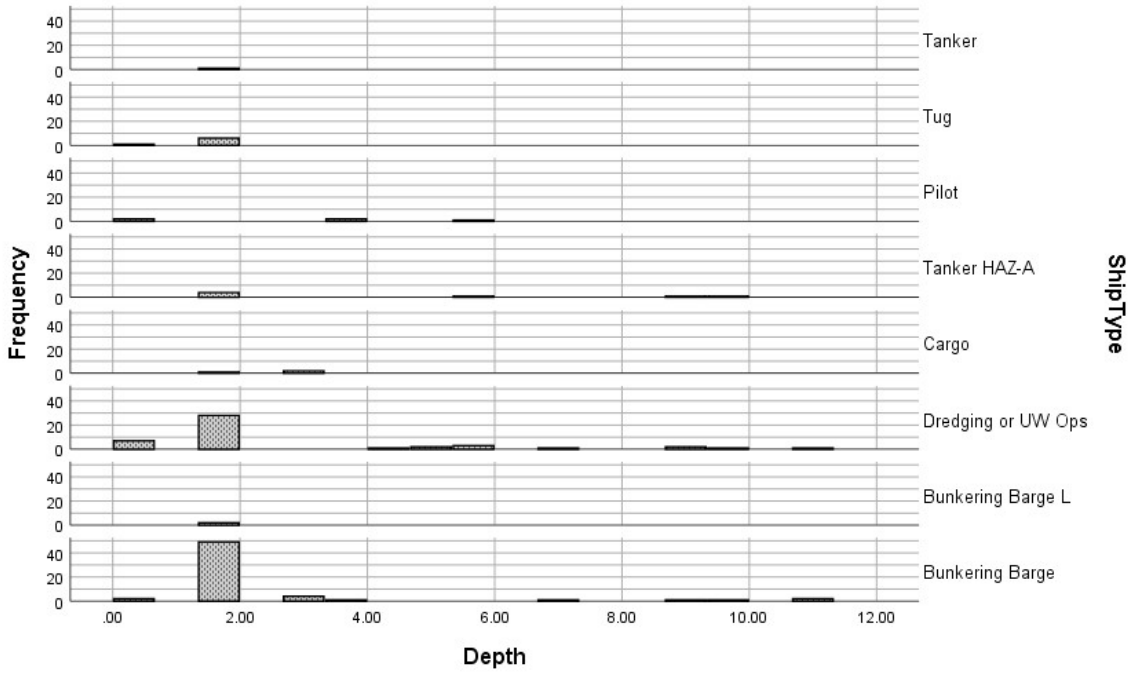


Figure 3.8. Frequency of vessels corresponding to under-predicted current magnitude values predicted by the hydrodynamic-wave forecast model, 'Marine Forecasting System' per water depth level.

3.4 Discussion

This study contributes evidence for the relevance of wind-driven forces on near-surface currents in this multiple user coastal environment. Moreover, it identifies occurrences of under-predicted currents in this Mediterranean bay and port area that could be explained by ship traffic and activity. Therefore, this work provides insight into the physical forces that could contribute towards sea current disturbances and that would help describe the hydrodynamics in this busy port area. These findings contribute knowledge about the relevance of natural and anthropogenic forces on water movement surrounding inshore marine fish farms in the Mediterranean. This provides an appreciation for the distinct wind and ship-influenced hydrodynamic effects, considering the implications for the development of marine aquaculture and spatial planning in similar coastal spaces.

3.4.1 Wind-influenced currents

These findings revealed variable hydrography between the different ADCP deployment sites and across a vertical water profile. Firstly, spatial differences in water movement around the cages would be anticipated due to the infrastructure and the orientation of the fish farm

(Harstein et al., 2021). The shadowing effects of cages have different implications for waste dispersion, production (Harstein et al., 2021) and fish behaviour (Johansson et al., 2014), within the farm. The established correlation between wind and near-surface water current velocities attests to the influence of this forcing factor on water movement in this bay. The temporal differences in hydrography, particularly in the near-surface waters, show that these currents are modulated by the seasonality of their driving force in Marsaxlokk Bay. This corresponds with the variable winds that have been described for this area, and substantiates findings of dominant wind-induced currents and inconsiderable tidal influences (Svašek Hydraulics, 2007). These computed predictions are corroborated by the relationship established between the observed current measurements and the wind forecast of the MARIA/Eta model in the present study. Elsewhere in the Mediterranean region, complex and heterogeneous flows have also been described in bays where near-surface currents are predominantly influenced by wind (Grifoll et al., 2014; Llebot et al., 2014; Cerralbo et al., 2015; Balsells et al., 2020).

These observations of wind-driven near-surface currents have important implications for the development of marine aquaculture. Intermittent and strong water currents driven by strong wind events could influence the traditional circular schooling behaviour of caged fish if current velocities are altered within the cage (Johansson et al., 2014). This could elicit behavioural response with potential effects on welfare and production efficiency (Johansson et al., 2014). Moreover, strong wind effects on near-surface currents could drive differences in water quality within the water column (Harstein et al., 2021) or accentuate flows to improve exchange rates (Holmer, 2010) and disperse wastes (Holmer, 2010; Klebert et al., 2013). This observational study also corroborates predictions that winds are not likely to influence near-seabed currents (Svašek Hydraulics, 2007). However, vertical variations in hydrography with strong current occurrences closer to the seabed and low coefficients of determination indicate external processes or forces that could act to drive or influence these currents. These currents could have distinctly different impacts, such as the mobilisation and suspension of sediments, and important implications for marine aquaculture that would need consideration. At least, an understanding of these effects on hydrodynamics and their implications for cage culture and production can be a support tool for optimised feeding practices and management strategies.

3.4.2 Ship-related currents

Although dominated by the most powerful and perpetual driving factor (wind), hydrodynamic behaviour and response depend on a combination of forcing factors (De Marchis et al., 2014; Grifoll et al., 2014), some of which considered in this study for a more precise

description of the system. Intermittent and specific under-predicted currents that were not explained by meteorological effects were associated with different ship and boat typologies that were operating, navigating or manoeuvring in the bay. The passage of deep-draft cargo ships involved in transhipment and bunkering, and dredging operations near the fish farm in the Marsaxlokk Bay could be cause for ship-generated movement that include near-seabed disturbances. Notwithstanding the possibility of propeller-induced suspended sediments, near-seabed currents could also cause sediment resuspension (Cromey et al., 2012). The short-term effects of suspended sediments on water quality, fish stress and behaviour are well-documented (Kjelland et al., 2015). Therefore, validated ship-generated near-seabed hydrodynamics could have considerable implications for aquaculture production and management, especially in heavily industrialised coastal areas.

The magnitude of impact of suspended sediments on fish depends on factors including sediment tolerance, exposure duration and frequency, sediment type and toxicity, life stage of fish and environmental conditions (Kjelland et al., 2015). Open-water fish farms, particularly in port areas like the study site, may face more frequent and longer periods of exposure to resuspended sediments and distinct hydrodynamic disturbances due to a higher frequency of ship passages. Resuspension may elevate concentrations of metals and other contaminants, eliciting physiological stress responses, altering behaviour, and possibly causing stress and death (Iwama et al., 2004). This presents challenges for fish farms that rely on inshore locations to provide sheltered nursery sites for farmed fish in their juvenile stages. Among the various species-specific effects, exposure to contaminated sediments, that are not uncommon in industrialized bays and port areas, can cause protein degradation and molecular interference in juvenile gilt-head sea bream (*Sparus aurata*) (Ribecco et al., 2011). Even in low quantities, contaminated sediments can induce physiological damage on sea bream when resuspended (Ribecco et al., 2011) and highlight the important regulatory requirements for effective management of co-existing industries, as in the case of Marsaxlokk Bay, and their impacts on the environment and each other.

At the surface and within the water column, different hydrodynamic disturbances could not only have direct effects on fish behaviour and welfare inside the cage (Klebert et al., 2013; Johansson et al., 2014) but also characterise floating collar and net deformations, and cause nuisance to farm structures in terms of engineering and economic effects (Klebert et al., 2013; Faltinsen and Shen, 2018). Distinct hydrodynamic effects within the vertical water gradient depend on ship characteristics (e.g. vessel type, size hull shape) that influence the magnitude and behaviour of currents in shipping waterways (Bellafiore et al., 2018; Mao and Chen, 2020; Mao et al., 2020). The vessels identified in Marsaxlokk Bay present different ship typologies that could potentially have distinct hydrodynamic effects within the

vertical gradient and that can have different interactions with the surrounding environment and aquaculture, which have yet to be understood. Fish farmers lack the freedom to choose their farm locations or adjust the orientation and layout of existing farms to enhance fish welfare, hence it is crucial to have stable currents and favourable water conditions at the farm site to ensure fish welfare and production. Therefore, understanding the conflicting interactions with ship traffic, monitoring the impacts on aquaculture, and having co-existing activities managed effectively by spatial planning principles and regulations is essential.

Considering these hydrodynamic effects is crucial for marine aquaculture development, especially in multiple-use areas and where challenged by pre-existing, traditional and socioeconomically significant maritime industries. As coastal cities urbanize, inshore areas will remain important sheltered locations for aquaculture, intensifying competition. The potential impacts on farmed fish welfare and fish farm infrastructure highlight the significant challenges that ship-influenced disturbances can pose to the development of aquaculture. Current disturbances influenced by navigation and dredging may add to the complexities of these coastal environments and may require additional consideration for monitoring and management, especially within the context of expansion of the maritime industries. This identifies the need for further research on interactions between different coastal users, the hydrodynamic environment, and their implications for cage aquaculture. To address these gaps, marine spatial planning is advocated as a means of planning and managing activities in crowded coastal areas, and models play a vital role in finding appropriate trade-offs to make better use of space and resources that promote aquaculture expansion (Falconer et al., 2023).

These findings support decision-making tools in providing a reliable evidence-based approach for coastal management and development. Albeit important to maintain the general applicability of decision-making tools and to appreciate the limits and uncertainty of modelling approaches especially when implemented under simplified conditions, this research helps advance knowledge on the effects of different natural and anthropogenic forces on water movement. This is relevant to describe the complexities of multiple user marine spaces like Marsaxlokk Bay, particularly in terms of site and event specific forcing that influence and drive hydrodynamic variability. This realistic description of coastal dynamics helps to address functionality gaps in decision-making tools when applied under similar scenarios. Therefore, resolving water movement variability under realistic forcing conditions and then understanding the implications for marine aquaculture is essential knowledge that benefits the environmental, economic and social aspects linked with the development of the industry. Moreover, it helps to meet expectations of reliable and effective management and optimisation of site and resource use, at farm and bay scale.

Nevertheless, the effective management and development of marine aquaculture does not only rely on a true and detailed hydrodynamic description, but also decision-support tools that account for site-specific complexities associated with farm management and husbandry practices.

3.5 Conclusion

This study reveals variable seawater currents in dynamic coastal spaces where multiple maritime activities can influence farm-scale hydrodynamics. This research describes causes for distinct hydrodynamic disturbances that may need to be embraced for a more detailed description of seawater current complexities. Dominant and perpetual wind forcing, which is already a fundamental component of hydrodynamic models, influenced near-surface currents in this semi-enclosed bay. However, intermittent seawater current disturbances could not be explained by meteorological influences but were associated with maritime traffic and operations in this crowded coastal space. Therefore, to provide a detailed account of local farm-scale hydrodynamics, anthropogenically-derived forcing variables on water movement through fish farms should be accounted for in these dynamic environments.

Decision-support tools should consider these real-world complexities in processes and policies for the effective management and development of marine aquaculture in these complex coastal areas. Under these circumstances, marine aquaculture can be influenced by different wind and ship-influenced impacts that need to be assessed further to understand how to predict and mitigate these hydrodynamic effects effectively. Where the expansion of marine aquaculture is increasingly challenged by strong wind events and coastal maritime activity, these variable and dynamic forcing factors need further representation.

Chapter 4. Real-world waste dispersion modelling for benthic Integrated Multi-Trophic Aquaculture

Abstract

In real-world situations, marine fish farms accommodate multiple fish species and cohorts within the farm, leading to diverse farm layouts influenced by cage dimensions, configurations, and intricate arrangements. These cage management practices are essential to meet production demands, however, farm-level complexities can impact model predictions of waste deposition and benthic impact near fish cages. This is of particular importance when the cages are used for integrated multi-trophic aquaculture (IMTA) with benthic feeders, where this waste not only affects environmental conditions but also provides a potential food source. The Cage Aquaculture Particulate Output and Transport (CAPOT) model incorporated multiple species, cohorts, and cage arrangements to estimate waste distribution from a commercial fish farm in the Mediterranean between October 2018 and July 2019. This spreadsheet model estimated dispersion for individual fish cages using a grid resolution of 5 m x 5 m. The study categorized discrete production periods for each fish cage every month, aligning with intermittent changes in biomass and food inputs due to different cage management practices throughout production. This approach facilitated the use of detailed input data and enhanced model representativeness by considering variations in cage biomass, food types, settling velocities, and configurations. Model outputs, represented in contour plots, indicated higher deposition directly below fish cages that varied monthly throughout fish production cycles. Deposition footprints reflected changes in cage biomass, food inputs, and farm-level practices reflecting this real-world scenario where aquaculture does not follow a production continuum. Moreover, cohort dynamics and cage movements associated with the cage management practices of the fish farm influenced the quantity and fate of wastes distributed around fish cages, revealing variability in deposition footprints. Clearly, these findings have important implications for the design of benthic IMTA systems, with species such as sea cucumber and polychaetes. Variability in waste deposition creates challenges in identifying where the benthic organisms should be placed to allow optimal uptake of waste to meet their food requirements and increase survivability. Evidently, models have an important role to play and this study emphasizes the need for representative input data to describe actual food inputs, cage biomass changes, and management practices for more representative farm-scale modelling and essentially to improve particulate waste management. To effectively mitigate benthic impacts through IMTA, models must quantify and resolve particulate waste distribution and impact around fish farms to maintain a balanced system with net removal of wastes. Resolving farm-level complexities provides vital information about the variability of food availability and quality for extractive organisms that helps improve recycling of organic wastes in integrated systems, demanding a more representative modelling approach.

4.1 Introduction

Marine fish farms vary considerably in terms of size, husbandry techniques and management practices. Farm layouts can be highly variable between species, production intensity, and location. Different sizes and shapes of cages are used, and even within individual farms, there can often be complex or irregularly organised cage systems (Magill et al., 2006; Ferreira et al., 2008). The organisation of cages within a farm is one of the major factors that influences impact of waste on the surrounding environment, and a better understanding of different farm layouts could help reduce environmental impact (McIntosh et al., 2022). Further layers of complexity arise in many countries, where multiple fish species are farmed at the same site with minimal organisation of species and size classes within the farm (Magill et al., 2006; Ferreira et al., 2008; Cromey et al., 2012). When multiple cohorts of different species and sizes of fish are stocked at different times in adjacent cages on the same fish farm, a range of cage management practices (e.g. cage batch inputs, cage splitting, cage movement and re-organisation) are required to accommodate production demands. These management practices influence the standing biomass of farmed fish in fish cages and the feeding requirements at the fish farm. Due to these complexities, production is not constant and consequently particulate waste dispersion and deposition near fish cages varies. These practices can present new challenges for predicting waste deposition around these fish farms and have implications for management and mitigation of benthic impacts.

Models are used by the aquaculture industry and regulators to help ensure compliance with environmental regulations and evaluate production levels within the carrying capacity of the system (Ferreira et al., 2012; Ross et al., 2013). Particle dispersion models simulate the fate and transport of particulate wastes from marine fish cages (Cromey et al., 2002; Corner et al., 2006) and predict the benthic impacts of farmed species, including Atlantic salmon (*Salmo salar*) (Cromey et al., 2002; Stigebrandt et al., 2004; Chamberlain and Stucchi, 2007), and gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) (Jusup et al., 2009; Cromey et al., 2012). These waste dispersion models are often used as decision-support tools that inform aquaculture planning and licensing processes, providing insight into how a farm might impact the environment and what production level may be acceptable within regulatory limits (Luthman et al., 2019). Models have been used to predict waste deposition from marine fish farms for many years (Gowen et al., 1989), and there have been many advances since the first applications. However, models have limitations when fish farm management practices like cage movement are not represented in detail (Cromey et al., 2009).

Waste dispersion models tend to use whole farm summaries of feed input, and short-term and averaged data to predict benthic flux from the production of multiple fish species and sizes, within the same farm (Cromey et al., 2002; Chamberlain and Stucchi, 2007; Cromey et al., 2012; Riera et al., 2017). Summarised husbandry information can limit model data inputs, and while still valid for simplified production scenarios, detailed input data helps to improve the representativeness of established farm-scale models (Cromey et al., 2012; Chang et al., 2014). Similarly, the use of species-specific information over single averaged data inputs provides better representation of simulated waste deposition from multiple species and cohort fish farms (Magill et al., 2006; Cromey et al., 2012). Where fish farm production is not constant, discrete changes influence the deposition footprint (Chary et al., 2021; Chary et al., 2022). Since the deposited waste material is consumed by benthic integrated multi-trophic aquaculture (IMTA) species, the modelled footprint is an indication of food availability and environmental conditions. Hence, simplified scenarios of cohort dynamics and changes in positions of the cage are not enough for accurate representation of fish farm deposition footprints needed when used in IMTA with bottom-dwelling extractive species.

Knowledge gaps exist in understanding the variability in fish farm deposition footprints that is associated with the complexities of real-world cage management practices, which has particular importance in setting up deposit-feeding lower trophic species within an IMTA system. The farm-scale spreadsheet-based Cage Aquaculture Particulate Output and Transport (CAPOT) model provides the flexibility to account for complex cage configurations and management practices (Telfer et al., 2022). In this chapter, the model was used to predict waste deposition from multiple species from different cohorts of fish at the fish farm. The distribution of sediment carbon was predicted for discrete periods of production every month established to account for cage management operations carried out at individual cages on the fish farm during production. In our predictions of waste deposition, simulations were based on real-time hydrodynamic conditions, species-specific literature data, discrete food input and cage biomass information, and accounts of cage movements. In the present study, emphasizing the variability in waste deposition around complex fish farming practices contributes towards better predictions of deposition footprints. The work has important implications for effective management and mitigation of benthic impact, particularly in placement and management of deposit-feeding IMTA systems, such as sea cucumbers.

4.2 Materials and methods

4.2.1 Study site

The study was set up in Marsaxlokk Bay, Malta (Fig. 4.1 A), at the nearshore cage-based commercial fish farm (35°49'39.90" N, 14°32'30.73" E) (Fig. 4.1 B). At the time of the study, the fish farm reported a total annual production of 719 t and a feed conversion ratio of 1.7. During the study period, commercially available formulated feeds were used for the continual production of sea bream and sea bass juveniles. The juveniles had been transferred as hatchery-produced fingerlings (about 2 – 3 g) for grow-out at this nearshore aquaculture facility (approximately 13 months at the time of study), before being transferred at about 190 g to an offshore site in deeper waters where they are cultured for approximately 17 months until harvest (harvest size of 550 g). These juveniles are transferred from this shallow and sheltered site to an offshore site in deeper waters where they are cultured until harvest. Moreover, this nearshore fish farm produced small quantities of greater amberjack (*Seriola dumerili*) in one of the fish cages using chopped baitfish fed 2 – 3 times a week throughout the study period.

The fish farm has 20 round fish cages that are 12 m or 28 m in diameter. The fish cages have net depths that are between 7 m and 10 m. Fig. 4.1 C shows the cage dimensions and the irregular arrangement of fish cages. Throughout the study, the changes between cage positions in Fig. 4.1 B influenced fish production and altered the cage layout of the fish farm. Water depth was taken from *in-situ* measurements near each fish cage at the fish farm. The fish farm lies on an increasing downward slope into deeper waters in a south-west direction, so that cages 1 to 6 are in 12-13 m water depth, cages 7 to 12 are in 10-11 m, and cages 13 to 20 in 8-9 m.

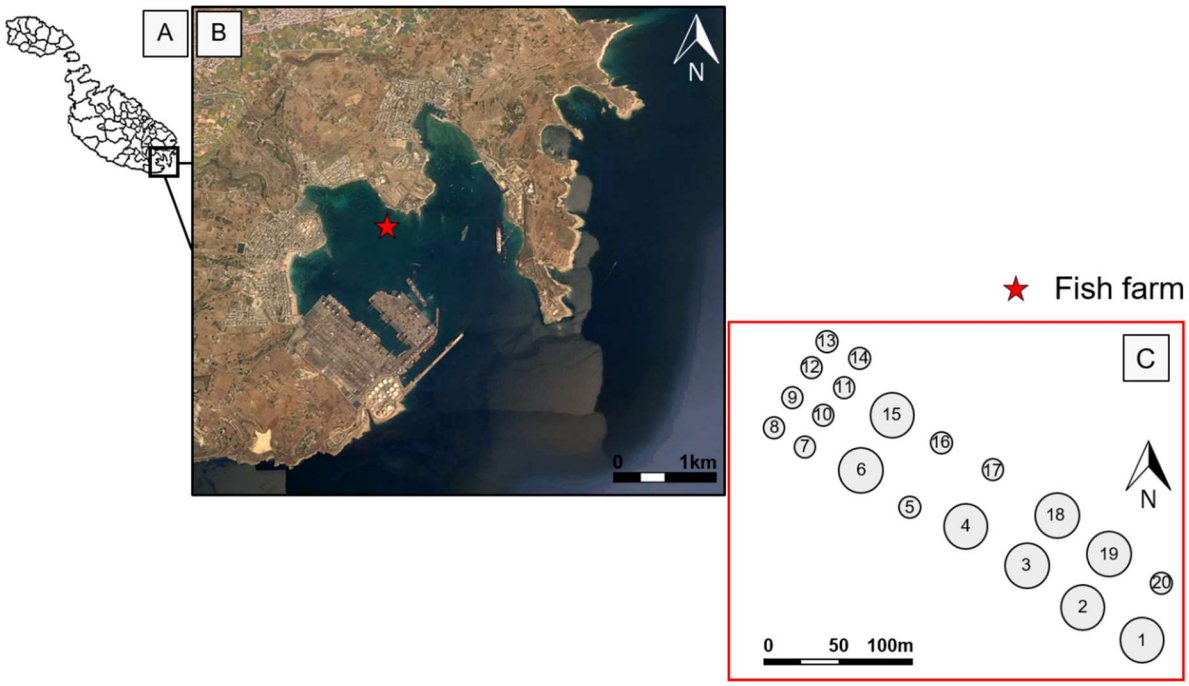


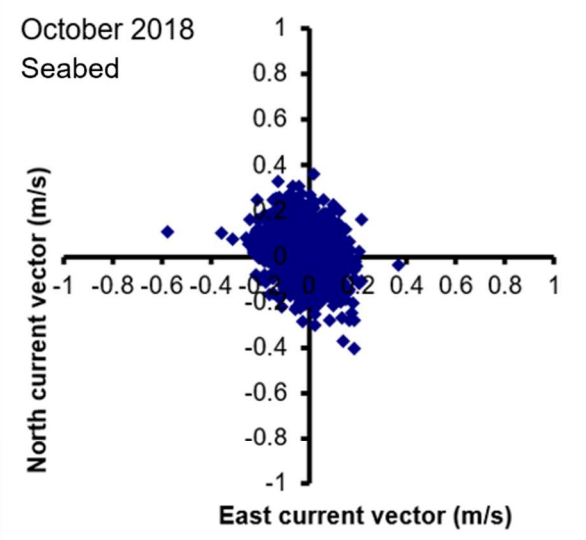
Figure 4.1. A. Location of the study site in the southeast of Malta, and B. the fish farm in Marsaxlokk Bay. C. Arrangement of fish cages at the existing aquaculture facility at the site. Grey circles represent fish cages of different dimensions and numbered for position reference. These positions are not always occupied.

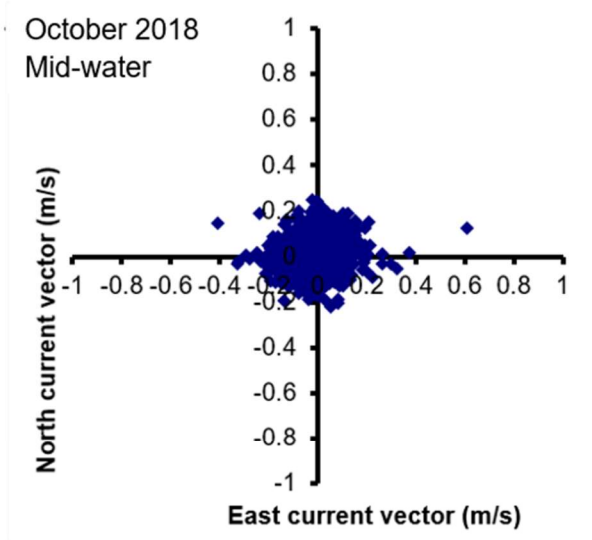
4.2.2 Waste dispersion

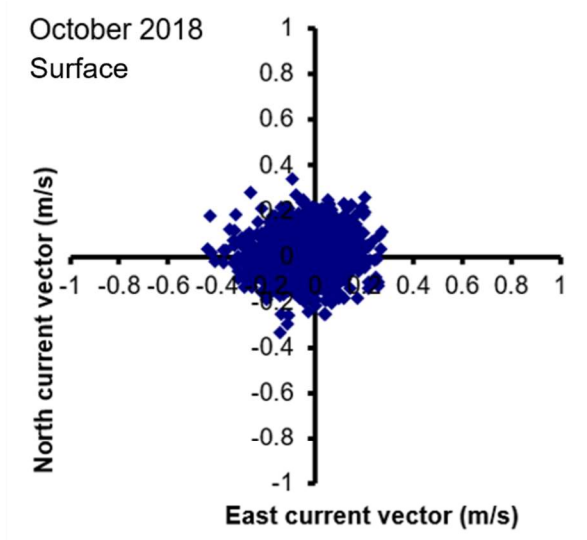
The dispersion of particulate wastes around the nearshore fish farm in Marsaxlokk Bay was modelled using the depositional model 'Cage Aquaculture Particulate Output and Transport' (CAPOT) (Telfer et al., 2022). CAPOT compares favourably with established models that are used for environmental regulation (e.g. Cromey et al., 2002). CAPOT models particulate waste input over a fish production period, using actual/planned feed input, biomass increase per fish cage, or an estimated Feed Conversion Ratio.

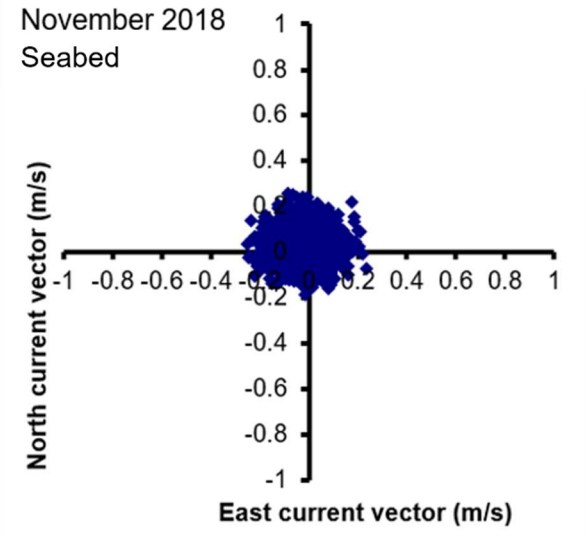
The model uses the following information: hydrographic data, food input and fish biomass data, depth of nets, water depth, and size and arrangement of cages. *In-situ* current speed (m s^{-1}) and direction (° N) taken from three depths in the water column represented near surface, mid-water, near seafloor currents. Hydrographic data was collected continuously between ten locations around the farm, and always within 20 m from the nearest fish cage, over a 16-month period between May 2018 and August 2019. Data was extracted every month from the positions around the fish cages described in Chapter 3). Current speed and direction were taken at 20-minute intervals for whole months throughout the study. Water currents were predominantly in an east to north direction through the fish farm most of the time and variable, particularly at different depths (as presented in Fig. 4.2). Plots show

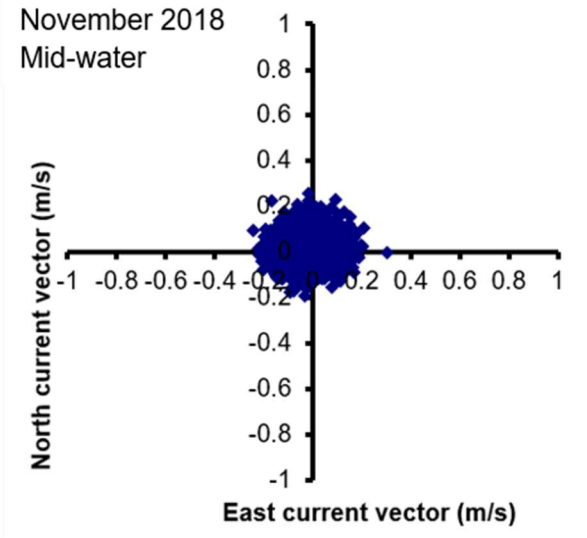
currents at the near-seafloor depth (3 m), at the near-surface depth (i.e. 7 m in October 2018, 13 m in November 2018, 9 m in December 2018, 8 m in January 2019, 12 m in February 2019, 10 m in March 2019, 9 m in April and May 2019, 11 m in June and July 2019), and the respective mid-water depths. Especially considering the variability in local hydrographic conditions at the site (Chapter 3), simulations of waste distribution were based on detailed whole-month current datasets. This hydrographic data corresponded with the production data that was modelled every month to represent the path of initial waste settlement. The model calculates average current speeds and directions across the depth range at each time-point, providing an approximate path for initial waste settlement through the water column.

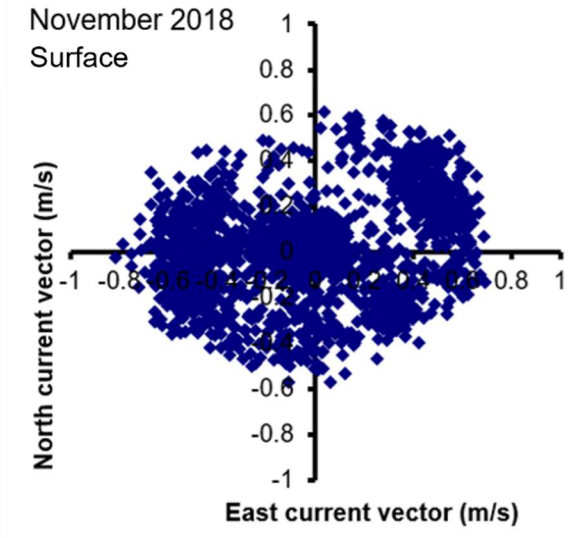

October 2018
Seabed
North current vector (m/s)
East current vector (m/s)

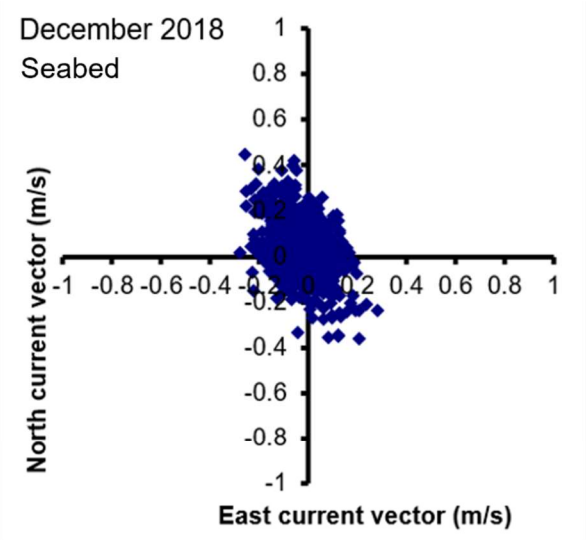

October 2018
Mid-water
North current vector (m/s)
East current vector (m/s)

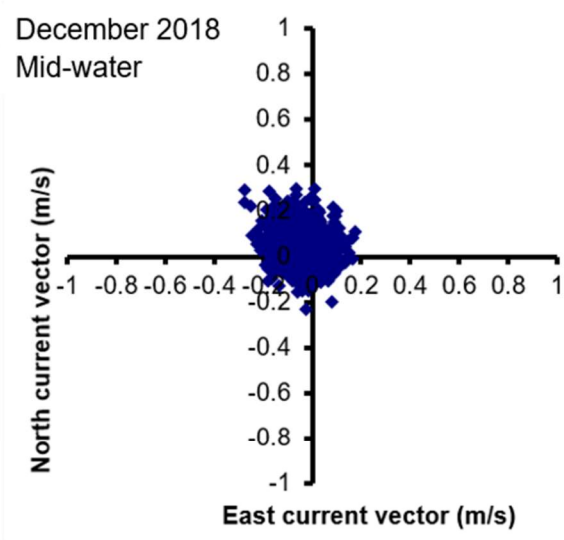

October 2018
Surface
North current vector (m/s)
East current vector (m/s)

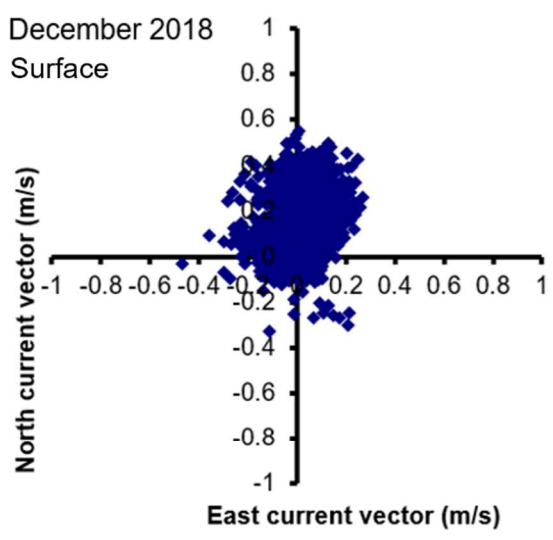

November 2018
Seabed
North current vector (m/s)
East current vector (m/s)

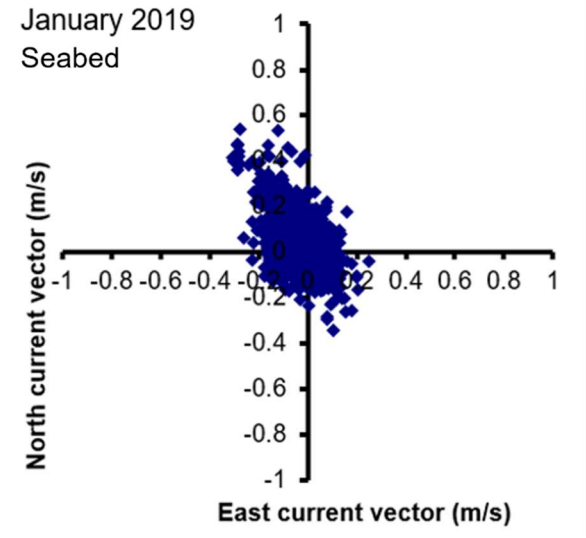

November 2018
Mid-water
North current vector (m/s)
East current vector (m/s)

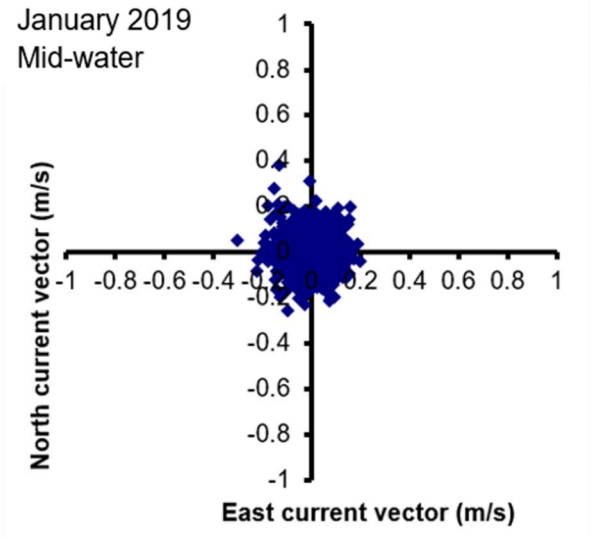

November 2018
Surface
North current vector (m/s)
East current vector (m/s)

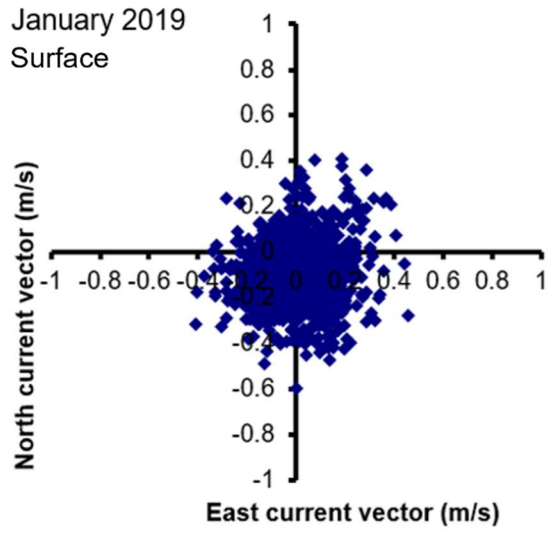

December 2018
Seabed
North current vector (m/s)
East current vector (m/s)

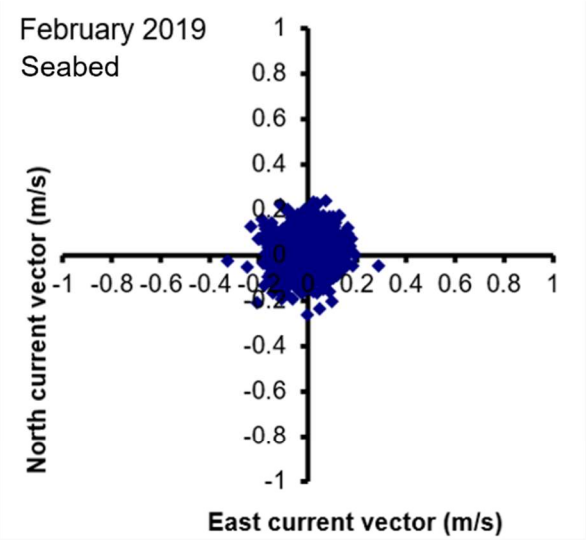

December 2018
Mid-water
North current vector (m/s)
East current vector (m/s)

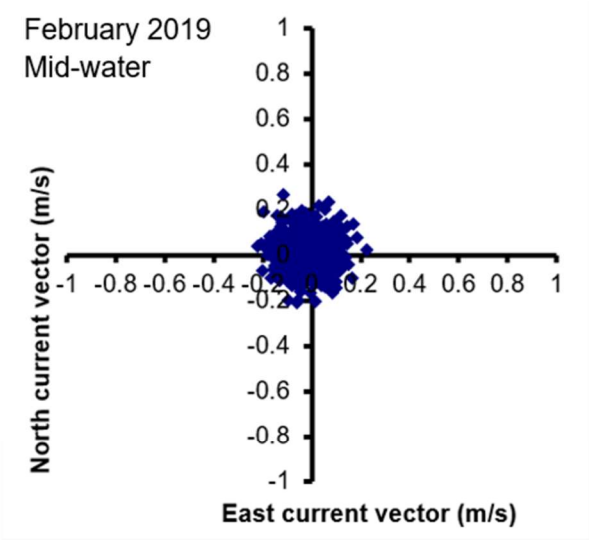

December 2018
Surface
North current vector (m/s)
East current vector (m/s)

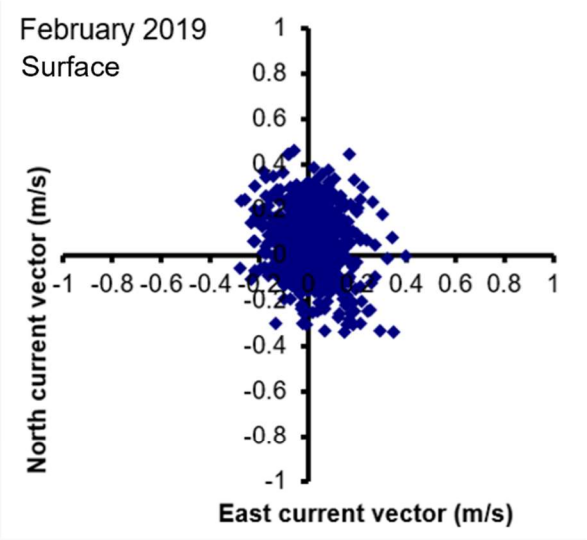

January 2019
Seabed
North current vector (m/s)
East current vector (m/s)

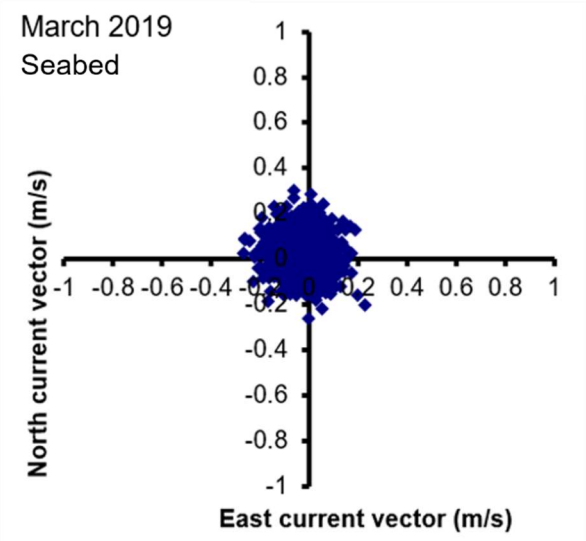

January 2019
Mid-water
North current vector (m/s)
East current vector (m/s)

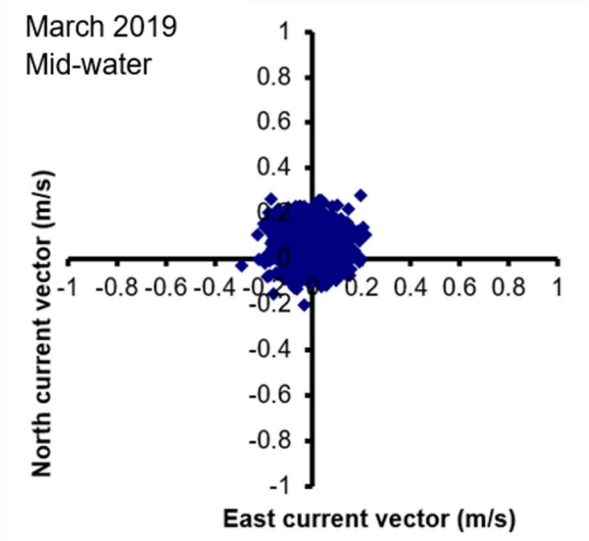

January 2019
Surface
North current vector (m/s)
East current vector (m/s)

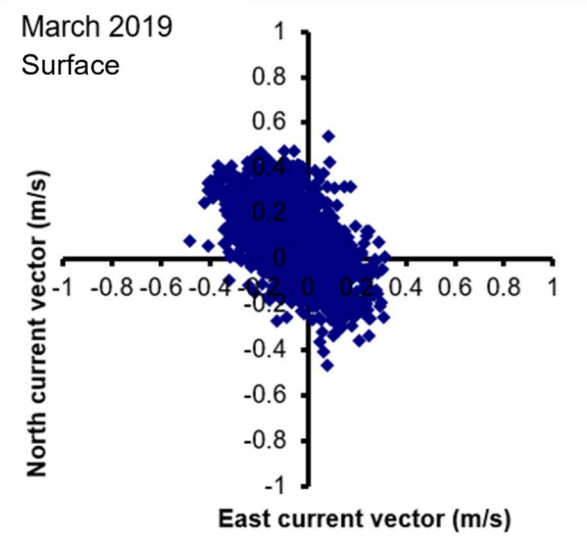

February 2019
Seabed
North current vector (m/s)
East current vector (m/s)

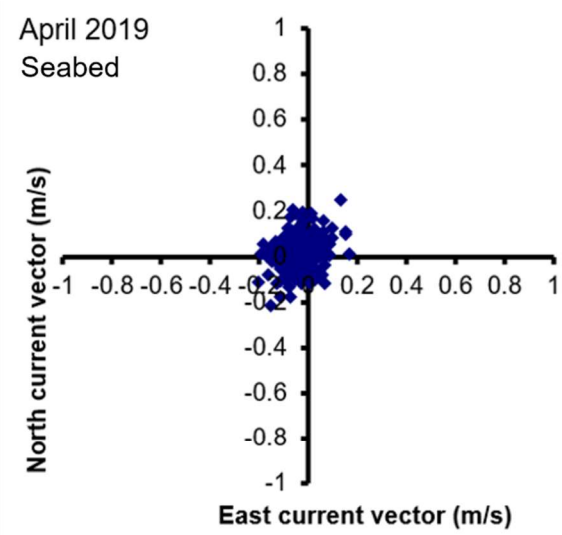

February 2019
Mid-water
North current vector (m/s)
East current vector (m/s)

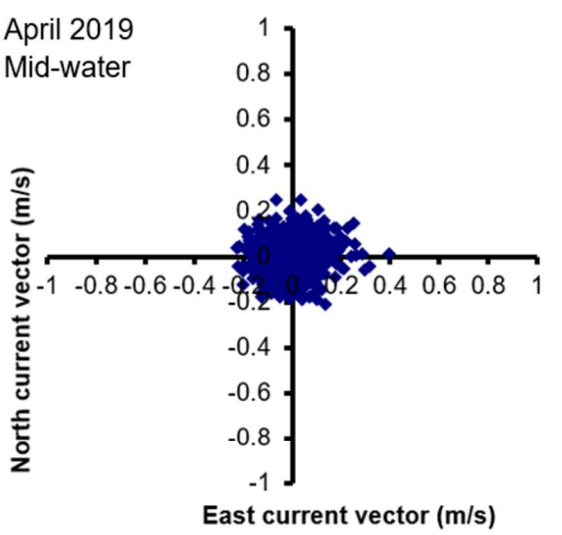

February 2019
Surface
North current vector (m/s)
East current vector (m/s)

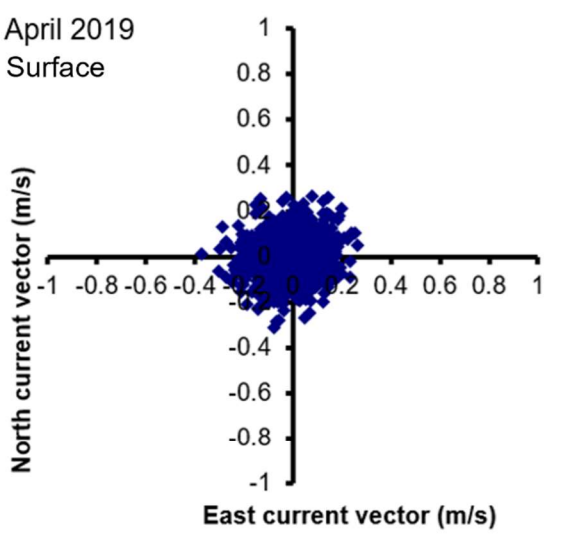

March 2019
Seabed
North current vector (m/s)
East current vector (m/s)

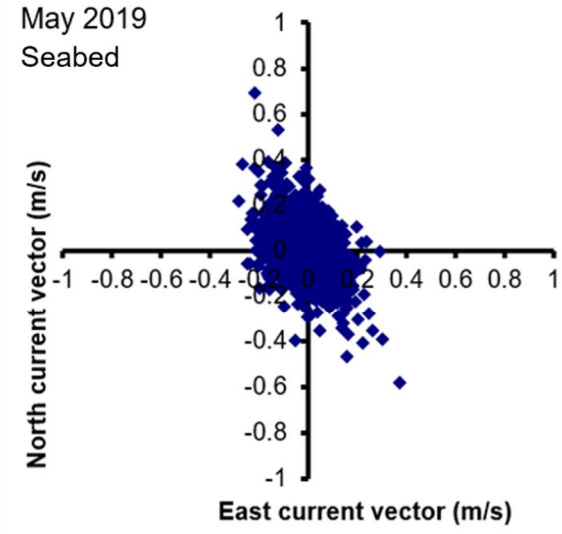

March 2019
Mid-water
North current vector (m/s)
East current vector (m/s)

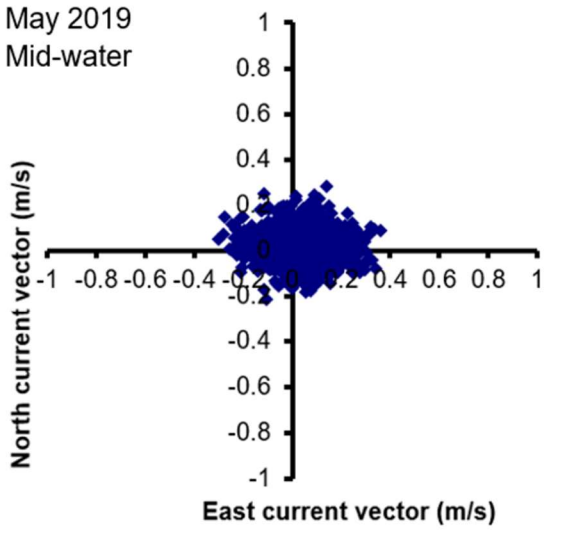

March 2019
Surface
North current vector (m/s)
East current vector (m/s)

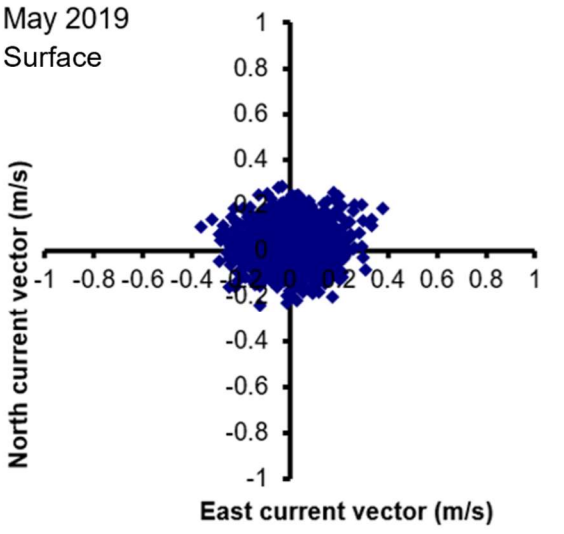

April 2019
Seabed
North current vector (m/s)
East current vector (m/s)


April 2019
Mid-water
North current vector (m/s)
East current vector (m/s)


April 2019
Surface
North current vector (m/s)
East current vector (m/s)


May 2019
Seabed
North current vector (m/s)
East current vector (m/s)


May 2019
Mid-water
North current vector (m/s)
East current vector (m/s)


May 2019
Surface
North current vector (m/s)
East current vector (m/s)

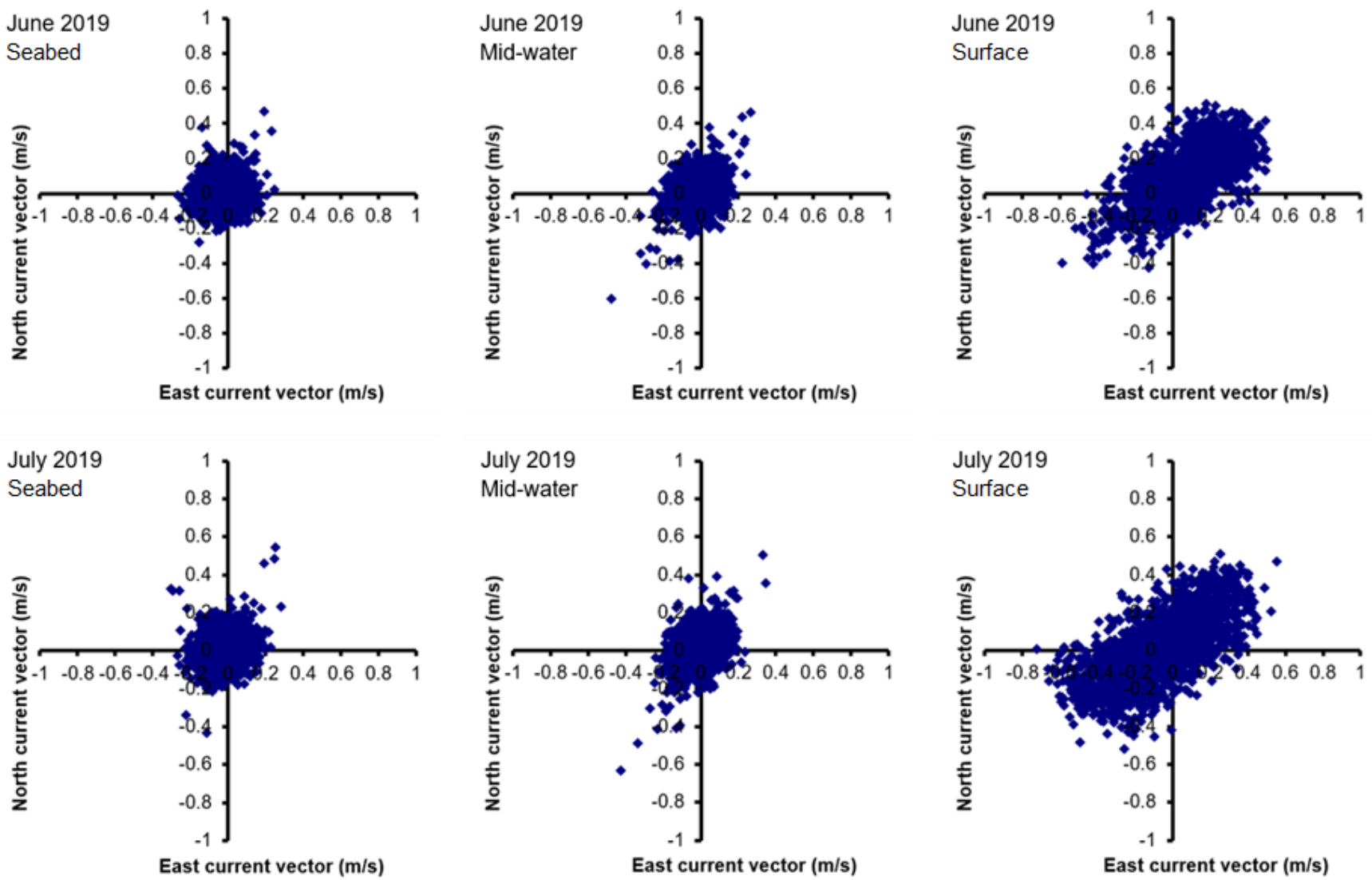


Figure 4.2. Plots for currents measured near surface, mid-water and near seabed depths at different positions around the fish farm throughout the study period between October 2018 and July 2019 shown in Fig. 3.1 C.

Particulate waste dispersion modelling involves two phases that include the estimation of dispersion of waste settling to the seabed and the calculation of the quantity and form of waste released into the environment. Within the waste dispersion model, mass balance equations were used to determine the amount of organic carbon and the form of waste dispersed from the fish farm to the surrounding environment (Telfer et al., 2022). Feed wastage, actual feed input and cage biomass data for sea bream, sea bass and amberjack culture were used within the model to estimate dispersion from fish cages for these farmed species.

Waste dispersion for each fish cage was assessed monthly, utilizing the actual feed input and cage biomass data, if the cage management practices did not affect the production process. However, when these practices caused changes in fish cage biomass (excluding mortalities) beyond the typical increment associated with regular production, waste dispersion was estimated using distinct data about cage biomass and food input. From October 2018 to July 2019, specific production periods were determined and modelled for each fish cage every month. These periods aligned with intermittent instances when the biomass within the cages shifted due to reasons beyond the usual growth associated with standard cage production, as shown in Fig. 4.3. This categorization into ‘discrete’ production periods mirrors the modifications triggered by differing management strategies at the fish cage during the study period. As a result, multiple modelled periods were created under these conditions. Discrete input data captured the variations in cage biomass, food types and quantities, settling velocities of food and faecal matter, and cage dimensions and configurations. This data was collected for each cage on a monthly basis throughout the production period, as detailed in Table 4.1.

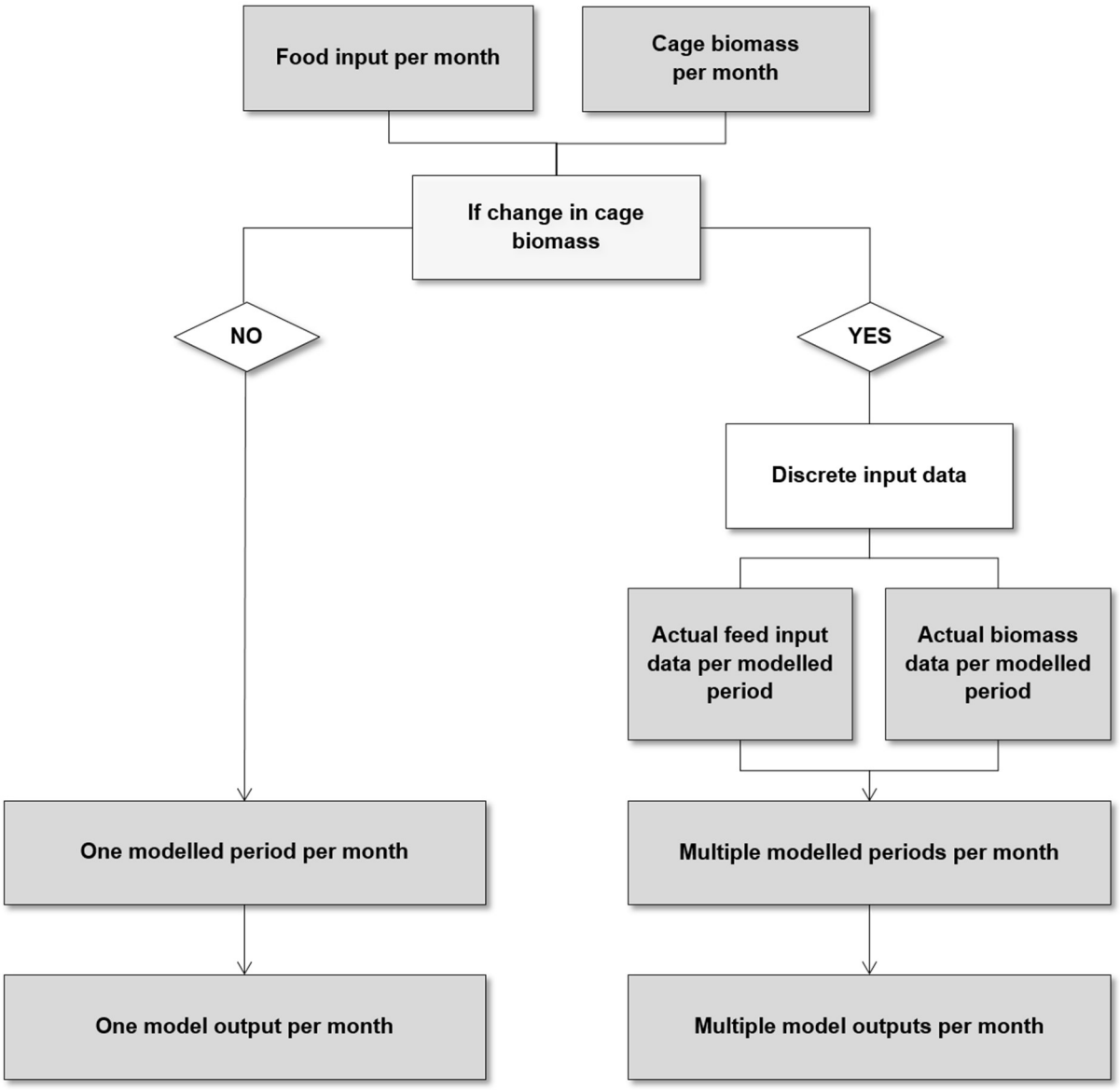


Figure 4.3. Flow diagram of the modifications in the process used to model waste dispersion per cage. Arrows indicate model outputs.

In more detail, cage management practices included ‘cage input’ when fish batches were added to existing fish cages, ‘cage repositioning’ when existing fish cages were moved into new positions within the grid layout, ‘cage splitting’ that split fish batches in existing cages into multiple fish batches, ‘cage joining’ that combined fish batches from different cages, ‘site transfer’ when fish batches or cages were moved from the nursery facility to the offshore site for grow-out, and cage harvesting (partial or complete). These practices influenced the arrangement of fish cages at the fish farm, and the cage biomass and feed inputs. To account for changes in production, the fate of particulate solids was modelled for each cage during discrete periods of constant production and fixed cage arrangement for every month.

When cage management practices influenced cage biomass in fish cages at the fish farm, discrete input data was modelled instead of monthly food and cage biomass information. The consequent changes in fish farm cage positioning were accounted for by repositioning grid cells for cages in the model. Fish farm management practices were identified in husbandry data provided by the farm manager and discrete periods of production were modelled when cage biomass was constant prior to and following intervals in production (Table 4.1). Therefore, cage production was considered closed and followed by the onset of a discrete period of production at intermittent intervals when cage management practices influenced cage biomass. These distinctly different cage production setups were modelled separately. For instance, discrete periods of production were modelled separately around fish farm operations when the cage biomass was split or combined. The approach of modelling discrete periods of production for fish cages that were influenced by cage management practices facilitated the use of detailed input data to improve the representativeness of fish farm production. Moreover, discrete model outputs represented distinct periods of consistent change in cage biomass and food input, and fixed cage arrangement, and therefore accounted for change in production and waste dispersion.

Table 4.1. Cage production at the fish farm with changes in cage biomass and feed inputs between October 2018 and July 2019.

Cage No.	Species	Start Date	End Date	Opening Biomass (kg)	Closing Biomass (kg)	Period Transfer (-) (kg)	Period Transfer (+) (kg)	Period Stocking (kg)	Cage Move From	Cage Move To	Transaction Type	Net Size	Actual Feed Input (kg)	Feed Type
October 2018														
1	*S. aurata*	30/09/2018	31/10/2018	2705.69	4425.84							SS	2515	StartPrem -1.5mm, PreGrower-2.0mm
2	*S. aurata*	30/09/2018	31/10/2018	12567.59	16517.32							SS	6050	Supreme-3.0mm, PreGrower-2.0mm
3	*S. dumerili*	30/09/2018	31/10/2018	172652.68	172924.00							MS	1800	Baitfish
4	*S. aurata*	30/09/2018	31/10/2018	28685.82	34034.93							MS	11968.75	Supreme-3.0mm, PreGrower-2.0mm
5	*S. aurata*	23/10/2018	31/10/2018	0.00	58948.73		57890.1				Cage splitting	MS	4531.25	Supreme-4.5mm
	S. aurata	30/09/2018	22/10/2018	10296.79	0.00	-13971			5	7	Cage repositioning	SS	4306.25	Supreme-3.0mm, PreGrower-2.0mm
6	*S. aurata*	30/09/2018	22/10/2018	110864.52	115765.67	-57890.1			6	5	Cage splitting	MS	18125	Supreme-4.5mm
	S. aurata	23/10/2018	31/10/2018	57967.35	58527.52							MS	1875	Supreme-4.5mm
7	*S. aurata*	22/10/2018	31/10/2018	0.00	14652.31		13971				Cage repositioning	SS	1718.75	Supreme-3.0mm
9	*S. aurata*	30/10/2018	31/10/2018	0.00	974.71			917.5			Cage input	SS	70	StartPrem -1.0mm
10	*S. aurata*	30/09/2018	31/10/2018	4933.41	7041.65							SS	3000	StartPrem -1.5mm, PreGrower-2.0mm
11	*S. aurata*	30/09/2018	31/10/2018	4259.02	6326.85							SS	3000	StartPrem -1.5mm, PreGrower-2.0mm
12	*S. aurata*	30/10/2018	31/10/2018	0.00	875.99			817.5			Cage input	SS	70	StartPrem -1.0mm
13	*S. aurata*	30/09/2018	31/10/2018	5250.72	7821.24							SS	3750	PreGrower-2.0mm, StartPrem -1.5mm
14	*S. aurata*	30/09/2018	31/10/2018	6831.02	8736.69							SS	3500	PreGrower-2.0mm, StartPrem -1.5mm
15	*S. aurata*	30/09/2018	31/10/2018	9258.46	11617.93							SS	4893.75	PreGrower-2.0mm, Supreme-3.0mm
16	*S. aurata*	30/09/2018	31/10/2018	10922.82	14137.70							SS	4556.25	Supreme-3.0mm, PreGrower-2.0mm
17	*S. aurata*	30/09/2018	31/10/2018	12593.49	13140.08							SS	4868.75	Supreme-3.0mm, PreGrower-2.0mm
18	*S. aurata*	30/09/2018	31/10/2018	8323.95	12420.13							SS	4868.75	PreGrower-2.0mm, Supreme-3.0mm
19	*S. aurata*	30/09/2018	31/10/2018	2608.93	4337.34							SS	2515	StartPrem -1.5mm, PreGrower-2.0mm
20	*S. aurata*	30/09/2018	31/10/2018	73324.19	81120.75							MS	17187.5	Supreme-3.0mm, Supreme-4.5mm
November 2018														
1	*S. aurata*	31/10/2018	30/11/2018	4425.84	5358.55							SS	2235	PreGrower-2.0mm, StartPrem -1.5mm
2	*S. aurata*	21/11/2018	30/11/2018	34866.85	36098.80		16508.9				Cage joining	MS	2656.3	Supreme-3.0mm
	S. aurata	31/10/2018	20/11/2018	16517.32	18357.85							MS	7656.25	Supreme-3.0mm
3	*S. dumerili*	31/10/2018	30/11/2018	172924.00	173348.51							MS	2300	Baitfish
4	*S. aurata*	31/10/2018	30/11/2018	34034.93	44581.13							MS	9593.75	PreGrower-2.0mm, Supreme-3.0mm
5	*S. aurata*	31/10/2018	30/11/2018	58948.73	62989.13							MS	14218.75	Supreme-4.5mm, Sup-Winter-4.5mm
6	*S. aurata*	31/10/2018	30/11/2018	58527.52	60399.06							MS	6406.25	Supreme-4.5mm, Sup-Winter-4.5mm
7	*S. aurata*	22/11/2018	30/11/2018	0.00	1512.60			1244.92			Cage input	SS	350	StartPrem -1.0mm, StartPrem -1.5mm
	S. aurata	31/10/2018	21/11/2018	14652.31	0.00	-16508.86			7	2	Cage joining	SS	4062.5	Supreme-3.0mm
8	*S. aurata*	13/11/2018	30/11/2018	0.00	2456.05			1622.27			Cage input	SS	690	StartPrem -1.5mm

9	*S. aurata*	31/10/2018	30/11/2018	974.71	2028.15					SS	1290	StartPrem -1.0mm, StartPrem -1.5mm
10	*S. aurata*	31/10/2018	30/11/2018	7041.65	10206.58					SS	2800	PreGrower-2.0mm
11	*S. aurata*	31/10/2018	30/11/2018	6326.85	8704.89					SS	2775	PreGrower-2.0mm
12	*S. aurata*	31/10/2018	30/11/2018	875.99	1939.38					SS	1290	StartPrem -1.0mm, StartPrem -1.5mm
13	*S. aurata*	31/10/2018	30/11/2018	7821.24	10512.57					SS	3812.5	PreGrower-2.0mm, Supreme-3.0mm
14	*S. aurata*	31/10/2018	30/11/2018	8736.69	10913.41					SS	3812.5	PreGrower-2.0mm, Supreme-3.0mm
15	*S. aurata*	31/10/2018	30/11/2018	11617.93	13056.44					SS	4026.875	Supreme-3.0mm, PreGrower-2.0mm, StartPrem -1.5mm
16	*S. aurata*	31/10/2018	30/11/2018	14137.70	16124.32					SS	4687.5	Supreme-3.0mm
17	*S. aurata*	31/10/2018	30/11/2018	13140.08	15100.52					SS	4687.5	Supreme-3.0mm
18	*S. aurata*	31/10/2018	30/11/2018	12420.13	14063.36					SS	4046.875	Supreme-3.0mm, PreGrower-2.0mm
19	*S. aurata*	31/10/2018	30/11/2018	4337.34	5189.90					SS	2235	PreGrower-2.0mm, StartPrem -1.5mm
20	*S. aurata*	31/10/2018	30/11/2018	81120.75	87067.34					MS	12656.25	Supreme-3.0mm
December 2018												
1	*S. aurata*	30/11/2018	31/12/2018	5358.55	7031.89					SS	3587.5	PreGrower-2.0mm
2	*S. aurata*	30/11/2018	31/12/2018	36098.80	40223.83					MS	11250	Supreme-3.0mm
3	*S. dumerili*	30/11/2018	31/12/2018	173348.51	173945.01					MS	4950	Baitfish
4	*S. aurata*	30/11/2018	31/12/2018	44581.13	47998.87					MS	10781.25	Supreme-3.0mm
5	*S. aurata*	30/11/2018	31/12/2018	62989.13	66395.41					MS	9843.75	Sup-Winter-4.5mm, Supreme-4.5mm
6	*S. aurata*	30/11/2018	31/12/2018	60399.06	62011.05					MS	7343.75	Sup-Winter-4.5mm, Supreme-4.5mm
7	*S. aurata*	30/11/2018	31/12/2018	1512.60	2261.90					SS	1305	StartPrem -1.5mm, StartPrem -1.0mm, PreGrower-2.0mm
8	*S. aurata*	30/11/2018	31/12/2018	2456.05	3276.06					SS	1620	StartPrem -1.5mm, PreGrower-2.0mm
9	*S. aurata*	30/11/2018	31/12/2018	2028.15	3161.72					SS	1765	StartPrem -1.5mm, StartPrem -1.0mm, PreGrower-2.0mm
10	*S. aurata*	30/11/2018	31/12/2018	10206.58	11609.16					SS	4125	PreGrower-2.0mm
11	*S. aurata*	30/11/2018	21/12/2018	8704.89	0.00	-9472.24	11	20	Cage repositioning	SS	2175	PreGrower-2.0mm
12	*S. aurata*	30/11/2018	31/12/2018	1939.38	2938.49					SS	1755	StartPrem -1.5mm, StartPrem -1.0mm, PreGrower-2.0mm
13	*S. aurata*	30/11/2018	31/12/2018	10512.57	12023.08					SS	4512.5	PreGrower-2.0mm, Supreme-3.0mm
14	*S. aurata*	30/11/2018	31/12/2018	10913.41	12298.48					SS	4337.5	PreGrower-2.0mm, Supreme-3.0mm
15	*S. aurata*	30/11/2018	31/12/2018	13056.44	14598.32					SS	5512.5	PreGrower-2.0mm, Supreme-3.0mm
16	*S. aurata*	30/11/2018	31/12/2018	16124.32	19454.78					SS	5546.88	Supreme-3.0mm
17	*S. aurata*	30/11/2018	31/12/2018	15100.52	23429.16					SS	5546.88	Supreme-3.0mm
18	*S. aurata*	30/11/2018	31/12/2018	14063.36	15587.15					SS	5512.5	PreGrower-2.0mm, Supreme-3.0mm
19	*S. aurata*	30/11/2018	31/12/2018	5189.90	6521.87					SS	3137.5	PreGrower-2.0mm
20	*S. aurata*	30/11/2018	20/12/2018	87067.34	0.00	-91283.88	20	M30	Site transfer	MS	8593.75	Supreme-3.0mm, Supreme-4.5mm, Sup-Winter-4.5mm

	S. aurata	21/12/2018	31/12/2018	0.00	9845.00		9472.24			Cage repositioning	SS	1350	PreGrower-2.0mm
January 2019													
1	*S. aurata*	31/12/2018	31/01/2019	7031.89	6.22						SS	2940.00	PreGrower-2.0mm, AllerBlueEX-3.0mm, StartPrem -1.0mm
2	*S. aurata*	31/12/2018	31/01/2019	40223.83	6.53						MS	8125.00	Supreme-3.0mm
3	*S. dumerili*	31/12/2018	31/01/2019	173945.01	28.25						MS	5100.00	Baitfish
4	*S. aurata*	31/12/2018	31/01/2019	47998.87	7.80						MS	8125.00	Supreme-3.0mm
5	*S. aurata*	31/12/2018	31/01/2019	66395.41	10.78						MS	6843.75	Supreme-4.5mm, Sup-Winter-4.5mm
6	*S. aurata*	31/12/2018	31/01/2019	62011.05	10.07						MS	6818.75	Supreme-4.5mm, Sup-Winter-4.5mm
7	*S. aurata*	31/12/2018	31/01/2019	2261.89	2.00						SS	1300.00	PreGrower-2.0mm, StartPrem -1.0mm, StartPrem -1.5mm
8	*S. aurata*	31/12/2018	31/01/2019	3276.06	2.90						SS	1395.00	PreGrower-2.0mm, StartPrem -1.0mm, StartPrem -1.5mm
9	*S. aurata*	31/12/2018	31/01/2019	3161.72	2.80						SS	1365.00	StartPrem -1.0mm, PreGrower-2.0mm, StartPrem -1.5mm
10	*S. aurata*	31/12/2018	31/01/2019	11609.16	10.26						SS	3814.07	PreGrower-2.0mm, Supreme-3.0mm, AllerBlueEX-3.0mm
12	*S. aurata*	31/12/2018	31/01/2019	2938.49	2.60						SS	1365.00	PreGrower-2.0mm, StartPrem -1.0mm, StartPrem -1.5mm
13	*S. aurata*	31/12/2018	31/01/2019	12023.08	10.63						SS	4425.00	Supreme-3.0mm, AllerBlueEX-3.0mm, PreGrower-2.0mm
14	*S. aurata*	31/12/2018	31/01/2019	12298.48	10.87						SS	4353.13	Supreme-3.0mm, AllerBlueEX-3.0mm, PreGrower-2.0mm
15	*S. aurata*	31/12/2018	31/01/2019	14598.32	12.91						SS	4765.63	Supreme-3.0mm
16	*S. aurata*	31/12/2018	31/01/2019	19454.78	17.20						SS	4843.75	Supreme-3.0mm
17	*S. aurata*	31/12/2018	31/01/2019	23429.16	20.72						SS	4843.75	Supreme-3.0mm
18	*S. aurata*	31/12/2018	31/01/2019	15587.15	13.78						SS	4765.63	Supreme-3.0mm
19	*S. aurata*	31/12/2018	31/01/2019	6521.87	5.77						SS	2890.00	PreGrower-2.0mm, AllerBlueEX-3.0mm, StartPrem -1.0mm
20	*S. aurata*	31/12/2018	31/01/2019	9845.00	8.70						SS	3664.06	PreGrower-2.0mm, Supreme-3.0mm, AllerBlueEX-3.0mm
February 2019													
1	*S. aurata*	31/01/2019	28/02/2019	8609.05	8944.45						SS	2175	PreGrower-2.0mm, Proactive-2.0mm – S&H
2	*S. aurata*	31/01/2019	28/02/2019	53977.91	56377.17						MS	7812.5	Supreme-3.0mm
3	*S. dumerili*	31/01/2019	07/02/2019	174310.23	174368.78	-165682.1		3	M35	Site transfer	MS	3300	Baitfish
	S. dumerili	08/02/2019	28/02/2019	8685.07	8813.06							2200	Baitfish
4	*S. aurata*	31/01/2019	28/02/2019	51211.99	54638.44						MS	7933.75	Supreme-3.0mm
5	*S. aurata*	31/01/2019	12/02/2019	79772.68	80156.35						MS	11406.25	Sup-Winter-4.5mm, Supreme-4.5mm
	S. aurata	13/02/2019	28/02/2019	161094.00	162533.18		80938.1			Cage joining	MS	8750	Sup-Winter-4.5mm, Supreme-4.5mm

6	*S. aurata*	31/01/2019	13/02/2019	80538.31	0.00	-80938.12		6	5	Cage joining	MS	2656.25	Sup-Winter-4.5mm, Supreme-4.5mm
	S. aurata	14/02/2019	28/02/2019	0.00	45304.80		43691.8			Cage joining	MS	4062.5	Supreme-3.0mm
7	*S. aurata*	31/01/2019	28/02/2019	2840.41	2968.18						SS	1205	StartPrem -1.5mm, PreGrower-2.0mm, Proactive-2.0mm – S&H
8	*S. aurata*	31/01/2019	28/02/2019	3832.24	5238.80						SS	1390	PreGrower-2.0mm, StartPrem -1.5mm, Proactive-2.0mm – S&H
9	*S. aurata*	31/01/2019	28/02/2019	3765.62	5449.78						SS	1230	StartPrem -1.5mm, PreGrower-2.0mm, Proactive-2.0mm – S&H
10	*S. aurata*	31/01/2019	28/02/2019	12603.01	17455.73						SS	2915.63	Supreme-3.0mm, PreGrower-2.0mm, Proactive-2.0mm – S&H
12	*S. aurata*	31/01/2019	18/02/2019	3554.11	0.00	-4775.72		12	15	Cage repositioning	SS	730	StartPrem -1.5mm, Proactive-2.0mm – S&H
13	*S. aurata*	31/01/2019	28/02/2019	13107.43	13537.08						SS	3675	PreGrower-2.0mm, Supreme-3.0mm, Proactive-2.0mm – S&H
14	*S. aurata*	31/01/2019	28/02/2019	17230.47	18204.57						SS	3906.25	Supreme-3.0mm
15	*S. aurata*	31/01/2019	14/02/2019	19406.84	0.00	-20192.70		15	6	Cage joining	SS	2031.25	Supreme-3.0mm
	S. aurata	18/02/2019	28/02/2019	0.00	4943.94		4775.72			Cage repositioning	SS	520	StartPrem -1.5mm, Proactive-2.0mm – S&H, PreGrower-2.0mm
16	*S. aurata*	31/01/2019	06/02/2019	20844.95	0.00	-21245.04		16	20	Cage joining	SS	781.25	Supreme-3.0mm
17	*S. aurata*	31/01/2019	04/02/2019	25623.03	0.00	-25822.02		17	20	Cage repositioning	SS	468.75	Supreme-3.0mm
	S. aurata	04/02/2019	28/02/2019	0.00	15406.56		10891.1			Cage repositioning	SS	2621.872	Supreme-3.0mm, PreGrower-2.0mm, Proactive-2.0mm – S&H
18	*S. aurata*	31/01/2019	14/02/2019	22957.38	0.00	-23499.11		18	6	Cage joining	SS	2031.25	Supreme-3.0mm
19	*S. aurata*	31/01/2019	28/02/2019	7776.73	8230.36						SS	2200	PreGrower-2.0mm, Proactive-2.0mm – S&H
20	*S. aurata*	04/02/2019	28/02/2019	0.00	49577.73		47067.1			Cage joining	MS	6996.874	Supreme-3.0mm, PreGrower-2.0mm
	S. aurata	31/01/2019	04/02/2019	10810.25	0.00	-10891.09		20	17	Cage repositioning	SS	328.124	Supreme-3.0mm, PreGrower-2.0mm
March 2019													
1	*S. aurata*	15/03/2019	31/03/2019	0.00	34355.99		33436.6			Cage joining	MS	3656.25	Proactive-2.0mm – S&H, Supreme-3.0mm, PreGrower-2.0mm
	S. aurata	28/02/2019	15/03/2019	8944.45	0.00	-12280.70		1	17	Cage repositioning	SS	1250	PreGrower-2.0mm, Proactive-2.0mm – S&H
2	*S. aurata*	28/02/2019	31/03/2019	56377.17	62957.83						MS	9531.25	Supreme-3.0mm
3	*S. dumerili*	28/02/2019	31/03/2019	8813.06	9170.73						SS	4100	Baitfish
4	*S. aurata*	28/02/2019	31/03/2019	54638.44	63702.38						SS	9531.25	Supreme-3.0mm
5	*S. aurata*	28/02/2019	31/03/2019	162533.18	164980.16						MS	15625	Supreme-4.5mm, Sup-Winter-4.5mm
6	*S. aurata*	28/02/2019	31/03/2019	45304.80	48830.88			6	19	Cage joining	MS	7578.125	Supreme-3.0mm
7	*S. aurata*	28/02/2019	31/03/2019	2968.18	5012.26						SS	1660	StartPrem -1.5mm, PreGrower-2.0mm, Proactive-2.0mm – S&H
8	*S. aurata*	28/02/2019	19/03/2019	5238.80	0.00	-5487.83		8	16	Cage repositioning	SS	850	PreGrower-2.0mm, Proactive-2.0mm – S&H
9	*S. aurata*	28/02/2019	31/03/2019	5449.78	6137.14						SS	1700	PreGrower-2.0mm, Proactive-2.0mm – S&H
10	*S. aurata*	28/02/2019	15/03/2019	17455.73	17728.50	-17728.50		10	1	Cage joining	SS	1150	PreGrower-2.0mm, Proactive-2.0mm – S&H

13	*S. aurata*	28/02/2019	26/03/2019	13537.08	0.00	-16864.15			13	19	Cage joining	SS	3515.625	Supreme-3.0mm
14	*S. aurata*	28/02/2019	25/03/2019	18204.57	0.00	-19056.88			14	19	Cage repositioning	SS	3359.375	Supreme-3.0mm
15	*S. aurata*	28/02/2019	31/03/2019	4943.94	5651.60							SS	1745	PreGrower-2.0mm, StartPrem -1.5mm, Proactive-2.0mm – S&H
16	*S. aurata*	19/03/2019	31/03/2019	0.00	5881.55		5487.83				Cage repositioning	SS	750	Proactive-2.0mm – S&H, PreGrower-2.0mm
17	*S. aurata*	28/02/2019	15/03/2019	15406.56	0.00	-15708.10			17	1	Cage joining	SS	1225	PreGrower-2.0mm, Proactive-2.0mm – S&H
	S. aurata	15/03/2019	31/03/2019	0.00	12772.87		12280.7				Cage repositioning	SS	2196.875	Proactive-2.0mm – S&H, Supreme-3.0mm
18	*S. aurata*	25/03/2019	31/03/2019	0.00	12255.21		12033.2				Cage repositioning	SS	1093.75	Supreme-3.0mm
19	*S. aurata*	26/03/2019	31/03/2019	0.00	36245.81		359210				Cage joining	MS	2031.25	Supreme-3.0mm
	S. aurata	28/02/2019	25/03/2019	8230.36	0.00	-12033.24			19	18	Cage repositioning	SS	2275	PreGrower-2.0mm, Proactive-2.0mm – S&H, Supreme-3.0mm
20	*S. aurata*	28/02/2019	31/03/2019	49577.73	53827.94							SS	9218.75	Supreme-3.0mm
								April 2019						
1	*S. aurata*	31/03/2019	30/04/2019	34355.99	36271.91							MS	6825	Supreme-3.0mm, PreGrower-2.0mm
2	*S. aurata*	31/03/2019	30/04/2019	62957.83	66131.56							MS	10781.25	Supreme-3.0mm, Sup-Winter-4.5mm, Supreme-4.5mm
3	*S. dumerili*	31/03/2019	30/04/2019	9170.73	9371.50							MS	4560	Baitfish
4	*S. aurata*	31/03/2019	30/04/2019	63702.38	67964.38							MS	8750	Supreme-3.0mm
5	*S. aurata*	31/03/2019	30/04/2019	164980.16	167258.50							MS	14531.25	Supreme-4.5mm, Sup-Winter-4.5mm, Supreme-3.0mm
6	*S. aurata*	31/03/2019	30/04/2019	48830.88	52618.13							MS	7443.75	Supreme-3.0mm, PreGrower-2.0mm
7	*S. aurata*	31/03/2019	30/04/2019	5012.26	5855.65							SS	2100	PreGrower-2.0mm, Proactive-2.0mm – S&H
8	*S. aurata*	30/04/2019	30/04/2019	0.00	781.47			772.50			Cage input	SS	20	StartPrem -1.5mm, StartPrem -1.0mm
9	*S. aurata*	31/03/2019	30/04/2019	6137.14	9841.42							SS	2125	PreGrower-2.0mm, Proactive-2.0mm – S&H
10	*S. aurata*	09/04/2019	30/04/2019	0.00	581.30			378.978			Cage input	SS	420	StartPrem -1.5mm, StartPrem -1.0mm
11	*S. aurata*	09/04/2019	30/04/2019	0.00	563.67			366.94			Cage input	SS	420	StartPrem -1.5mm, StartPrem -1.0mm
13	*D. labrax*	11/04/2019	30/04/2019	0.00	1482.95			1038.87			Cage input	SS	830	Proactive-2.0mm – S&H, PreGrower-2.0mm, StartPrem -1.5mm
14	*D. labrax*	13/04/2019	30/04/2019	0.00	1382.15			981.96			Cage input	SS	730	PreGrower-2.0mm, StartPrem -1.5mm
15	*S. aurata*	31/03/2019	30/04/2019	5651.60	8089.11							SS	2125	PreGrower-2.0mm, Proactive-2.0mm – S&H
16	*S. aurata*	31/03/2019	30/04/2019	5881.55	6306.84							SS	2025	PreGrower-2.0mm, Proactive-2.0mm – S&H
17	*S. aurata*	31/03/2019	16/04/2019	12772.87	0.00	-13285.49			17	18	Cage joining	SS	2187.5	Supreme-3.0mm
	S. aurata	30/04/2019	30/04/2019	0.00	781.59			772.5			Cage input	SS	20	StartPrem -1.5mm, StartPrem -1.0mm
18	*S. aurata*	16/04/2019	30/04/2019	26093.00	26901.84		13285.5				Cage joining	MS	2837.5	Supreme-3.0mm, Proactive-2.0mm – S&H, PreGrower-2.0mm
	S. aurata	31/03/2019	15/04/2019	12255.21	12808.90							MS	5025	Supreme-3.0mm, Proactive-2.0mm – S&H, PreGrower-2.0mm
19	*S. aurata*	31/03/2019	30/04/2019	36245.81	38170.66							MS	7131.25	Supreme-3.0mm, PreGrower-2.0mm

20	*S. aurata*	31/03/2019	30/04/2019	53827.94	57699.95						MS	9062.5	Supreme-3.0mm, Sup-Winter-4.5mm, Supreme-4.5mm
						May 2019							
1	*S. aurata*	30/04/2019	15/05/2019	36271.91	0.00	-37706.51		1	M23	Site transfer	MS	4575	Supreme-3.0mm, PreGrower-2.0mm
2	*S. aurata*	17/05/2019	31/05/2019	535.35	744.41		535.36			Cage input	SS	330	StartPrem -1.0mm, StartPrem -1.5mm
	S. aurata	30/04/2019	14/05/2019	66131.56	0.00	-68603		2	M34	Site transfer	MS	5468.75	Supreme-4.5mm, Supreme-3.0mm
3	*S. dumerili*	02/05/2019	31/05/2019	3873.26	4270.89					Harvest	MS	4300	Baitfish
	S. dumerili	30/04/2019	01/05/2019	9371.50	9389.39					Harvest	MS	4900	Baitfish
4	*S. aurata*	30/04/2019	31/05/2019	67964.38	77528.02						MS	13693.75	Supreme-3.0mm, PreGrower-2.0mm
5	*S. aurata*	30/04/2019	11/05/2019	167258.50	0.00	-181139.9		5	M35	Site transfer	SS	5000	Supreme-4.5mm
6	*S. aurata*	30/04/2019	31/05/2019	52618.13	61560.86						MS	11812.5	Supreme-3.0mm, PreGrower-2.0mm
7	*S. aurata*	30/04/2019	31/05/2019	5855.65	6668.64						SS	2325	Proactive-2.0mm – S&H, PreGrower-2.0mm
8	*S. aurata*	30/04/2019	31/05/2019	781.47	1527.60						SS	1010	StartPrem -1.5mm, StartPrem -1.0mm
9	*S. aurata*	30/04/2019	31/05/2019	9841.42	10838.66						SS	2950	Proactive-2.0mm – S&H, PreGrower-2.0mm
10	*S. aurata*	30/04/2019	31/05/2019	581.30	1008.77						SS	710	StartPrem -1.0mm, StartPrem -1.5mm
11	*S. aurata*	30/04/2019	31/05/2019	563.67	987.56						SS	710	StartPrem -1.5mm, StartPrem -1.0mm
13	*D. labrax*	30/04/2019	31/05/2019	1482.95	2298.35						SS	1375	StartPrem -1.5mm, PreGrower-2.0mm
14	*D. labrax*	30/04/2019	31/05/2019	1382.15	2116.50						SS	1375	PreGrower-2.0mm, StartPrem -1.5mm
15	*S. aurata*	30/04/2019	31/05/2019	8089.11	9173.21						SS	2950	Proactive-2.0mm – S&H, PreGrower-2.0mm
16	*S. aurata*	30/04/2019	31/05/2019	6306.84	6770.41						MS	2350	Proactive-2.0mm – S&H, PreGrower-2.0mm
17	*S. aurata*	30/04/2019	31/05/2019	781.59	1561.57						SS	1010	StartPrem -1.5mm, StartPrem -1.0mm
18	*S. aurata*	30/04/2019	31/05/2019	26901.84	28425.11						MS	8437.5	Supreme-3.0mm, PreGrower-2.0mm
19	*S. aurata*	30/04/2019	31/05/2019	38170.66	47133.49						MS	10325	PreGrower-2.0mm, Supreme-3.0mm
20	*S. aurata*	17/05/2019	31/05/2019	535.35	745.30		535.36			Cage input	SS	330	StartPrem -1.0mm, StartPrem -1.5mm
	S. aurata	30/04/2019	11/05/2019	57699.95	0.00	-59254.43		20	M36	Site transfer	MS	3750	Supreme-3.0mm, Supreme-4.5mm
						June 2019							
1	*S. aurata*	07/06/2019	30/06/2019	0.00	8554.35	7417.73				Cage repositioning	SS	3325	PreGrower-2.0mm
2	*S. aurata*	31/05/2019	30/06/2019	744.41	1758.21						SS	1220	StartPrem -1.0mm, StartPrem -1.5mm
3	*S. dumerili*	31/05/2019	30/06/2019	4270.89	4677.74						MS	3300	Baitfish
4	*S. aurata*	03/06/2019	30/06/2019	0.00	23126.63	20148.4				Cage joining	MS	8525	PreGrower-2.0mm, Supreme-3.0mm
5	*S. aurata*	14/06/2019	30/06/2019	0.00	701.53		369.90			Cage input	SS	425	StartPrem -1.0mm, StartPrem -1.5mm
6	*S. aurata*	31/05/2019	30/06/2019	61560.86	70611.11						MS	19843.75	Supreme-3.0mm, Supreme-4.5mm
7	*S. aurata*	31/05/2019	30/06/2019	6668.64	7323.75						SS	3925	PreGrower-2.0mm
8	*S. aurata*	31/05/2019	30/06/2019	1527.60	2372.44						SS	1995	StartPrem -1.5mm, PreGrower-2.0mm
9	*S. aurata*	31/05/2019	03/06/2019	10838.66	0.00	-10911.02		9	4	Cage joining	SS	200	PreGrower-2.0mm
10	*S. aurata*	31/05/2019	30/06/2019	1008.77	1730.68						SS	1170	StartPrem -1.5mm, StartPrem -1.0mm
11	*S. aurata*	31/05/2019	30/06/2019	987.56	1473.00						SS	1200	StartPrem -1.5mm, StartPrem -1.0mm

13	*D. labrax*	31/05/2019	30/06/2019	2298.35	4293.40							SS	2632.5	PreGrower-2.0mm, StartPrem -1.5mm
14	*D. labrax*	31/05/2019	30/06/2019	2116.50	4058.26							SS	2632.5	StartPrem -1.5mm, PreGrower-2.0mm
15	*S. aurata*	14/06/2019	30/06/2019	0.00	663.28			369.90			Cage input	SS	425	StartPrem -1.0mm, StartPrem -1.5mm
	S. aurata	31/05/2019	03/06/2019	9173.21	0.00	-9237.38			15	4	Cage joining	SS	200	PreGrower-2.0mm
16	*S. aurata*	31/05/2019	07/06/2019	6770.41	0.00	-7417.73			16	1	Cage repositioning	SS	600	PreGrower-2.0mm
17	*S. aurata*	31/05/2019	30/06/2019	1561.57	2277.31							SS	1995	StartPrem -1.5mm, PreGrower-2.0mm
18	*S. aurata*	31/05/2019	30/06/2019	28425.11	33926.85							MS	11562.5	Supreme-3.0mm
19	*S. aurata*	31/05/2019	30/06/2019	47133.49	53362.40							MS	14881.25	Supreme-3.0mm, PreGrower-2.0mm
20	*S. aurata*	31/05/2019	30/06/2019	745.30	1770.54							SS	1220	StartPrem -1.0mm, StartPrem -1.5mm
							July 2019							
1	*S. aurata*	30/06/2019	31/07/2019	8554.35	11169.16							SS	6575	PreGrower-2.0mm, Supreme-3.0mm
2	*D. labrax*	12/07/2019	31/07/2019	0.00	15048.41		10015.3				Cage joining	MS	6846.875	PreGrower-2.0mm, Supreme-3.0mm
	D. labrax	30/06/2019	11/07/2019	1758.21	0.00	-2305.16			2	19	Cage repositioning	SS	795	PreGrower-2.0mm, StartPrem -1.5mm, StartPrem -1.0mm
3	*S. dumerili*	30/06/2019	14/07/2019	4677.74	4841.07						Harvest	MS	2900	Baitfish
	S. dumerili	15/07/2019	18/07/2019	2800.69	2838.71						Harvest	MS	200	Baitfish
	S. dumerili	19/07/2019	31/07/2019	2689.40	2781.25						Harvest	MS	800	Baitfish
4	*S. aurata*	30/06/2019	31/07/2019	23126.63	28406.69							MS	12506.25	PreGrower-2.0mm, Supreme-3.0mm
5	*S. aurata*	30/06/2019	31/07/2019	701.53	2073.23							SS	1770	StartPrem -1.0mm, StartPrem -1.5mm
6	*S. aurata*	30/06/2019	31/07/2019	70611.11	77283.26							MS	19531.25	Supreme-3.0mm, Supreme-4.5mm
7	*S. aurata*	30/06/2019	31/07/2019	7323.75	10228.85							SS	6121.88	PreGrower-2.0mm, Supreme-3.0mm
8	*S. aurata*	30/06/2019	31/07/2019	2372.44	4854.06							SS	3730	StartPrem -1.5mm, PreGrower-2.0mm, StartPrem -1.0mm
10	*S. aurata*	30/06/2019	31/07/2019	1730.68	3321.85							SS	2407.5	StartPrem -1.5mm, PreGrower-2.0mm, StartPrem -1.0mm
11	*S. aurata*	30/06/2019	31/07/2019	1473.00	3111.44							SS	2502.5	PreGrower-2.0mm, StartPrem -1.5mm, StartPrem -1.0mm, Proactive-1.5mm – S&H
12	*S. aurata*	03/07/2019	31/07/2019	0.00	1816.31			532.5			Cage input	SS	1645	StartPrem -1.0mm, StartPrem -1.5mm, Proactive-1.0mm – S&H, Proactive-1.5mm – S&H
13	*D. labrax*	30/06/2019	12/07/2019	4293.40	0.00	-5104.70			13	2	Cage joining	SS	1175	PreGrower-2.0mm
14	*D. labrax*	30/06/2019	12/07/2019	4058.26	0.00	-4910.60			14	2	Cage joining	SS	1225	PreGrower-2.0mm
15	*S. aurata*	30/06/2019	31/07/2019	663.28	2073.66							SS	1750	StartPrem -1.0mm, StartPrem -1.5mm, Proactive-1.0mm – S&H, Proactive-1.5mm – S&H
16	*S. aurata*	03/07/2019	31/07/2019	532.50	1813.02			532.50			Cage input	SS	1620	StartPrem -1.0mm, StartPrem -1.5mm
17	*S. aurata*	30/06/2019	31/07/2019	2277.31	4810.21							SS	3830	StartPrem -1.5mm, PreGrower-2.0mm, StartPrem -1.0mm, Proactive-1.0mm – S&H, Proactive-1.5mm – S&H
18	*S. aurata*	30/06/2019	31/07/2019	33926.85	45437.23							MS	17031.25	Supreme-3.0mm, Supreme-4.5mm
19	*S. aurata*	11/07/2019	31/07/2019	2305.16	3678.22		2305.16		2	19	Cage repositioning	SS	2027.5	PreGrower-2.0mm, StartPrem -1.5mm

	S. aurata	30/06/2019	11/07/2019	53362.40	0.00	-55225.01	19	M27	Site transfer	MS	5000	Supreme-3.0mm
20	*S. aurata*	30/06/2019	31/07/2019	1770.54	3678.96					SS	2822.5	StartPrem -1.5mm, PreGrower-2.0mm, StartPrem - 1.0mm

SS indicates 12 m diameter fish cages, MS indicates 28 m diameter fish cages

Actual feed input data included 1 mm (StartPrem; Alltech Coppens, Germany), 1.5 mm (StartPrem; Alltech Coppens, Germany), 2 mm (Pre-Grower 16; Alltech Coppens, Germany) and (Proactive 2; Veronesi, Italy), 3 mm (Supreme; Alltech Coppens, Germany) and AllerBlueEX; Aller Aqua, Denmark), and 4.5 mm (Supreme; Alltech Coppens, Germany) feeds that were administered in succession or in combination at the fish farm. Generally, the extruded feeds that were supplemented to sea bream and sea bass included marine sources, terrestrial plant-based sources and the derivatives of terrestrial animals. Moreover, thawed and chopped Atlantic mackerel (*Scomber scombrus*) was used as baitfish supplemented to amberjack at the fish farm.

Settlement velocities of formulated feeds used in sea bream and sea bass production in the study were deduced from literature (Table 4.2). The settling rate of baitfish used in amberjack production was based on estimates in Fernandes et al. (2007). The faecal pellet velocities were 0.005 m s^{-1} in sea bream and 0.007 m s^{-1} in sea bass, according to Magill et al. (2006). Settling velocities were consistent for various faecal pellet sizes from different fish sizes (Chen, 2000, Magill et al., 2006, Pérez et al., 2014). The settling velocity for the faecal material of amberjack (0.005 m s^{-1}) was assumed according to estimations for baitfish faeces in Fernandes et al. (2007).

Table 4.2. Feed settling velocities for sea bream, sea bass and amberjack.

Feed type	**Settling velocity (m s^{-1})**
1 mm feed pellet	0.04 [a, b]
1.5 mm feed pellet	0.062 [a, b]
2 mm feed pellet	0.079 [a, b]
3 mm feed pellet	0.087 [c]
4.5 mm feed pellet	0.103 [c]
Chopped baitfish	0.07 [d]

[a] Chen, 2000,

[b] Cromey et al., 2012,

[c] Vassallo et al., 2006,

[d] Fernandes et al., 2007.

In the mass-balance model, default nutrient input parameters were defined for the production of sea bream, sea bass, and amberjack, according to literature, listed in Table 4.3. Values for nutrient uptake by fish were changed for the feed used (e.g. pellet size), the farmed species, and size of fish.

Assumptions were made as some species and feed-specific information was limited particularly for amberjack production. The wastage of baitfish in amberjack production was adopted from the estimated wastage of trash fish supplemented to the areolate grouper (*Epinephelus areolatus*) in open-sea cages elsewhere (Leung et al., 1999) (Table 4.3). Waste deposition is based on estimates of particulate wastes emanating from the cages as a mixture of wasted feed and fish faeces. Food wastage for the farmed species in the farm were taken to be 33% for sea bream and 38% for sea bass (Brigolin et al., 2014) and amberjack (Leung et al., 1999) (Table 4.3). General mass balance estimates for carbon in the food-fish-waste system were adopted from Gowen et al. (1988) and Chen (2004). The assumed respired fraction also accounts for losses through urea (Gowen et al., 1988).

Table 4.3. Nutrient input parameters and assumptions for sea bream, sea bass and amberjack.

Parameter	**Sea bream**	**Sea bass**	**Amberjack**
Relative food wastage	33% [a]	38% [a]	38% [b]
Carbon content of feed	48.6 – 50.1% [c]	49.2 – 50.9% [c]	46.56%
Carbon content of fish tissue	15% [d, e]	15% [d, e]	15% [d, e]
Relative respired carbon	60% [d, f]	60% [d, f]	60% [d, f]
Carbon content of faeces	36.7% [c]	33.1% [c]	36.7%

[a] Brigolin et al., 2014,

[b] Leung et al., 1999,

[c] Ballester-Moltó et al., 2017,

[d] Gowen et al., 1988,

[e] Chen, 2000,

[f] Olsen et al., 2008.

After mass balance estimations, the horizontal dispersion of waste within the model, prior to initial settlement, was based on hydrographic data, settlement velocities, water depth and horizontal distance dispersed (Gowen et al., 1988). The location and orientation of grid cells on the worksheet of the spreadsheet model represent the position and layout of cages at the fish farm. In the model spreadsheet, dispersion is estimated for individual fish cages as described in Telfer et al. (2022) and adapted for 5 m intervals. The 12 m fish cages were represented by five grid cells and the 28 m fish cages represented by 25 cells in this study. For each discrete modelled period, grid cells were repositioned to reflect cage movements

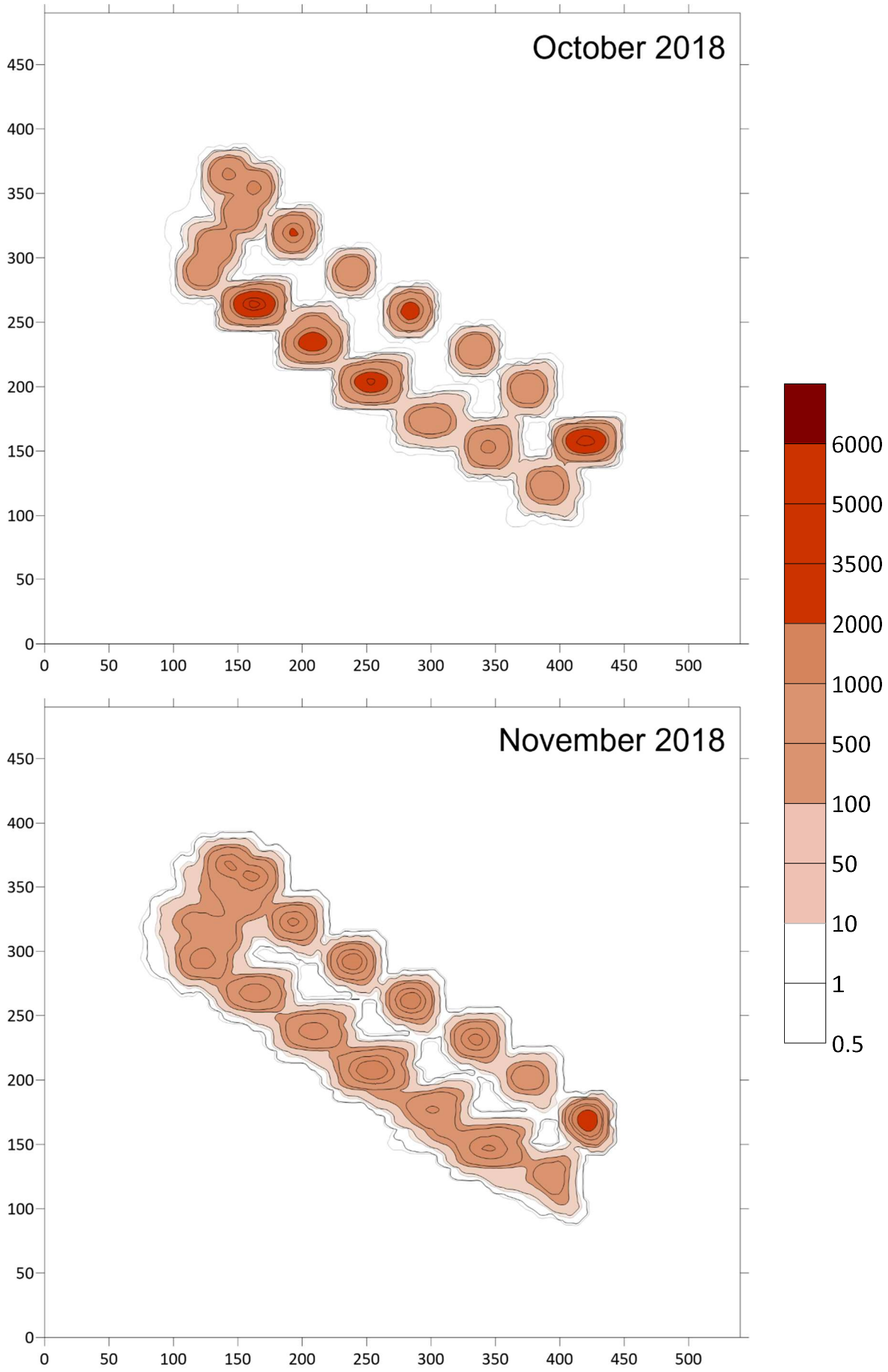
and configurations. The amount of particulate waste released by fish cages at the site was estimated as deposition of organic carbon in 5 x 5 m grid cells, within sectors along eight compass axes (North, Northeast, East, Southeast, South, Southwest, West, and Northwest). Each sector is defined as a 45-degree wide arc centered on the specified directions. The total waste input for each sector is converted to input distributed across the corresponding grid cells within the sectors. The settlement associated with each cage was individually entered into an excel spreadsheet or 'layer' and the final data outputs of the dispersion models for each cage were overlaid as layers of individual worksheets to form a single worksheet for every month.

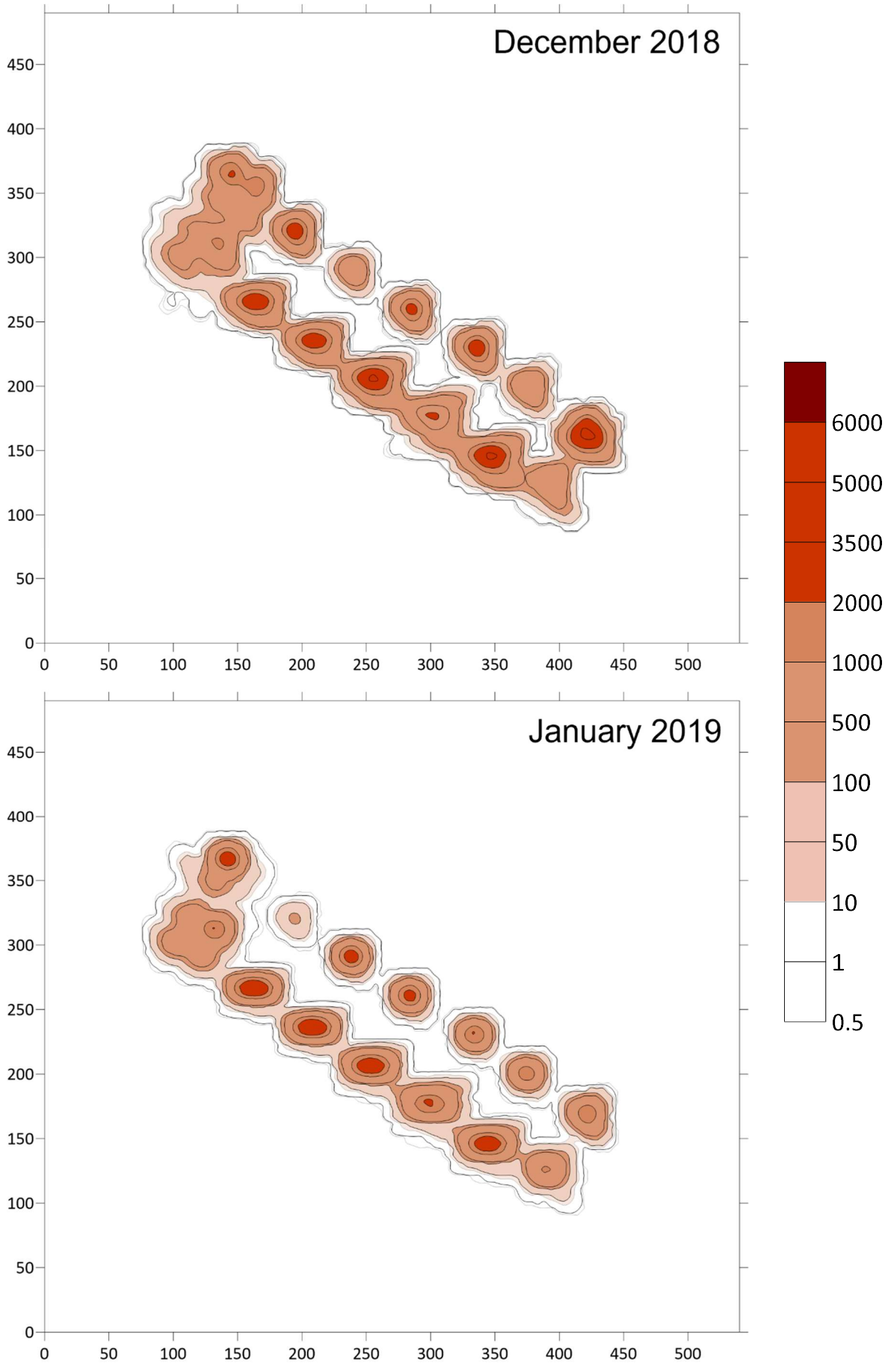
The combined data output was imported into Surfer™ 16 (Golden Software Inc., USA) to produce two-dimensional contour maps for a visual representation of waste dispersion around the fish farm per month. The spreadsheet data plotted in Surfer™ produced contour models of organic carbon deposition (g m^{-2}) per month around, based on the biomass and growth of multiple farmed species in Marsaxlokk Bay.

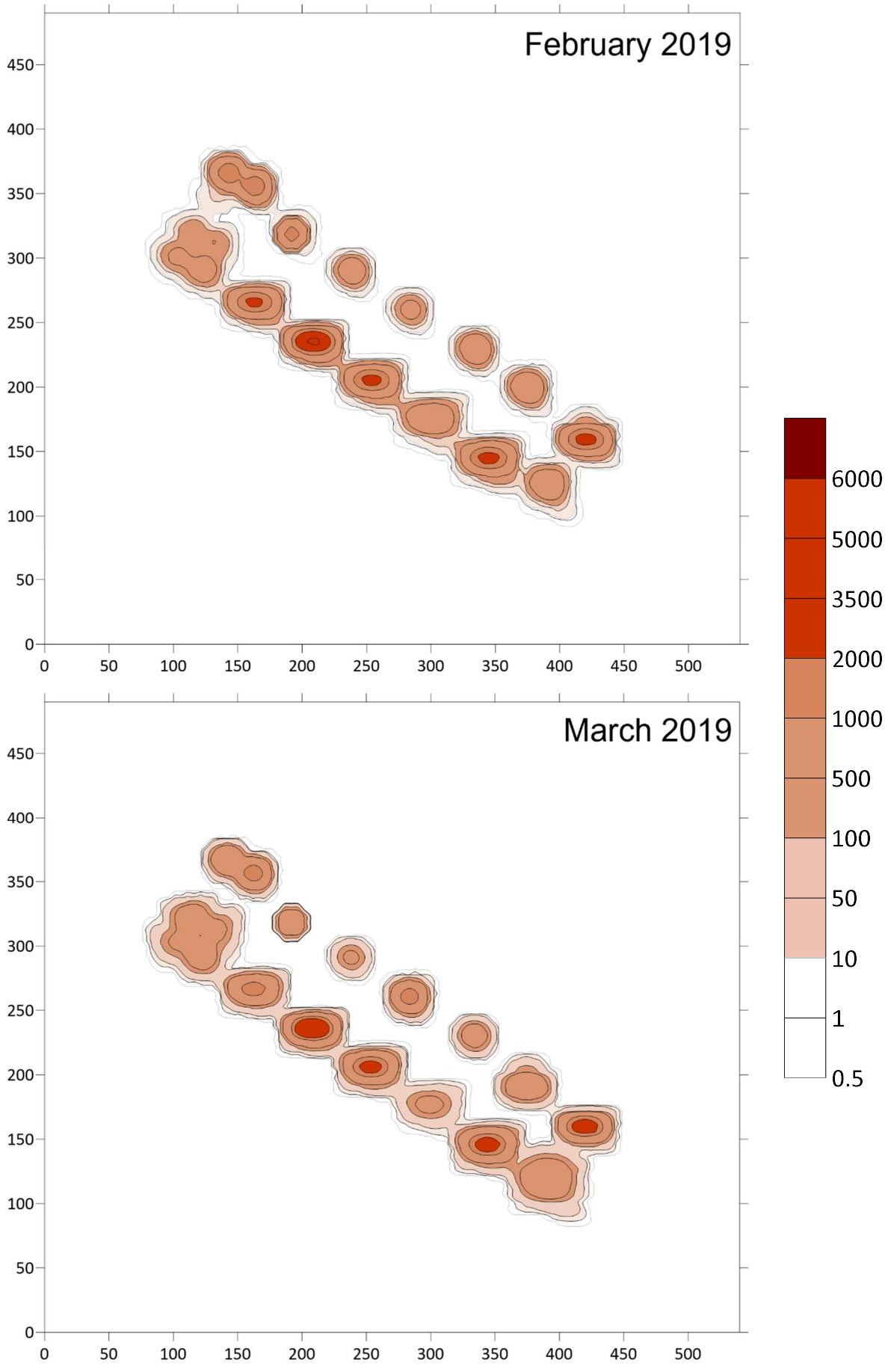
4.3 Results

4.3.1 Particulate waste dispersion

The production of sea bream, sea bass and amberjack was modelled using actual feed and cage biomass for each individual cage at the fish farm. Model estimates and patterns of sediment carbon (gC m^{-2}) deposition near cages at the fish farm represent the spatial and temporal effects of cage management practices on waste dispersion. Model outputs produced as contour plots in Surfer™ are presented in Fig. 4.4.


October 2018
November 2018
0
50
100
150
200
250
300
350
400
450
500
6000
5000
3500
2000
1000
500
100
50
10
1
0.5


December 2018
January 2019
0
50
100
150
200
250
300
350
400
450
500
6000
5000
3500
2000
1000
500
100
50
10
1
0.5


February 2019
March 2019
0
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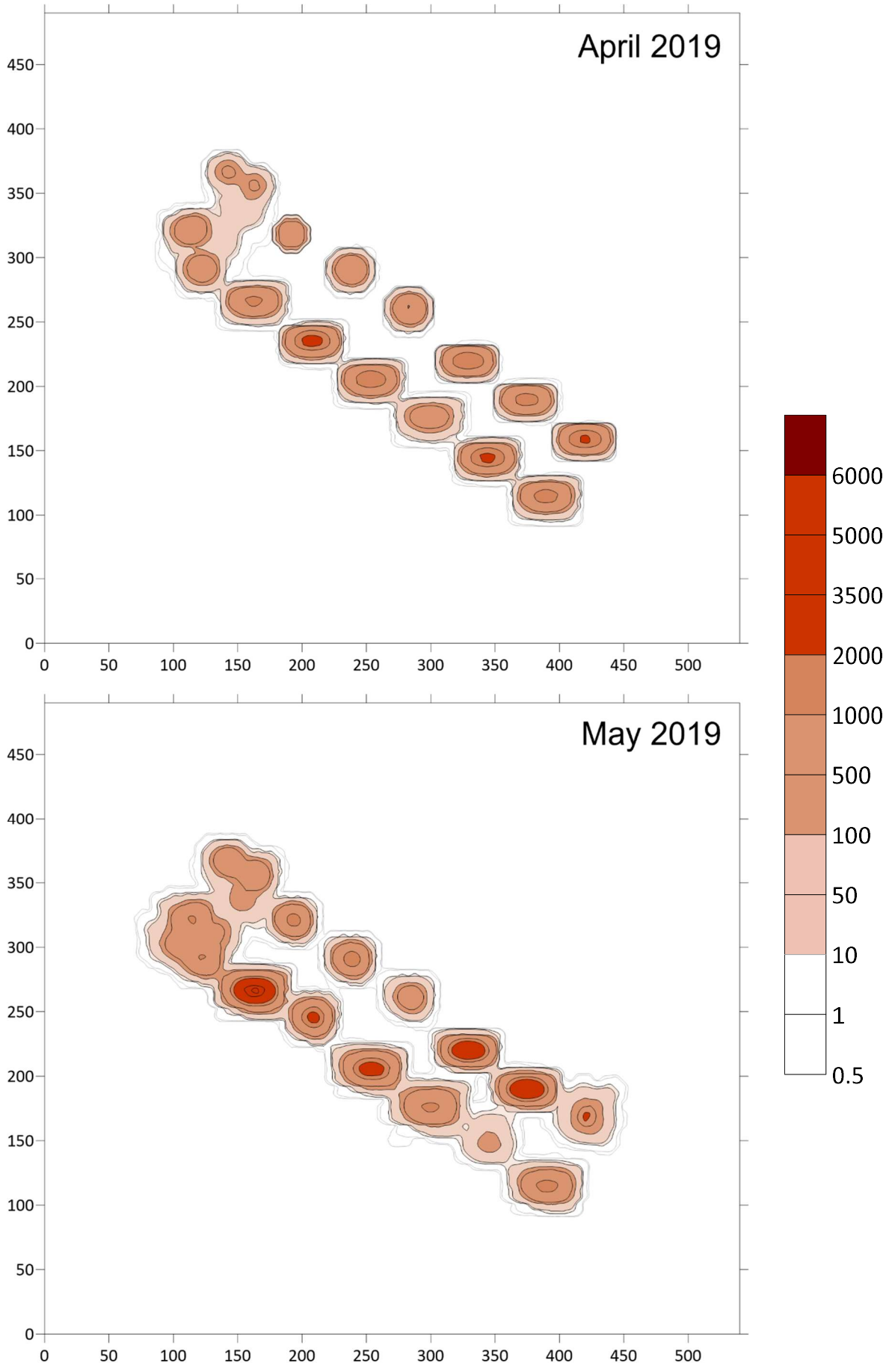
April 2019
May 2019
0
50
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0.5

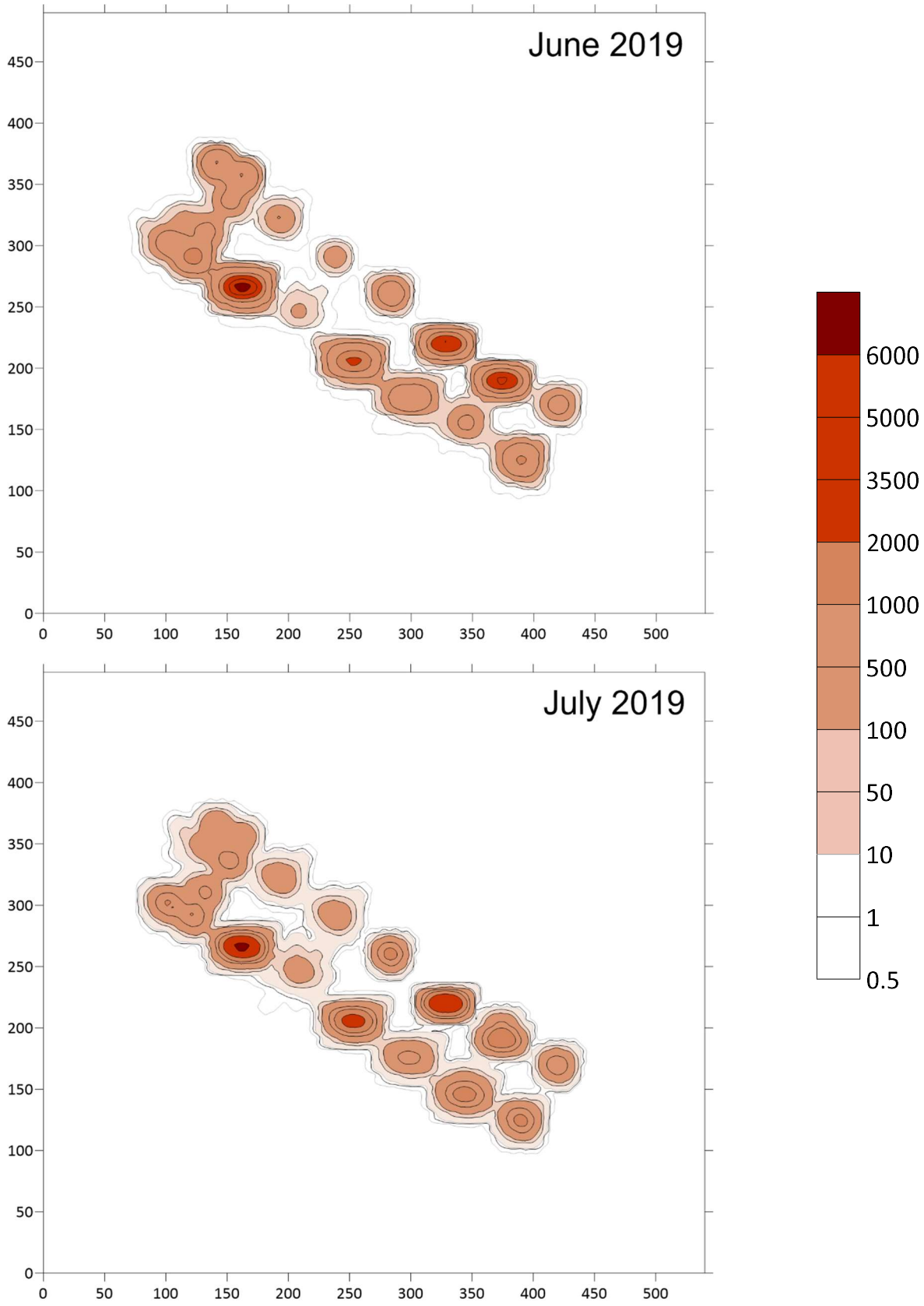

Figure 4.4. Particulate waste dispersion from fish in fish cages to the seabed on monthly basis over a twelve-month period (October 2018 to July 2019). Axis units are in metres North (Y-axis) and East (X-axis), and deposition contours are in total gC m^{-2} accumulation over the month.

Model outputs revealed higher deposition directly below the fish cages that was localised and decreased with increasing distance from the fish cages. Deposition rates and patterns exhibit variation that is presumably associated with alteration in cage biomass and food input, and modifications in cage sizes and arrangements throughout production, which are attributed to farm-level management practices (Table 4.1). The deposition footprints and the maximum deposition rate, F_{max}, reveal cage-level differences in deposition during the same periods (Table 4.4). For instance, the deposition in June 2019 changed from a negligible F_{max} to the highest F_{max} (6858.3 gC m^{-2}) recorded. Below fish cages, the maximum rates of deposition fluctuated between different modelled months during the 10-month period. F_{max} does not represent a production continuum and trends in waste distribution attest to changes in cage biomass and food data input throughout production.

Table 4.4. Maximum deposition rates (F_{max}) predicted directly below fish cage positions every month between October 2018 and July 2019.

	F_{max} (gC m^{-2})									
Cage number	**October**	**November**	**December**	**January**	**February**	**March**	**April**	**May**	**June**	**July**
1	835.1	411.3	1332.3	1384.2	979.3	1881.4	1530.2	1778.8	1358.7	1513.4
2	1581.1	1311.3	4574.1	2877.7	2421.6	3981.3	2137.9	488.3	520.5	1788.1
3	796.6	1007.5	2087.1	2201.4	1367.4	630.9	629.2	1627.4	939.8	1005.8
4	3822.8	1824.3	4251.4	2858.7	2548.5	3516.2	1824.8	2694.0	2711.0	3398.7
5	2691.1	1026.3	3011.3	2842.7	4410.1	5254.1	2311.6	1992.5	117.8	431.1
6	5641.3	1299.7	3204.1	2835.8	2366.7	1995.0	1387.1	5898.3	6858.3	6148.4
7	746.9	944.2	100.4	543.1	529.3	779.3	615.3	1158.8	1633.1	1187.4
8	0.6	56.5	445.2	581.7	319.1	407.1	1.6	465.7	543.7	1162.3
9	13.9	101.2	337.1	549.9	287.1	802.6	695.2	1170.0	105.1	10.1
10	936.2	238.5	1508.4	1728.4	504.0	589.7	98.4	324.7	355.0	1011.2
11	948.5	553.7	877.2	10.4	0.6	5.4	98.6	273.8	352.8	796.8
12	14.7	449.5	396.1	428.8	50.9	1.9	10.3	0.3	0.2	409.8
13	1164.4	1163.5	1998.3	2178.4	517.1	1177.6	387.1	641.6	1011.2	356.3
14	1119.7	1282.2	1560.9	54.1	1589.4	1728.7	291.2	605.7	1006.3	370.0
15	1694.1	1338.6	2441.4	135.2	984.6	781.9	617.9	1179.4	299.1	459.2
16	816.6	1645.2	651.2	2051.3	598.8	322.9	762.2	1341.1	126.2	407.6
17	2355.3	1677.3	2004.9	2017.6	408.3	1747.5	917.4	465.1	543.3	1452.1
18	936.8	1442.7	2445.6	2000.7	813.7	572.9	1787.1	3287.3	3798.7	2646.0
19	823.1	597.7	1098.7	1289.5	913.8	1095.6	1385.7	3555.5	4098.8	1704.3
20	4934.5	3012.3	3337.6	1784.3	2463.1	3582.4	2018.6	2188.3	501.1	1012.1

4.4 Discussion

This study illustrates some of the complexities in marine fish farming and the implications of real-world farming practices. Cohort dynamics and cage movements associated with the aquaculture management practices of the fish farm influenced the dispersion of wastes. Deposition footprints revealed variability that reflects the cage management complexities that affect the quantity and fate of wastes distributed around fish cages. Better representation of these farm-specific dynamics can improve the accuracy of farm-scale model estimations. This study shows that representative input data is needed to describe actual food inputs and cage biomass changes, and to account for cage management practices.

The predicted deposition around multiple species and cohorts reflects real-world aquaculture that does not follow a production continuum but F_{max} reveals variation as a function of the multitude of husbandry settings described throughout production. While models do not always have the capability to adapt to different sites and production settings (Chary et al., 2019), when applied to this fish farm, this farm-scale model also resolved important spatial variability in sediment carbon enrichment. Model studies that considered different cohort and feed input data in individual cages at the same farm improved model accuracy (Magill et al., 2006; Cromey et al., 2012) whereas others applied to different aquaculture scenarios revealed that farm management practices were important criteria for better predictions (Burić et al., 2020; Chary et al., 2021). Where management practices complicate cage arrangements and change cage biomass and food inputs to divert production trends, effective monitoring and management of environmental impact need a finer assessment modelling approach to account for the variability revealed at cage level.

There are natural and anthropogenic dynamics that influence water movement and consequently can affect the distribution and fate of wastes (Chapter 3). Therefore, these dynamics need to be considered when trying to obtain a representative footprint for setting up of bottom dwelling IMTA. A detailed description of local currents in real time accounts for episodic events of severe weather and other real-world complications that are not necessarily represented by short-term or averaged hydrography data. Though there have been considerable advances in development and use of two- or three-dimensional hydrodynamic models to simulate aquaculture waste dispersion (Wu et al, 2014; Broch et al, 2017), modelling complex coastal areas at sufficient resolution is still a challenge (Ward et al., 2023). With any environmental sampling and modelling, there are limitations as neither models nor *in-situ* measurements can capture the full complexity of complex environments (Skogen et al., 2022). Still, there needs to be sufficient information to

recognise the dynamic nature of the environment and represent the farm environment beyond oversimplistic generalisations. Decision-support models used in aquaculture planning and licensing need reliable data that capture the true extent and severity of impact as production changes with farm-level management decisions. Based on findings from the present study, it is recommended that waste dispersion models use hydrographic inputs, farm layouts, and simulate realistic production practices. Since models can have an important role in aquaculture planning, licensing and regulation (Falconer et al., 2023), it important that models are realistic, so that representative computations of deposition footprints are obtained, supporting production levels within capacity limits.

Factors attributing to waste deposition vary between farms due to different practices with seasonal variations and site-specific characteristics. Incorporating the effects of wild fish feeding on pellets improved model performance in others part of the Mediterranean (Cromey et al., 2012). It is anticipated that incorporating any influence of wild fish feeding on feed wastage could reduce the contribution of high feed wastage to seabed flux assumed to be constant throughout production (Table 4.3), especially during high-feed-input summer months, thus affecting waste deposition patterns. Furthermore, consideration for deposition that may occur from wild fish faeces or resuspension effects if critical erosion thresholds (10 cm s^{-1}) for fish farm wastes (not considered in this study) are exceeded, may improve model representativeness (Cromey et al., 2012). Considering the limitations in the available data regarding feed wastage and nutrient information in the mass balance model, and feed-specific information, particularly for the greater amberjack, assumptions were made to model deposition within the entire fish farm. More comprehensive species-specific data can improve the waste deposition outputs from CAPOT. In addition, wasted feed fractions are highly variable depending on operating conditions and production period, and should account for both uneaten feed and feed losses by chewing in the case of sea bream (Ballester-Moltó et al., 2017). Improving the representativeness of deposition is facilitated by the capability of CAPOT to adjust model input data according to diverse production scenarios. This adaptability, demonstrated through its flexibility in depicting complex cohort dynamics and cage movements linked to intricate aquaculture management practices, contributes to enhancing the representativeness of deposition.

Waste dispersion models also have an important role to play in IMTA research and development as models are required to understand the potential nutrient transfer, environmental interactions, and production consequences of co-cultivating species under different production scenarios (Reid et al., 2018; Falconer et al., 2023). For a balanced system that contributes to the removal of wastes, models need to quantify and resolve the distribution and impact of particulate wastes around the fish farm (Troell et al., 2009).

Resolving farm-level complexities can provide essential information about the variability of food availability and quality for extractive organisms to recycle aquaculture-derived organic wastes effectively and to maximise production. For example, understanding how the carbon deposition footprint changes would help producers to site and manage extractive organisms (e.g. deposit-feeding sea cucumbers) properly near fish cages. In practice, to optimise IMTA production, farm management practices should account for the variability in waste distribution associated with cage production and the effects on benthic conditions, so the physiological requirements of extractive species throughout its entire grow-out production can be adequately satisfied. Models such as the one used in this study, can be used to help identify appropriate locations for placement of the extractive IMTA species, but only if they are representative of the site. Clearly, generalisations of site characteristics could under or over-estimate the amount of waste available for the extractive species with implications for survivability and commercial viability. Refining waste dispersion models is crucial. *In situ* studies on extractive species' growth performance and survivability in IMTA can help incorporate real-world data into models, grounding predictions in empirical findings to optimise IMTA systems by maximising the uptake of organic matter of aquaculture-derived wastes throughout production.

4.5 Conclusion

Where multiple species, cohort dynamics and irregular cage arrangements influence production continuity in intensive cage production, variability in deposition footprints reveals cage-level complexities that need to be considered for improved model predictions. In this study, the farm-scale model resolved variation in the initial particulate waste settlement as a function of cage-level considerations. This finer modelling approach towards predicting waste distribution and benthic impact is down to detailed input data and farm-specific considerations for cage level variability. For licensing and environmental regulation, predicting the magnitude of the deposition footprint and the influence of cage management practices can contribute towards more representative assessments and effective management of benthic impacts. While environmental impact assessments and monitoring efforts are typically staggered one-time or one-point occasions, cage production is variable and even if different model scenarios are considered, in practice real-world complexities need to be accounted for to have effective management. Then, to mitigate benthic impacts efficiently through IMTA, the availability and quality of particulate organic wastes in seafloor sediments during cage production needs detailed representation. The feasibility and

profitability of IMTA, especially at commercial scale, depends on informed decisions of IMTA producers towards holistic management practices and benthic waste management.

Chapter 5. Culturing the sea cucumber *Holothuria poli* in Integrated Multi-Trophic Aquaculture at an inshore Mediterranean fish farm

Published work edited based on chapter:

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Abstract

The survival and growth of the sea cucumber *Holothuria poli* were assessed during a 12-month field study when cultured at a commercial fish farm in Malta as part of an integrated multi-trophic aquaculture (IMTA) system. Sea cucumbers were cultured directly below a fish cage at 0 m, E0, at 10 m (E10) and 25 m (E25) distances from the cage and at two reference sites (R1 and R2) located over 800 m away from the fish farm. Mass mortalities were recorded at E0 within the first month of the study due to smothering by settled wastes. All individuals died at one of the reference sites, R1, by the end of the study. After deducting missing sea cucumbers, survival rates at E10 (23%) and E25 (33%) from the fish cage were similar to the remaining reference site (R2) (27%). Stocking density and physical disturbances to the sea cucumber cage setup were also probable cause for the low survival rates. The relative weight gain (RWG) and specific growth rates (SGR) of *H. poli* varied significantly between sites close to the fish farm and the reference site. The SGR of *H. poli* at E10 (0.18 ± 0.02% day^{-1}) and E25 (0.20 ± 0.01% day^{-1}) was positive over the whole study period while no average growth was recorded at the reference site (−0.04 ± 0.07% day^{-1}) over the same period. Differences in RWG and SGR were recorded throughout the study. The overall growth observed in *H. poli* by January was followed by a drop in growth rate across all sites and an increase in SGR at E25 in July. Slower growth rates were observed as water temperature approached 15 °C. The results indicated that the sediments near the commercial fish cage provided an enriched source of food that supported significantly better growth in *H. poli*. This suggests that *H. poli* in IMTA might have the potential to uptake organic farm waste and increase aquaculture production, albeit with important considerations for setup design and stocking density.

5.1 Introduction

In the dynamic open-water environment, effective management of aquaculture requires a comprehensive understanding of the interactions between fish farm activities and the surrounding ecosystem. Crowded coastal areas introduce complexities that require representative data and decision-support tools capable of untangling these intricate dynamics to allow strategies to minimise adverse effects while maximising ecological and economic benefits. Amid the competitive constraints on space, existing production sites may need to be optimised through innovative and novel approaches for further aquaculture expansion (Barrington et al., 2009). However, it is equally crucial not to overlook the pressing need to introduce strategies that can help aquaculture grow while addressing the widely documented effects of waste deposition from intensive marine fish farming that include loss of benthic biodiversity (Mazzola et al., 2000; Kalantzi and Karakassis, 2006; Tomassetti et al., 2016) and changes in sediment chemistry, such as increased organic carbon and oxygen depletion (Karakassis et al., 2000; Porello et al., 2005; Papageorgiou et al., 2010).

The uptake and conversion of fish farm waste into marketable biomass by extractive organisms co-cultured with fed species through integrated multi-trophic aquaculture (IMTA) is often recommended as a way to diversify and maximise production while reducing the environmental impact of intensive farming (Troell et al., 2009; Chopin et al., 2012). While previous studies have shown the viability and efficiency of IMTA in land-based systems or laboratory trials, the fewer reported cases of open-water IMTA have reported contradictory growth performances for different fed-extractive species combinations (Mazzola and Sarà, 2001; Cheshuk et al., 2003; Handå et al., 2012; Irisarri et al., 2013, 2014; Jiang et al., 2013; Giangrande et al., 2020). This demonstrates the challenges of coastal and nearshore IMTA in the real-world environment.

Deposit-feeding sea cucumbers have been proposed as potential candidates to take up and reduce the excess accumulation of organic nutrients in seafloor sediment from farm production (Cubillo et al., 2016; Zamora et al., 2016; Chary et al., 2020). The potential of deposit-feeders to mitigate organic pollution emerges from their capacity to rework as much as 10,590 kg dry sediment m^{-2} $year^{-1}$, while feeding selectively on enriched sediments (Lee et al., 2018). Sea cucumbers are not only recommended for use in IMTA for their waste biomitigation potential but also for their high economic value on the seafood market (Toral-Granda et al., 2008; Purcell, 2015). High value sea cucumber species are sold as premium products to meet the increasing demands of luxury seafood markets in Asia. In the Mediterranean region, active fisheries exist for commercially important sea cucumber

species with *Holothuria poli* and the similar shallow water holothurian, *Holothuria tubulosa* becoming increasingly popular target species (González-Wangüemert et al., 2018a) and favourable candidates for aquaculture (Rakaj et al., 2019). Recently, these species have been increasingly harvested along the Italian coast leading to a moratorium on sea cucumber fishing in Italy.

The little data available in literature on the growth and natural density of this species for populations present in other regions of the Mediterranean is limited by variation in geographic and bathymetric distribution (Francour, 1989). Similarly, the population density of the related *H. tubulosa* varies across the Mediterranean within the range of 0.1 – 3.77 individuals m^{-2} (Coulon and Jangoux, 1993; Kazanidis et al., 2010). In the wild, *H. poli* is found in soft marine sediment where naturally occurring organic content is low. Consequently, the foraging behaviour and digestive capacity of *H. poli* allow this sea cucumber to effectively select and assimilate organic-rich particles (Mezali and Soualili, 2013; Belbachir et al., 2014). This underlies the potential importance of this species for nutrient recycling. *H. poli* is also considered a benthic indicator sensitive to pollution (Harmelin et al., 1981; Mezali, 2008). While high organic inputs may be an opportunity for value-added production, additional nutrient inputs from a fish farm could have detrimental effects on the physiological performance of the selected species.

In recent studies in laboratory settings and pilot-scale field experiments, sea cucumbers were able to survive and grow on organic waste from finfish culture (Nelson et al., 2012; Yu et al., 2012; Hannah et al., 2013; MacDonald et al., 2013; Yokoyama, 2013; Yu et al., 2014a, b). In the Mediterranean, the potential of *H. tubulosa* to reduce organic waste in fish farm biodeposits has been shown by the three-fold growth difference when placed under an open-water commercial monoculture farm in sediment with organic carbon content 30 times higher than that present in natural environment (Tolon et al., 2017a). The capacity of individual holothurians to ingest up to 15.25 kg dry weight sediment m^{-2} yr^{-1} and reduce as much as 74.09% of organic carbon per mean total drained weight of 196 g, should lead to further research into the suitability of deposit-feeding sea cucumbers to recycle and remediate organically rich sediments in nearshore IMTA (Neofitou, 2019). Studies on the reduction rate and absorption efficiency of organic carbon and *in situ* growth rates of *H. poli* are lacking. Nonetheless, the proposed species exhibited similar growth rates as *H. tubulosa* under laboratory conditions (Tolon, 2017) with comparable capacities for particle selectivity (Mezali and Soualili, 2013) and higher adaptability to elevated salinities (Tolon, 2017). This suggests *H. poli* could also be a good candidate for IMTA.

This study introduces *H. poli* as an alternative candidate to open-water IMTA in the Mediterranean and aims to provide baseline information on the survival and growth performance of this sea cucumber when cultured in a commercial fish farm.

5.2 Materials and methods

5.2.1 Sea cucumber cage siting

The study was set up at the nearshore commercial fish farm in Marsaxlokk Bay (35°49'39.90" N, 14°32'30.73" E) described in Chapter 2 (Fig. 5.1 A). The reference sites, R1 (35°49'55.1" N, 14°32'59.3" E) and R2 (35°49'53.5" N, 14°32'54.5" E), were located over 800 m north east of the fish farm in 8 m water depth. No aquaculture activity was present within a 600 m radius from the nursery site (Fig. 5.1 B).

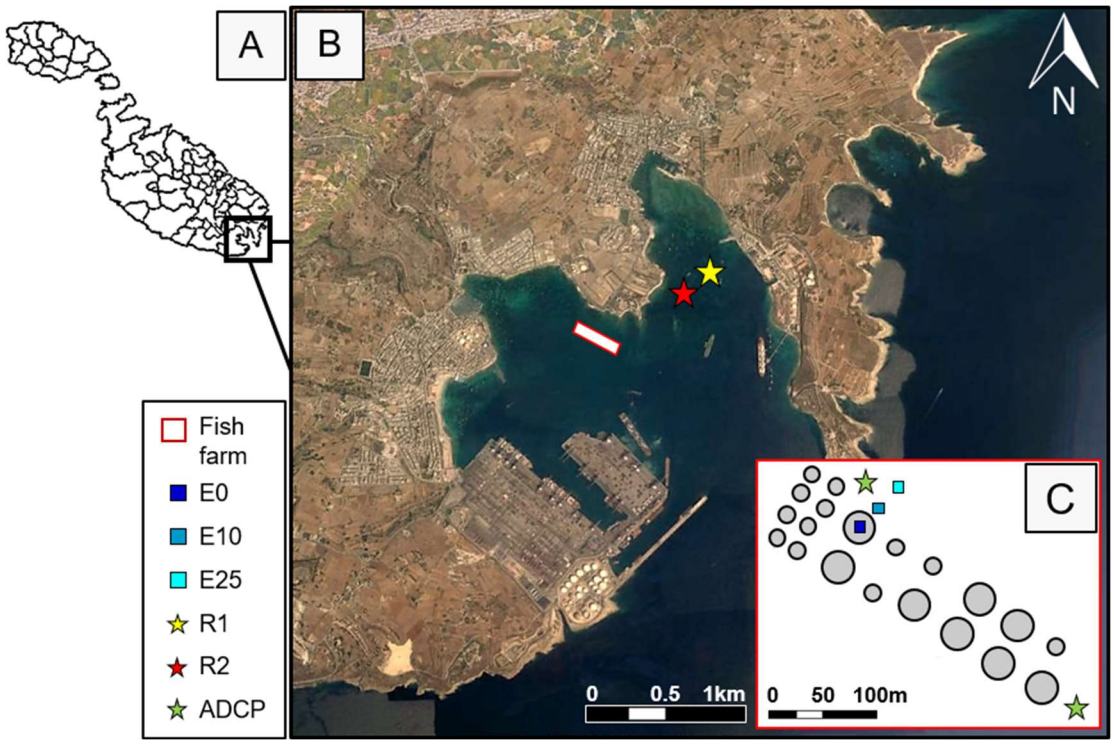


Figure 5.1. A. Location of the test site in Marsaxlokk Port, southeast Malta. B. Zoomed Google Earth image of location showing the IMTA and reference sites, R1 and R2. C. IMTA site showing the sea cucumber cage positions at each experimental site (E0, E10 and E25) and the deployment locations of the acoustic Doppler current profiler (ADCP).

The spatial arrangement of the IMTA setup at the farm site (Fig. 5.1 C) was based on the output of the particulate depositional model, 'Cage Aquaculture Particulate Output & Transport' (CAPOT) (Telfer et al., 2022), that predicted the amount of organic carbon and

the form of waste dispersed from the fish farm to the environment using a grid resolution of 5 m x 5 m, as described in Chapter 4. Production data was obtained from the producer. Water current data was collected at 8 m and 12 m depths over 6 weeks using a current meter (Aquadopp Profiler 400Hz; Nortek, Norway). The profiler was deployed on the seabed between May and June 2018, at two different locations both approximately 10 – 15 m from the fish cages (Fig. 5.1 C). Input data on food losses and settling velocities for feed and faecal particles were obtained from literature specified in Table 4.2 (Chapter 4). Model outputs plotted in Surfer 16® (Golden Software Inc, USA) were used for the appropriate siting of sea cucumber cages at the IMTA site to maximise potential waste uptake from the seabed.

Three cylindrical sea cucumber cages (1 m x 0.2 m (*d* x *h*)) made of 0.8 cm galvanised mesh wiring and a synthetic rope mesh bottom were set at 8 m depth directly below a commercial fish cage at 0 m (E0), another three cages at 10 m (E10) and then at 25 m (E25) from the fish cage. The same cage setup was used at the reference sites, R1 and R2 (Fig. 5.1 B). At each site, sea cucumber cages were spaced out evenly every 2 m, weighted at either side of each cage and moored to the seafloor in parallel to the fish cages.

5.2.2 Sea cucumbers

Juvenile *H. poli* specimens were collected in September 2018 by SCUBA diving Palermo, Sicily and shipped to a land-based facility in Malta. Sea cucumbers were acclimated in 500 L tanks that were supplied with flow-through ambient water from the nearshore study site. The juveniles were fed an artificial microalgal diet (Algamac Protein Plus, Pacific Trading, Ireland) for two weeks prior to the start of the experiment. Faeces and accumulated uneaten feed were removed every two days, and physicochemical parameters of water quality in the tanks were assessed daily. No evisceration or mortalities were recorded during the acclimation period. At the end of this period, specimens that showed no evident signs of disease and were of similar initial mean weight (± standard deviation, SD) (24.6 ± 2.1 g) were selected.

The field trials started in October 2018 and ran until September 2019. 150 sea cucumbers were selected for the experiment and each cage randomly stocked with 10 sea cucumber specimens with an initial mean stocking biomass of 313 ± 6.6 g m^{-2}. The initial coefficient of variation for sea cucumber weight was < 12% and without significant differences (p = 0.183) between sites.

5.2.3 Sea cucumber survival and growth

Every two months, SCUBA divers retrieved all the sea cucumbers from their cages to assess survival and growth performance. Missing sea cucumber individuals were considered as mortalities and deducted. In addition, apparent diseased sea cucumbers present in each cage were removed and considered dead, not to be used for any subsequent analyses. Any water, sediment, and detritus on the sea cucumbers' integument was removed and individuals were weighed to the nearest ± 0.1 g at 30 s of removal from seawater to allow sea cucumbers to drain. After weighing, the surviving sea cucumbers were redeployed to their respective cage.

The mean relative weight gain (RWG), growth rate (GR), specific growth rate (SGR) and survival rate of *H. poli* for each experimental cage were determined according to Equations 1 to 4 respectively.

$$RWG\ (\%) = 100 \times (W_2 - W_1)/ W_1 \quad \text{[Equation 1]}$$

$$GR\ (g\ day^{-1}) = W_2 - W_1/ t \quad \text{[Equation 2]}$$

$$SGR\ (\%\ day^{-1}) = 100 \times (\ln W_2 - \ln W_1)/ t \quad \text{[Equation 3]}$$

$$Survival\ rate\ (\%) = 100 \times (n_2/ n_1) \quad \text{[Equation 4]}$$

where W_2 and W_1 are final and initial mean wet weight (g); t is culture duration (days); and n_2 and n_1 are final and initial number of *H. poli* individuals in each cage.

5.2.4 Environmental parameters

Measurements of water quality were recorded at each experimental site. Temperature (°C), dissolved oxygen (DO; mg L^{-1}), and pH were measured on site from near-bottom water samples collected in Niskin bottles and measured by an HQD Intellical meter (HQ30d; HACH, US), over 12 months between October 2018 and September 2019. Duplicate 2 L water samples were collected and transferred to the laboratory in a cool box for the quantitative analysis of nutrients and total suspended solids (TSS). These water quality parameters were assessed monthly from the fourth month of the study (February 2019) when suitable equipment and method detection limits were applied. Samples for the analysis of $N\text{-}NH_4^+$ were preserved with sulphuric acid. The parameters and methods of analyses together with their limits of detection are presented in Table 5.1.

Table 5.1. Methods of analyses used to assess near-bottom water quality parameters.

Parameters	Principal analytical method	Standard methodology	Limit of detection
Ammonium Nitrogen	Spectrophotometry	APAT CNR IRSA 4030 A1 Man 29 2003	0.03 µmol L^{-1}
Nitrate	Spectrophotometry	APAT CNR IRSA 4040 A2 Man 29 2003	0.03 µmol L^{-1}
Nitrite	Spectrophotometry	APAT CNR IRSA 4050 Man 29 2003	0.03 µmol L^{-1}
Total Phosphorus	Spectrophotometry	APAT CNR IRSA 4110 A2 Man 29 2003	0.03 µmol L^{-1}
Total Suspended Solids	Gravimetry	APAT CNR IRSA 2090 B Man 29 2003	0.01 mg L^{-1}

Seafloor sediments were collected in triplicates using sediment cores (5 cm diameter) at the start of the experiment in October 2018, then in February, May and at the end of the study, in September 2019, from each individual sea cucumber cage site. The top 3 cm of sediment samples were sliced, extracted and rinsed with distilled water. Dried samples were ground and homogenised prior to the analysis of total organic carbon (TOC) and total nitrogen (TN). Sub-samples were weighed in tin capsules and then analysed using a FlashSmart NC ORG elemental analyser. TOC was determined by deduction after ashing samples at 600 ^{o}C for 12h, and analysing total inorganic carbon. TOC and TN contents were calculated by comparison to standard samples. Elemental C: N ratios are reported as weight ratios and expressed as mg mg^{-1}.

5.2.5 Data analysis

Statistical analysis was performed using SPSS v26.0 for Windows (SPSS Inc., Chicago, USA). Shapiro-Wilk's test was used to assess normality of variables whereas homogeneity of variances was determined using Levene's test. A significance level of 0.05 was assumed. Environmental parameters were assessed using a General Linear Model (GLM) (*site* x *time*) followed by pairwise comparisons with Bonferroni adjusted significance levels.

Survival and growth parameter data were analysed using a GLM (site x time) with pairwise comparisons with Bonferroni correction. Differences between sites at the same sampling time were assessed using one-way ANOVA followed by Tukey's *post hoc* test. A generalised linear model was used to assess data that violated the assumption of normality tested using Shapiro-Wilk's test. Data on growth rates were represented by the average

value for each cage for the different experimental sites. Zero values for growth parameter data were omitted from the analysis.

5.3 Results

5.3.1 Hydrography and waste dispersion

The hydrography collected from the two sites near the fish farm showed average near-seabed flows of 0.091 m s^{-1} in May and 0.149 m s^{-1} in June 2018, with slow residual current flows and a south westerly to westerly direction during these periods.

The dispersion of particulate waste from the fish cages was highest directly below the fish cage (E0) at 74.3 gC m^{-2} day^{-1} in May and 23.3 gC m^{-2} day^{-1} in June (Fig. 5.2). The predictions of sedimentation show that the deposition of organic carbon was local to the fish cage area with flux decreasing with increasing distance from the edge of the fish cage. The model shows that sea cucumbers were placed in an area of high organic waste deposition at E0 whereas sea cucumber cages placed at increasing distances from the fish cage were in areas of lower deposition of organic carbon. Model predictions showed similar estimated values for organic carbon at E10 in May (5.6 gC m^{-2}) day^{-1} and June (5.1 gC m^{-2} day^{-1}), and lowest deposition rates at 25 m (E25) (May: 0.02 gC m^{-2} day^{-1}; June: 0.4 gC m^{-2} day^{-1}).

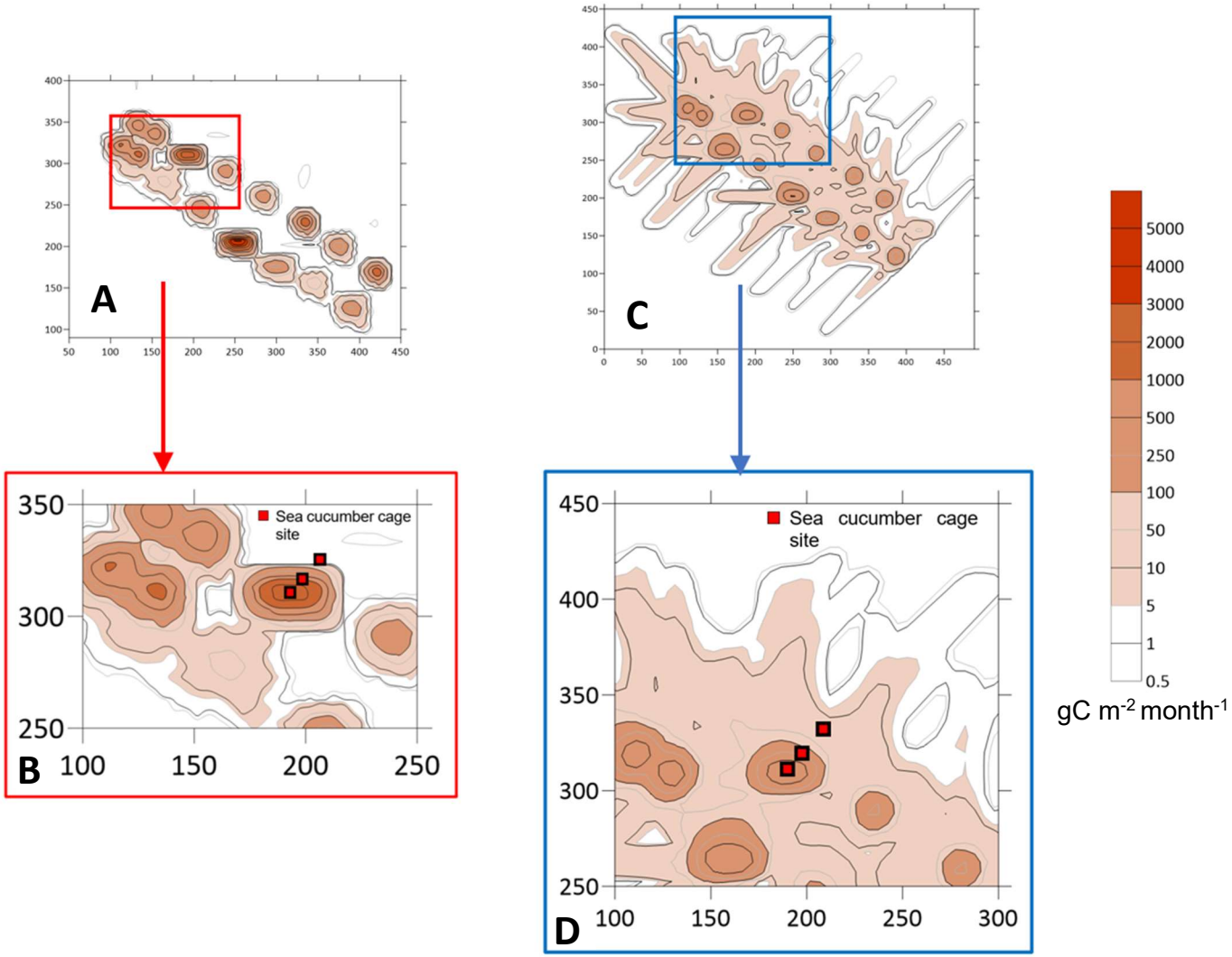


Figure 5.2. Modelled contour plot in 5m x 5 m grid resolution for carbon waste deposition (g m^{-2} $month^{-1}$) at the study site. Plot for deposition from A. all cages on the fish farm and B. fish cage used for co-culture of *Holothuria poli* in May 2018. C. Plot for deposition from all cages on the fish farm and D. fish cage used for co-culture of *Holothuria poli* in June 2018.

5.3.2 Environmental parameters

Throughout the trial period, temperature ranged between 14.9 ± 0.1 ºC and 28.3 ± 0.1 ºC with similar readings recorded across sites (p = 0.186) (Fig. 5.3 A). The lowest water temperature was recorded in February, and then increased steadily to reach peak readings in August. DO levels varied significantly over time (p < 0.001) in the range between 6.3 ± 0.3 mg L^{-1} and 10.5 ± 0.1 mg L^{-1} (Fig. 5.3 B). The lowest readings were recorded in proximity to the fish cages (E0 and E10) whereas the highest values were recorded at the reference sites (p < 0.001). DO levels were always above 5 mg L^{-1}. The water pH was consistent until March (8.18 ± 0.06 to 8.32 ± 0.01), and then levels decreased to reach the lowest recorded values (7.90 ± 0.03) at E0 in June (Fig. 5.3 C).

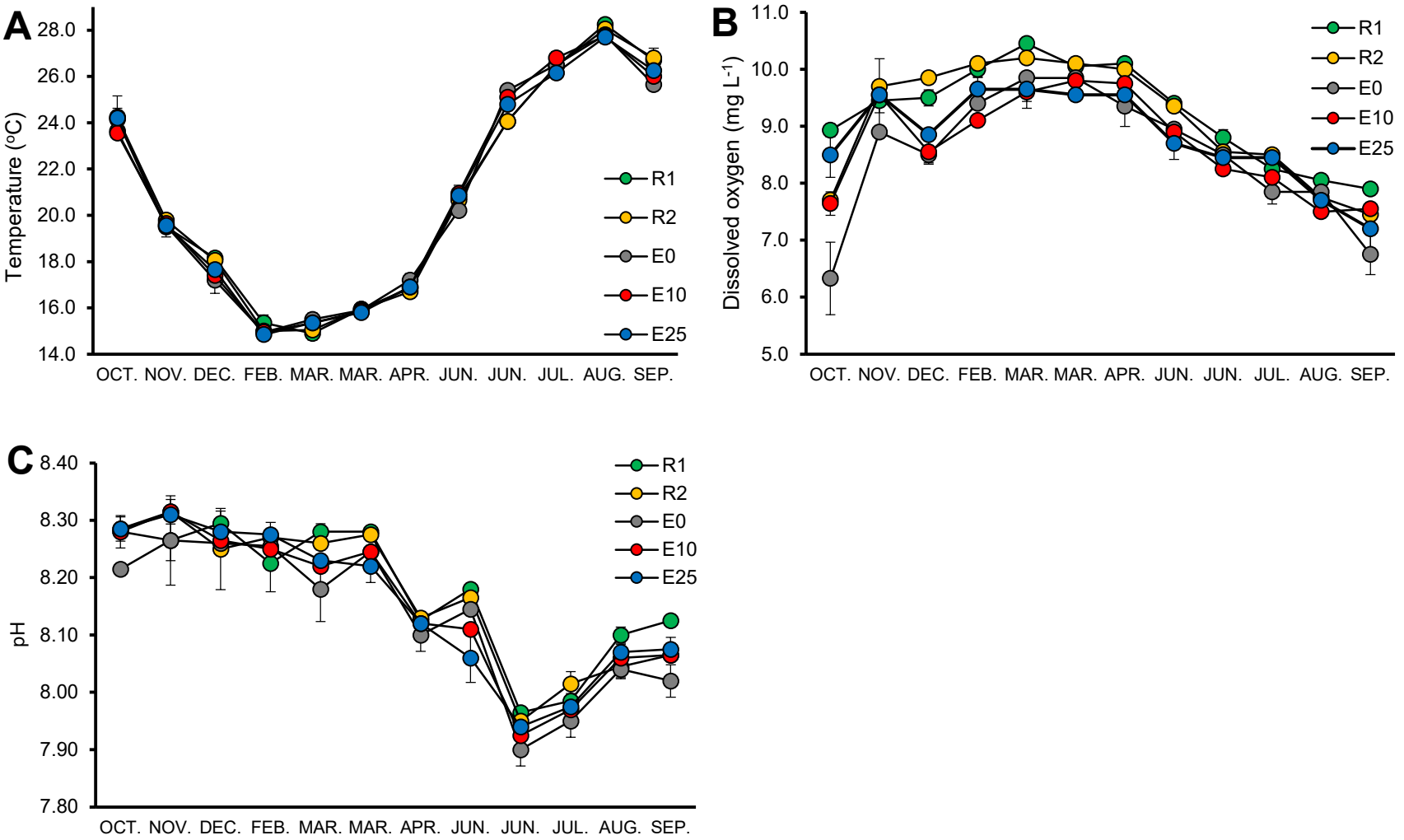
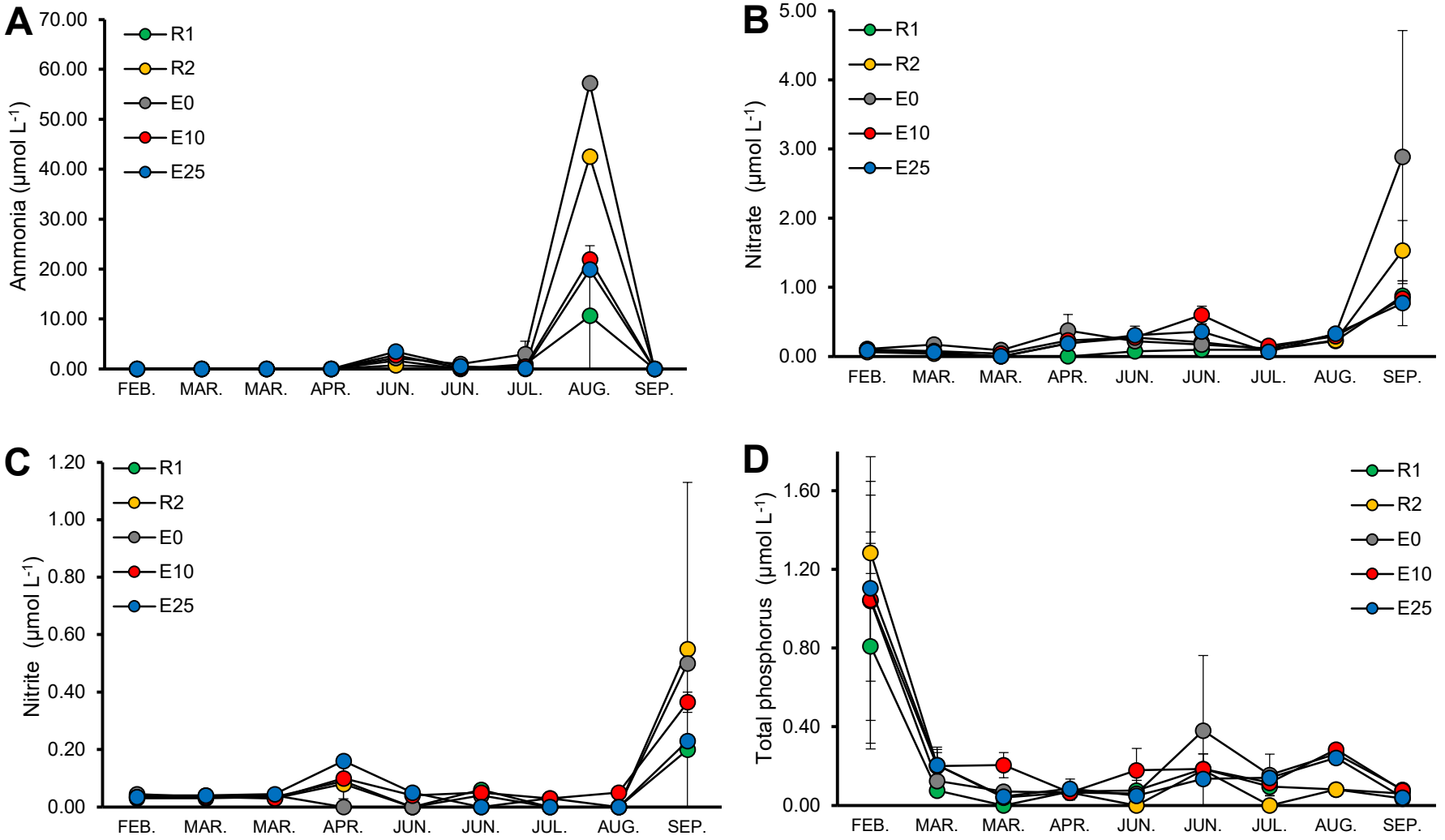


Figure 5.3. Temporal variation in near-bottom A. temperature, B. dissolved oxygen levels and C. pH, close to fish cages at E0, E10 and E25, and at reference sites R1 and R2. Values are given as mean ± SD (*n* = 2). Error bars plotted for standard deviation.

TSS and nutrients levels varied significantly across sampling sites ($p < 0.05$), with the exception of ammonia ($p = 0.186$). Levels of ammonia changed significantly over time ($p < 0.001$), particularly apparent were the peak level recorded in August ($p < 0.05$) (Fig. 5.4 A). Similarly, peak nitrate levels were recorded towards the end of summer, notably at E0 (2.89 ± 0.92 µmol L^{-1}) (Fig. 5.4 B). The nitrite levels were consistently below 0.05 µmol L^{-1} and similar across all sites (Fig. 5.4 C); however, concentrations increased significantly in April and September at these sites ($p < 0.05$). Differences in nitrate concentration were significant between E0 and the sites close to the fish cage, E10 and E25, and the reference sites, R1 and R2 ($p < 0.05$). On the other hand, marked differences in nitrite levels were only recorded between sites E0 and R1, and R1 and R2 ($p < 0.05$). TP levels dropped rapidly after February with significant differences across most sampling periods ($p < 0.05$) (Fig. 5.4 D). TSS levels increased to a peak concentration in late spring (33.38 ± 0.35 mg L^{-1}) (Fig. 5.4 E). Pairwise comparisons revealed marked variation in TSS levels across most sampling periods ($p < 0.05$). TSS levels under the fish cage (E0) were significantly higher than those recorded at E10 and R1 ($p < 0.05$).


A
Ammonia (µmol L-1)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
R1
R2
E0
E10
E25
FEB.
MAR.
MAR.
APR.
JUN.
JUN.
JUL.
AUG.
SEP.
B
Nitrate (µmol L-1)
5.00
4.00
3.00
2.00
1.00
0.00
C
Nitrite (µmol L-1)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
D
Total phosphorus (µmol L-1)
1.60
1.20
0.80
0.40
0.00

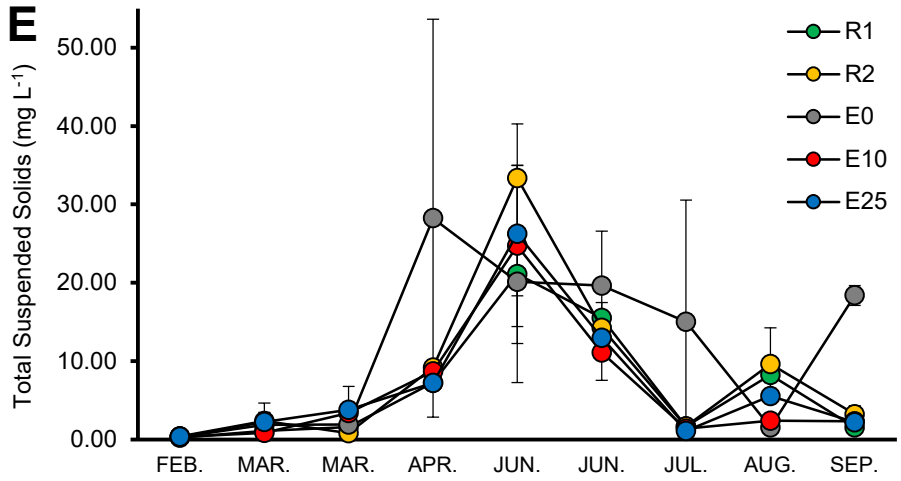


Figure 5.4. Temporal variation in near-bottom levels of A. ammonia, B. nitrate, C. nitrite, D. total phosphorus, and E. total suspended solids, close to fish cages at E0, E10 and E25, and at reference sites R1 and R2. Values are given as mean ± SD (n = 2). Error bars plotted for standard deviation.

The content of TN, TOC and C: N in surface sediments varied significantly between the different sites and over time ($p < 0.05$), except TN levels between the different sampling periods ($p = 0.437$). Sediments near the fish cages were generally more enriched in TN content than at the reference sites, with distinct spatial patterns (Fig. 5.5 A). TOC content in surface sediments did not show consistent spatial patterns between sites near fish cages and those at the reference location during the study (Fig. 5.5 B). Measured sedimentary organic carbon levels were lowest at all the sampled sites in May ($p < 0.001$), with the weight ratios of C: N showing similar temporal trends ($p < 0.001$). The C: N ratios in sediments were significantly lower near fish cages in October (Fig. 5.5 C).

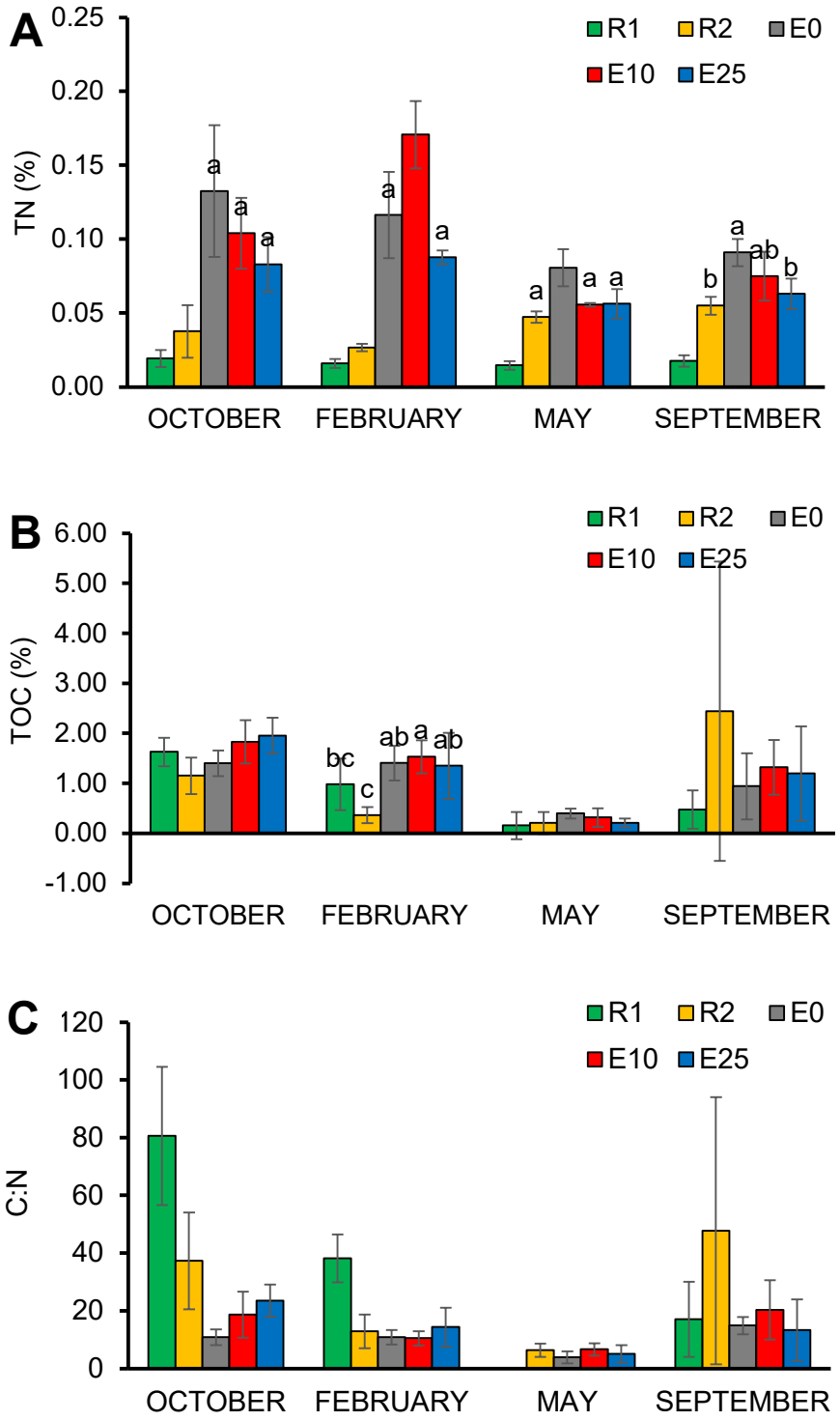


Figure 5.5. Temporal variation in the contents of A. total nitrogen (TN), B. total organic carbon (TOC) and C. weight ratio of carbon to nitrogen (C: N) in the surficial sediments (0 – 3 cm) close to fish cages at E0, E10 and E25, and at reference sites R1 and R2. Values are given as mean ± SD (n = 3). Error bars plotted for standard deviation. Different superscript labels indicate a significant difference (p < 0.05) between data for sites at the same sampling time.

5.3.3 Survival and growth

Within the first month of the study, all experimental cages on the seafloor directly below the fish cage (E0) were completely smothered with sediment consequently leading to mass mortality of the sea cucumbers (Fig. 5.6). At the other IMTA and reference sites, survival rates were relatively high until March, followed by a marked drop across all groups by May. Episodic storm events between March and May disturbed the sea cucumber cage setup and contributed to escapees and mortalities. At R1, all sea cucumbers were either missing or dead by September. The survival rates remained stable in the remaining groups until the end of the study. As a result of loss or mortality, the final survival rates at E10 and E25 were 23% and 33% respectively, similar to that of *H. poli* at R2 which was 27% (p = 0.706).

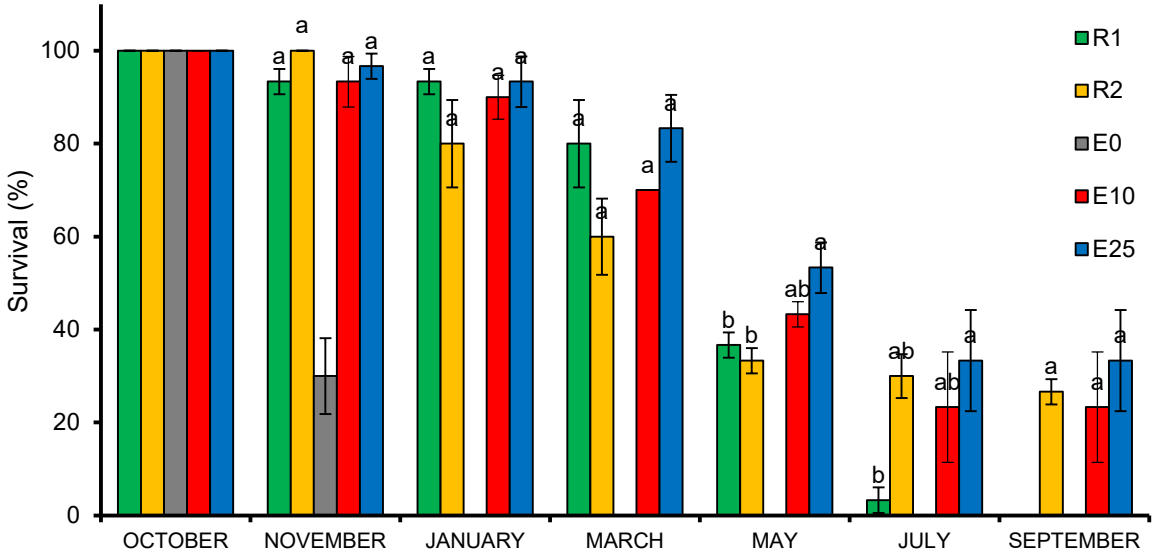


Figure 5.6. Percentage survival of *Holothuria poli* deployed at different IMTA (E0, E10 and E25) and reference (R1 and R2) sites over 12 months, between October 2018 (on deployment) and September 2019. Average survival for each site based on cage mean values. Standard deviation (n = 3) is represented by error bars. Different superscript labels indicate a significant difference (p < 0.05) between data for sites at the same sampling time.

The final weight of *H. poli* juveniles at E10 ranged between 38.5 g and 61.0 g (mean ± SD, 47.0 ± 7.0 g, n = 7), whereas that at E25 was between 42.5 g and 58.5 g (mean ± SD, 48.6 ± 5.0 g, n = 10). The final weight range of *H. poli* at R2 was between 11.4 g and 36 g with a mean of 20.5 ± 8.0 g (n = 8) (Fig. 5.7). Juvenile *H. poli* cultured at E10 and E25 showed an overall positive RWG with the greatest increase being the approximate two-fold increase at E25 over the culture period, though not statistically different from that of *H. poli* at E10 (87%). On the other hand, *H. poli* at R2 had suffered a loss in biomass by the end of the

experiment (-12%). The RWG differed significantly between sites (p = 0.008) with biomass gains in juvenile *H. poli* at E10 and E25 being significantly higher than those at R2.

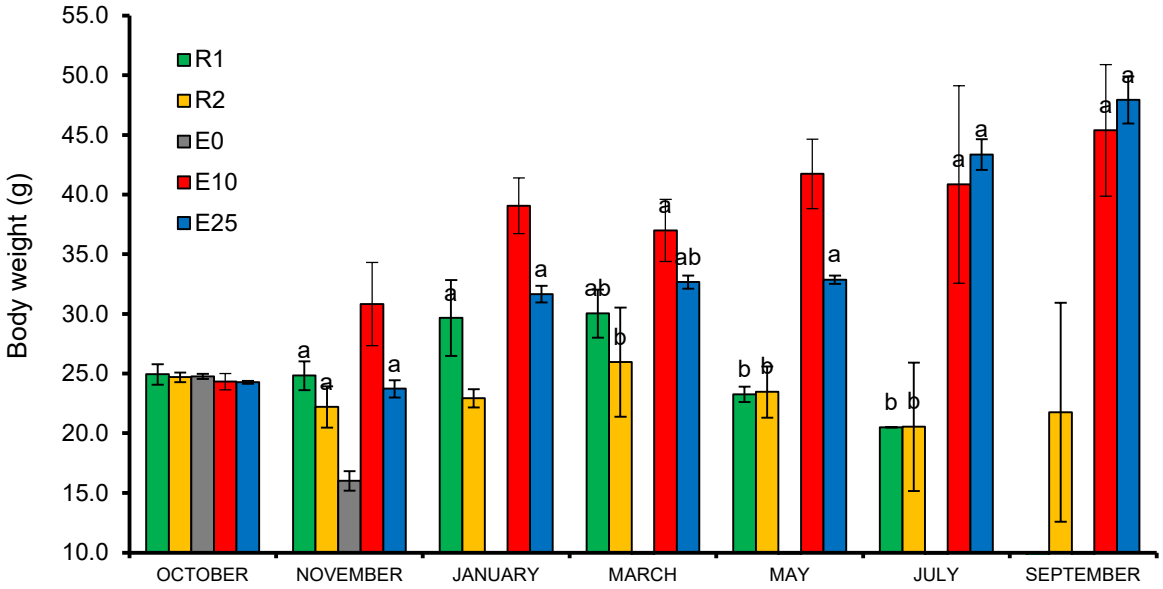


Figure 5.7. Mean wet body weight of *Holothuria poli* deployed at different IMTA (E0, E10 and E25) and reference (R1 and R2) sites over 12 months, between October 2018 (on deployment) and September 2019. Average weight for each site based on cage mean values. Standard deviation (n = 3) is represented by error bars. Zero values are not included. Different superscript labels indicate a significant difference (p< 0.05) between data for sites at the same sampling time.

The SGR of *H. Poli* at sites E10 and E25 was positive over the whole study period (E10 = 0.18 ± 0.02% day^{-1}; E25 = 0.20 ± 0.01% day^{-1}) whereas that at R2, was negative (-0.04 ± 0.07% day^{-1}). The most marked differences in growth rates were between E25 and R2 (p= 0.037) (Fig. 5.8). The growth rates were influenced by site during the study with multiple comparisons showing differences in SGR of *H. poli* between R1 and the IMTA sites, E10 and E25 (p < 0.001). Differences in growth rates were recorded with peak growth at E25 in July (SGR = 0.64% day^{-1}; GR = 0.24 ± 0.05 g day^{-1}) and E10 in November (SGR = 0.58 ± 0.1% day^{-1}; GR = 0.20 ± 0.01 g day^{-1}). The SGR of *H. poli* dropped significantly by March with little to no growth reported at the IMTA sites and negative growth rates at the references sites in the same period (p < 0.05), though not necessarily correlated with the temporal variability in the water quality parameters. The SGR of *H. poli* increased again towards the end of the experiment (p > 0.05).

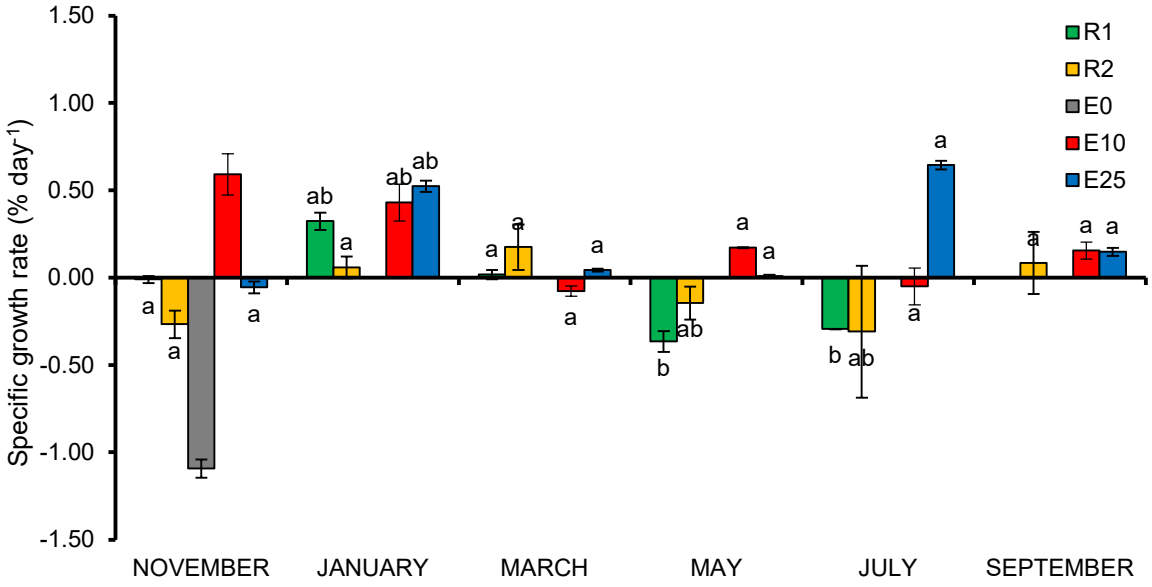


Figure 5.8. Specific growth rate of *Holothuria poli* deployed at different IMTA (E0, E10 and E25) and reference sites (R1 and R2) between successive sampling periods from October 2018 (on deployment) to September 2019. Average growth rates per site based on cage mean values. Standard error is represented (n = 3) by error bars. Zero values are not included. Different superscript labels indicate a significant difference (p < 0.05) between data for sites at the same sampling time.

5.4 Discussion

This work aimed to assess the survival and growth of *H. poli* on seafloor sediment that receives organic waste from uneaten fish feed and faecal pellets. Findings show that with proper siting, the deposit-feeding *H. poli* can survive and grow better on seafloor sediment near commercial fish farms compared to the natural environment.

The mass mortalities reported for sea cucumbers directly below the fish cage indicate unfavourable conditions for bottom culture of *H. poli*. The lower DO levels recorded under the fish cage may be attributed to a higher flux and accumulation of organic matter, but they were not at levels considered lethal in other species. Similar events of mass mortality were reported for *Holothuria leucospilota* cultured directly below the fish cage when DO levels were < 2.5 mg L^{-1} but survived when DO levels were above 3.2 mg L^{-1} (Yu et al., 2012). In this study, the concentrations of DO in near-bottom water were consistently above 5 mg L^{-1}, similar to conditions maintained in the laboratory culture of *H. poli* (Tolon, 2017). This sea cucumber species has been reported to survive and grow in laboratory conditions (Tolon, 2017) at similar water temperature experienced during the mass mortality at E0. Moreover, sea cucumbers were able to grow in similar temperature conditions at the other IMTA and

references sites therefore this is not expected to have contributed to the mass mortality at E0. Other water quality parameters measured would not be expected to bring about this level of mortality, which implies that the mortalities could be attributed to the conditions that prevailed in seafloor sediment below the fish cage and possibly farming activity that was only relevant to this area.

The particulate depositional modelling conducted prior to the present study showed that the estimated deposition of organic carbon was highest directly below the sea bream cage, at 2302.3 gC m^{-2} in May and 721.4 gC m^{-2} in June before *H. poli* juveniles were deployed under the fish farm. Several studies have reported excess sedimentation of organic matter below the fish cage that decreased with distance away from the cage (Pérez, et al. 2002; Sarà et al., 2004; Corner et al., 2006; Holmer et al., 2007). Findings suggest that the benthic community structure is affected when carbon flux (> 1 – 2 gC m^{-2} day^{-1}) exceed carbon loading tolerances (Holmer et al., 2005; Chamberlain and Stucchi, 2007). Based on these deductions, the modelled deposition of organic carbon in May and June may suggest changes to benthic conditions, at least during this period. These conditions would be expected to have effects on benthic sediment quality, among which elevated sediment oxygen demand (Wu, 1995; Holmer et al., 2005). The predicted load and accumulation of organic matter over a short period may be cause for the mass mortalities recorded below the fish cage. This suggests that integrating sea cucumber experimental sites within commercial monoculture requires consideration for suitable culture methods, appropriate setup design and siting considerations for IMTA systems.

The predicted levels of sedimentation decreased rapidly within a short distance from the fish cage. This gradient suggests that benthic enrichment may be localised at the studied farm site. The accumulation of organic particulates in surface sediments near the fish cages can be associated with the settlement of fish waste products and uneaten feed pellets (Sarà et al., 2004; Cromey et al., 2012). Waste from fish farms can be an additional organic-rich source that releases particulate nitrogen and carbon in varying proportions. The low C: N values near fish cages, particularly in October, follow trends of lower C: N ratios under sea bream and sea bass net cages in other Mediterranean countries (Holmer et al., 2007). In this study, nitrogen enrichment in sediments near fish cages and TN values that declined away from the farm also corroborates findings from isotopic studies in similar environments (Sarà et al., 2004; Holmer et al, 2007). The lack of consistent spatial patterns in sedimentary organic carbon content and lower than predicted values may be attributed to farm and site-specific variables like sedimentary metabolic processes that would require further research.

The growth performance of *H. poli* at E10 and E25, relative to that at E0 and the reference sites, indicates that the quantities and quality of organic content in seafloor sediment at these sites were suitable for sea cucumbers. Nevertheless, the reported escapees and low survival rates across all sites when compared to other field studies (Yokoyama, 2013; Yu et al., 2014a, b; Neofitou et al., 2019) suggest other critical factors may need to be considered for open-water culture of juvenile *H. poli*. Beyond the effects of rough sea conditions, which could have resulted in damage to cages facilitating escapees, the survival and growth of *H. poli* may have been influenced by the initial stocking density. The stocking density for juvenile *H. poli* in this experiment was higher than that (10 individuals m^{-2}) recommended by Nefitou et al. (2019) for *H. tubulosa*. The total stocking biomass used in this experiment (313 g m^{-2}) of *H. poli* was higher than that used in the laboratory culture of *H. poli* (270 g m^{-2}) by Tolon (2017) and that proposed for long-term culture of *H. tubulosa* (6 individuals m^{-2}, 253 g m^{-2}) (Tolon et al., 2017a) where no mortalities were recorded. Studies have reported survival rates up to 100% and higher growth rates for *A. japonicus* when cultured at low densities in similar studies (Yokoyama, 2013; Yu et al., 2014a, b). Conversely, survival rates in *H. tubulosa* showed a significant drop during a 30-day field trial at higher culture density (ca. 3300 g m^{-2}) (Neofitou et al., 2019) than that used in the present study. Competition is expected to have contributed to the missing *H. poli* individuals and consequentially, the low survival rates. This may be affirmed by the absence of individuals and escape attempts from experimental cages, in response to competition for space and food. This further demonstrates the need to use appropriate setup design for the bottom-culture of this deposit-feeding sea cucumber. While no published information is available on the local natural population densities of *H. poli*, the survival and growth performance of *H. poli* would be expected to improve at lower stocking densities.

Quantitative data on the growth performance of *H. poli* in the wild environment is limited and extensive trends of growth are unknown. The RWG of *H. poli* juveniles at E10 and E25 suggests a suitable food source for sea cucumbers, corroborating findings that deposit-feeders grow better in organic-rich sediments (Nelson et al., 2012; Yu et al., 2012; Hannah et al., 2013; MacDonald et al., 2013; Yokoyama, 2013; Yu et al., 2014a, b). These findings are consistent with other open-water studies that have demonstrated the ability of sea cucumbers to grow better in proximity to fish cages rather than at reference sites (Yokoyama, 2013; Yu et al., 2014a, b, Tolon et al., 2017b; Grosso et al., 2021). Nevertheless, even with favourable growth rates, poor survival rates at all sea cucumber culture sites suggest underlying factors, beyond those of overcrowding that presumably contributed to escapees, that led to apparent diseases and eventual mortality. Poor survival rates despite significant growth may signal conditions that are not conducive with the health

of the sea cucumbers and suggest underlying issues such as disease outbreaks, stress factors, poor environmental conditions near seafloor sediments, or nutritional imbalances. Consequently, these can have significant influence on the overall economic and environmental benefits of sea cucumbers in IMTA. The causes of this mortality and its effects commercial IMTA operations require further investigation. Understanding species-specific dietary requirements and tolerance ranges for environmental quality criteria is essential for effective management of sea cucumber health and performance in IMTA.

During a 3-month study by Tolon et al. (2017b), *H. tubulosa* grew an average of 0.32% day^{-1} below commercial cages in sediments enriched in organic carbon content (4.68 – 4.84%) and without record of mass mortalities. The average growth rate of *H. tubulosa* decreased (0.22% day^{-1}) at 70 m from the farm in sediments that were less influenced by organic carbon flux (3.64 – 4.2%) (Tolon et al., 2017b). Over 12 months, the growth rates of *H. poli* close to the fish cages (0.18 – 0.2% day^{-1}) were lower than the rates achieved by *H. tubulosa*. Nonetheless, peak growth rates of *H. poli* observed in this trial in November and July were higher than that reported by Tolon et al. (2017b) and comparable to those of *A. japonicus* in similar studies (Yu et al., 2014b). The SGR of *H. poli* at E10 and E25 increased until January but then decreased by March. This compares well with the growth trends of *A. japonicus* in open-water IMTA (Yokoyama, 2013; Yu et al., 2014b).

In a land-based setup, the average SGR of *H. poli* was 0.03% day^{-1} at 15 ^{o}C and 0.87% day^{-1} at 25 ^{o}C with juveniles able to survive and maintain weight through the winter (Tolon, 2017). In the present study, growth performance in *H. poli* generally decreased and subsequently stagnated to maintain weight as water temperatures approached 15 ^{o}C. While optimal water temperatures are species-specific, the seasonal fluctuations in growth performance of *H. poli* agree with conclusions of higher growth in the warmer months, and marginal or lack of growth for *H. tubulosa* and *A. japonicus* as water temperatures dropped to sub-optimal temperatures (Yokoyama, 2013; Günay et al., 2015; Tolon, 2017).

5.5 Conclusion

This 12-month field study showed that *H. poli* was able to grow better in locations near a commercial fish farm rather than at the reference sites, suggesting that sea cucumbers may have utilised nutrients from the waste released from the fish farm. Sea cucumbers cultured directly below the commercial fish cage were smothered by excess deposition of organic wastes from the fish cages and revealed the significance of farm-scale models to predict

the deposition footprints and inform proper placement of deposit-feeding organisms in IMTA. This attests to the important role that farm-scale models have in IMTA development.

Despite the positive sea cucumber growth, the effectiveness of fish-sea cucumber IMTA depends on better survival rates than those recorded and that require operational challenges associated with existing technology and infrastructure of open-water system to be addressed. Moreover, understanding and meeting the physiological and metabolic requirements of *H. poli* at different growth stages throughout production is paramount for the success of fish-sea cucumber IMTA. In an open-water environment, establishing connectivity and showing a trophic link between the fish farm and the sea cucumbers using biochemical tracers such as stable isotopes, can be an important step towards understanding the real potential of these extractive organisms to reduce organic waste derived from fish farms.

Chapter 6. Validation of trophic connectivity in fish-sea cucumber Integrated Multi-Trophic Aquaculture using stable isotope and fatty acid analysis

Published work edited based on chapter:

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Abstract

Stable isotope ratios, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), and fatty acids validated the trophic connection between farmed fish in a commercial nearshore fish farm and sea cucumbers in the Mediterranean Sea. This dual tracer approach evaluated organic matter transfer in integrated multi-trophic aquaculture (IMTA) and the ability of sea cucumbers to incorporate fish farm waste (fish faeces and uneaten artificial fish feed) into their tissue. Between October 2018 and September 2019, *Holothuria (Roweothuria) poli* Delle Chiaje, 1824, co-cultured at IMTA sites directly below one of the commercial fish cage, at 10 m and 25 m from the selected fish cage, and at two reference sites over 800 m from the fish farm. Sea cucumbers were sampled from each site in February, May, and September, except at 0 m due to mass mortalities recorded here in the first month of study. Isotopic mixing models revealed that fish farm organic waste was the dominant dietary source for *H. poli* in IMTA at 10 m and 25 m from the cage. The contribution of marine plant-derived organic matter, *Posidonia oceanica* leaves and rhizomes, was least important. The isotopic signatures of sea cucumber tissues at reference sites were not explained by the sampled food resources. Importantly, fatty acid profiling revealed a high abundance of individual terrestrial plant fatty acids, such as oleic (18:1*n*-9), linoleic (18:2*n*-6) and eicosenoic (20:1*n*-9) acids in sea cucumber tissue at 10 m and 25 m from the fish cage, presumably linked to the terrestrial plant oil content of the fish feeds. At the reference sites, sea cucumber tissues were characterised by higher relative abundance of arachidonic acid (20:4*n*-6) acid, and the natural marine-based eicosapentaenoic (20:5*n*-3) and docosahexaenoic (22:6*n*-3) acids. These analyses revealed important differences in the composition of *H. poli* between the IMTA and reference locations, driven by aquaculture-derived waste near fish cages. Moreover, this study revealed temporal variation in food availability and quality, and possible differences in the physiological responses of *H. poli*. Stable isotope analysis and fatty acid profiling provided complementary evidence for the important dietary preferences of *H. poli* and validated the potential of sea cucumbers to uptake aquaculture organic waste as part of inshore fish–sea cucumber IMTA. It reveals the important implications that an established trophic link has on the viability of using sea cucumbers for the development of IMTA and the sustainable expansion of aquaculture.

6.1 Introduction

Sea cucumbers can grow better on seafloor sediments near fish cages compared to other areas within the natural environment (Chapter 5) and provide promising evidence for the potential of establishing fish-sea cucumber integrated multi-trophic aquaculture (IMTA) systems under commercial aquaculture conditions within the dynamic marine environment. Similarly, earlier research demonstrated the capacity of sea cucumbers to utilise and reduce organic-rich aquaculture waste in experimental open-water IMTA (Yu et al., 2012; Yu et al., 2014a, b; Tolon et al., 2017b; Neofitou et al., 2019). However, if these IMTA systems are to be developed, there is a need to establish a trophic link between fed and extractive species and to verify the ability of sea cucumbers to assimilate organic matter from aquaculture-derived waste.

Deposit-feeding aspidochirotid holothurians can rework benthic sediment to ingest inorganic matter, detrital organic matter (algae, plants and decaying animals) and microorganisms (diatoms, protozoa and bacteria) (Belbachir and Mezali, 2018; 2020). These studies have shown that food sources for holothurians can be diverse and that uptake varies with season depending on the availability and nutritional quality of food items. Furthermore, these studies revealed that holothurians show preference for marine plant-derived organic matter, including dead and live *Posidonia oceanica* leaves. The ability of *Holothuria poli* to selectively ingest and assimilate organic-rich particles (Mezali and Soualili, 2013) promotes this species as a potential candidate to uptake nutrients from organic waste produced by commercial aquaculture. Moreover, as Mediterranean Sea cucumbers become increasingly popular target species to meet the demands of seafood markets in Europe and Asia, sea cucumber-fish IMTA may be novel means of production in the Mediterranean region.

The utilisation of different food resources by sea cucumbers has been assessed through traditional stomach and gut content observations (Belbachir and Mezali, 2018). However, this approach fails to account for indigestible particles (Dalsgaard et al., 2003) and to provide an accurate time-integrated depiction of food source contribution to the consumer diet. Moreover, this approach might not be suitable to process stomach and gut contents from small marine organisms because of the physical limitations in sampling smaller organisms (Gao et al., 2011). The use of stable isotope and fatty acid (FA) analysis is an alternative approach to this conventional technique to trace organic matter pathways in coastal systems and in food webs (Vizzini et al., 2002; Fry, 2006; Gao et al., 2011; Wen et al., 2016; Signa et al., 2017).

Stable isotopes are widely used in food web studies because isotopic ratios change in a predictable manner as nutrients are transferred across trophic levels (Fry and Sherr, 1989). This approach follows the basic principle that the isotopic ratio of consumer tissue reflects that of the assimilated food source and is relevant when sources are isotopically distinct. Stable isotopes can provide valuable information on organic matter and nutrient transfer from aquaculture waste to the natural environment due to isotopically distinct signatures of natural marine resources and aquaculture organic waste (Vizzini and Mazzola, 2004). Isotope analysis has been used to assess nutrient transfer from aquaculture to co-cultured species in IMTA (Gao et al., 2006; Yokoyama, 2013; Park et al., 2015). Particularly, isotopic studies have revealed the ability of the sea cucumber, *Apostichopus japonicus*, to take up organic matter from aquaculture wastes (Gao et al., 2006; Yokoyama, 2013). However, quantitative evaluation of food source contribution is difficult from stable isotope analysis alone, particularly when attempting to distinguish isotopically similar sources (Sun et al., 2013; Wen et al., 2016). The contextual use of other biochemical tracers, such as fatty acids (FAs), can help to overcome this constraint due to their higher biological specificity and trophic stability (Kelly and Scheibling, 2012; Signa et al., 2017). Moreover, the incorporation of terrestrial-based lipids in commercial aquaculture feeds provides the opportunity to use FAs to trace farm-derived sources in the marine environment (Redmond et al., 2010; Parrish, 2013; White et al., 2019). The incorporation of terrestrial-based lipid sources in commercial aquaculture feeds provides the opportunity to use FAs to trace fish farm-derived sources in the marine environment (Redmond et al., 2010; Parrish, 2013; White et al., 2019).

The simultaneous use of stable isotope and FA analyses overcomes the limitations of the individual techniques and provides a complementary approach to assess dietary source contribution (Gao et al., 2006). This dual indicator approach is still not widely applied to assess the assimilation of farmed finfish waste and the bioremediation potential of different co-cultured extractive components in open-water IMTA. This study assessed the ability of sea cucumbers to incorporate dietary organic matter from aquaculture waste into their tissue. The study used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic mixing models to estimate the relative contribution of different food sources to *H. poli* when co-cultured with fed fish in coastal IMTA in the Mediterranean Sea. This study complemented stable isotope techniques with FA profiling to assess dietary information of sea cucumbers in IMTA.

6.2 Materials and methods

6.2.1 Experimental setup and sampling

This study was carried out at the nearshore commercial fish farm in the Marsaxlokk Bay (35°49'39.90"N, 14°32'30.73"E), and the reference sites, R1 (35°49'55.1"N, 14°32'59.3"E) and R2 (35°49'53.5"N, 14°32'54.5"E), described in Chapter 2 (Fig. 2.1). The IMTA system that was set up in October 2018 at this fish farm as part of the experiment carried out in Chapter 5, was used to assess the uptake of organic wastes (uneaten fish feed and fish faeces) released from the commercial fish cage. The sea cucumbers cultured in four cylindrical cages directly below a fish cage (E0), then at 10 m (E10) and 25 m (E25) from the centre of the fish cage, as part of the IMTA trial described in Chapter 5, were used (Fig. 5.1). Similarly, sea cucumbers at R1 and R2 were used to assess dietary preferences of *H. poli* in the natural environment. At each site, three of the four cages were used to collect specimens for laboratory analyses of stable isotope and FAs, while the fourth additional cage was used to replace individuals sacrificed from the other three experimental cages to avoid density-dependent effects on growth. All the cages had been randomly stocked with 10 juvenile sea cucumbers each, for an initial cage biomass of 310 g m^{-2}.

Throughout 2019, sampling of sea cucumbers and potential food sources for stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and FA analyses were carried out simultaneously in February, May, and then at the end of the experiment in September. Two sea cucumbers were collected from each of the three experimental cages at each site and sampling time. Three farmed sea bream were collected from the fish cages to sample fish faeces by dissection. The commercial fish feeds administered to the farmed fish were also sampled. The 2 mm feeds, ‘A’ (Pre-Grower 16; Alltech Coppens, Germany) and ‘B’ (Proactive 2; Veronesi, Italy), and the 3 mm feed ‘C’ (Supreme; Alltech Coppens, Germany) were supplemented in succession or in combination as a mixture between sampling times throughout the study. Feeds ‘A’ and ‘C’ were administered before the first sampling time (i.e. February), feeds ‘A’ and ‘B’ between the first and second sampling time (i.e. February - May), whereas a combination of all feeds was then used until the end of the experiment (i.e. September). These feeds included blends of nitrogen-rich fish oils and terrestrial plant derivatives. The composition of feeds ‘A’ and ‘C’ included the marine sources, fishmeal and fish oils; terrestrial plant-based sources, sunflower meal, rape oil, wheat, maize gluten and soya protein; and the derivatives of terrestrial animals, poultry meal and blood meal, haemoglobin powder, and hydrolysed feather meal, in no particular order. Feed ‘B’ contained fishmeal, fish oil, wheat, wheat gluten meal, and manufactured using soya and corn. The seagrass *P. oceanica* was collected where present near the fish farm and at the reference sites. H. poli show

preference for marine plant-derived organic matter, including dead and live *P. oceanica* leaves (Belbachir and Mezali, 2018; 2020)) and is known to feed on seagrass detritus in the natural environment (Bocagni et al., 2019).

Suspended and seafloor sediments were collected in triplicates from the IMTA and reference sites using sediment traps and 5 cm diameter sediment corers, respectively, during the same sampling times. The sediment traps were deployed at three positions in a northeastern transect line away from the fish cage, directly below the fish cage at E0, E10, and at 25 m E25 (Fig. 5.1 C). Sediment traps were also deployed at R1 and R2. The sediment traps had four replicate tubes according to the design and method used by Chen (2000) (Fig. 6.1). The collection tube had a height to diameter ratio of 7.4: 1 (77.3 cm: 10.5 cm). The sediment trap assemblies were deployed as described in Telfer et al. (2022) and suspended 1 m off the seafloor bottom.

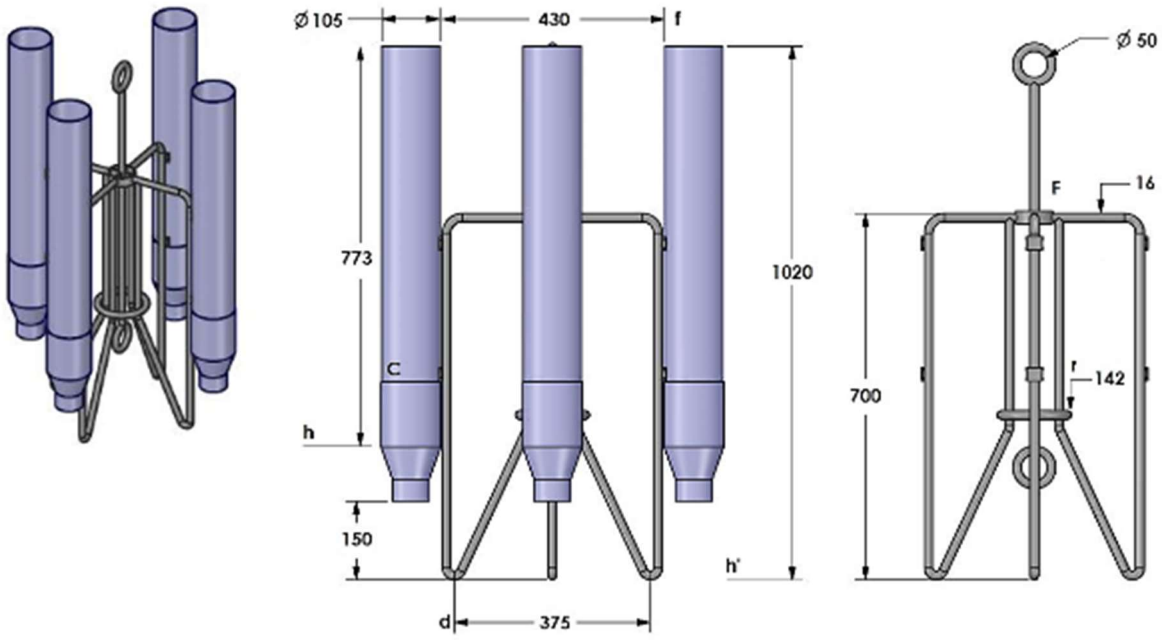


Figure 6.1. The design and measurements (in mm) of the sediment trap. C: PVC collection tube, F: stainless steel frame, h: collection tube height, f: distance between opposing trap feet, d: distance between traps on opposing legs, h^1: total height of collection tube to base of frame, r: diameter of the support ring.

Sediment traps were retrieved 6 days after deployment, or as otherwise necessary depending on weather conditions. Seagrass, suspended and seafloor sediments were sampled at the same time as the sea cucumbers. All samples were transferred in a cool box to the laboratory for processing.

6.2.2 Sample processing, isotopic and fatty acid analysis

In the laboratory, sea cucumber samples were processed to extract the body wall, specifically composed of connective tissue and muscle tissue together. Fish samples were dissected to extract faeces after careful gut dissection. The top 3 cm layer of sediment from the cores was extracted immediately whereas the entire particulate samples from sediment traps was retained. Suspended sediments and seafloor sediments samples collected from the sediment traps and the cores respectively, were homogenised separately. Seagrass samples were washed with distilled water and separated further into leaves and rhizomes, removing any epiphytes by scraping. *P. oceanica* leaves and rhizomes have different signatures (Vizzini et al., 2003) so their contribution to the sea cucumber diet was assessed separately. All samples were stored at -20 ^{o}C until further processing.

For isotope analysis, settling particulate matter and sediment sub-samples were first acidified dropwise using 2N HCl to eliminate carbonates and then washed to retain only the fraction of settling particulate organic matter (SPOM) and sedimentary organic matter (SOM). Sub-samples from each type of sample were then dried at 60 ^{o}C to constant weight and ground to fine powder using a micro-mill (Retsch MM200). Aliquots of each ground sample were packed in tin capsules and analysed for $\delta^{13}C$ and $\delta^{15}N$ using an isotope ratio mass spectrometer (Thermo Delta Plus XP) coupled with an elemental analyser (Thermo Flash EA 1112). The isotope ratios were defined in equation 1 as:

$$\delta X\ (‰) = [(R_{sample}/ R_{standard}) -1] \times 10^{3} \qquad \text{[Equation 1]}$$

where X is ^{13}C or ^{15}N and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. The values are expressed in the standard δ-unit notation (as parts per mil) on international reference standard scales (Vienna-Pee Dee Belemnite for $\delta^{13}C$; atmospheric N_2 for $\delta^{15}N$). Analytical precision was based on the standard deviation of internal standards (International Atomic Energy Agency IAEA-CH-6) replicates and within ± 0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$.

For lipid and FA analysis, frozen sub-samples of sea cucumber body wall, fish faeces, fish feeds, settling particulate matter, sediments, and *P. oceanica* leaves and rhizomes, were freeze-dried (ALPHA 1–4 LD plus, Martin Christ) and ground into fine powder. A modified Bligh and Dyer (1959) method was used to extract lipids from samples using a mixture of MilliQ distilled water, methanol and chloroform (1:2:1 *v*:*v*:*v*) with 0.01% BHT (butylated hydroxyl toluene) to avoid lipid oxidation. Samples were sonicated and centrifuged to separate the lipid and aqueous phases during extraction. Fatty acid methyl esters (FAMEs) were then isolated from the lipid extract through an acid-catalysed transesterification with

methanolic hydrogen chloride as described in Christie (1993). FAMEs were subsequently analysed by a gas chromatograph (GC-2010, Shimadzu) equipped with a BPX-70 capillary column (30 m length × 0.25 mm ID × 0.25 µm film thickness, SGE Analytical Science), and detected by a flame ionisation detector. Peaks were identified by using the retention times from mixed commercial standards (37FAME and BAME from Supelco; BR1 and QUALFISH from Larodan). Tricosanoic acid (C23:0) was used as an internal standard for FAME quantification. Lipid concentration and relative abundance of individual FAs were expressed as the percentage of the total FAs. These are presented as mg $100g^{-1}$ of dry sample.

6.2.3 Data analysis

$\delta^{13}C$ and $\delta^{15}N$ data were used to run Bayesian mixing models to estimate the median (± 95% credible interval) dietary contribution of food sources to *H. poli*, using the R (*v*. 4.0.2) (R Core Team, 2015) package MixSIAR (Stable Isotope Analysis in R) (Stock and Semmens, 2016; Stock et al., 2018) in RStudio (*v*. 1.3.1073).

The isotopic values for fish feed and fish faeces were pooled *a priori* when they were not statistically distinguishable and considered cumulatively as a farm-derived source of waste to allow mixing models to converge on a unique solution. This pooling increased isotopic variability and could influence the certainty in estimates of source contributions from the models (Phillips and Gregg, 2001). *P. oceanica* (leaves and rhizomes) and sediments (SPOM and SOM) were also used as potential food sources in the model to assess dietary contribution to *H. poli*. Before running the mixing models, significant differences in the $\delta^{13}C$ and $\delta^{15}N$ of sources were assessed separately per site for each sampling time in SPSS (*v.* 1.0.0.1327) using a general linear model (GLM) when residuals followed normal distribution, and a generalised linear model when data violated the assumption of normality. Data were assessed for normality with Kolmogorov–Smirnov test and homogeneity of variances using Levene's test.

Mixing models were fitted separately for each individual level of factor 'site' (E0, E10, E25, R1 and R2), and for each 'time point' (February, May, and September). TEF of 4.2 ± 0.5 ‰ for $\delta^{13}C$ from the body wall of sea cucumbers was used to account for calcareous spicules (Watanabe et al., 2013), and TEF of 3.4 ± 1.0 ‰ for $\delta^{15}N$ per trophic level was applied (Peterson and Fry, 1987; Post, 2002).

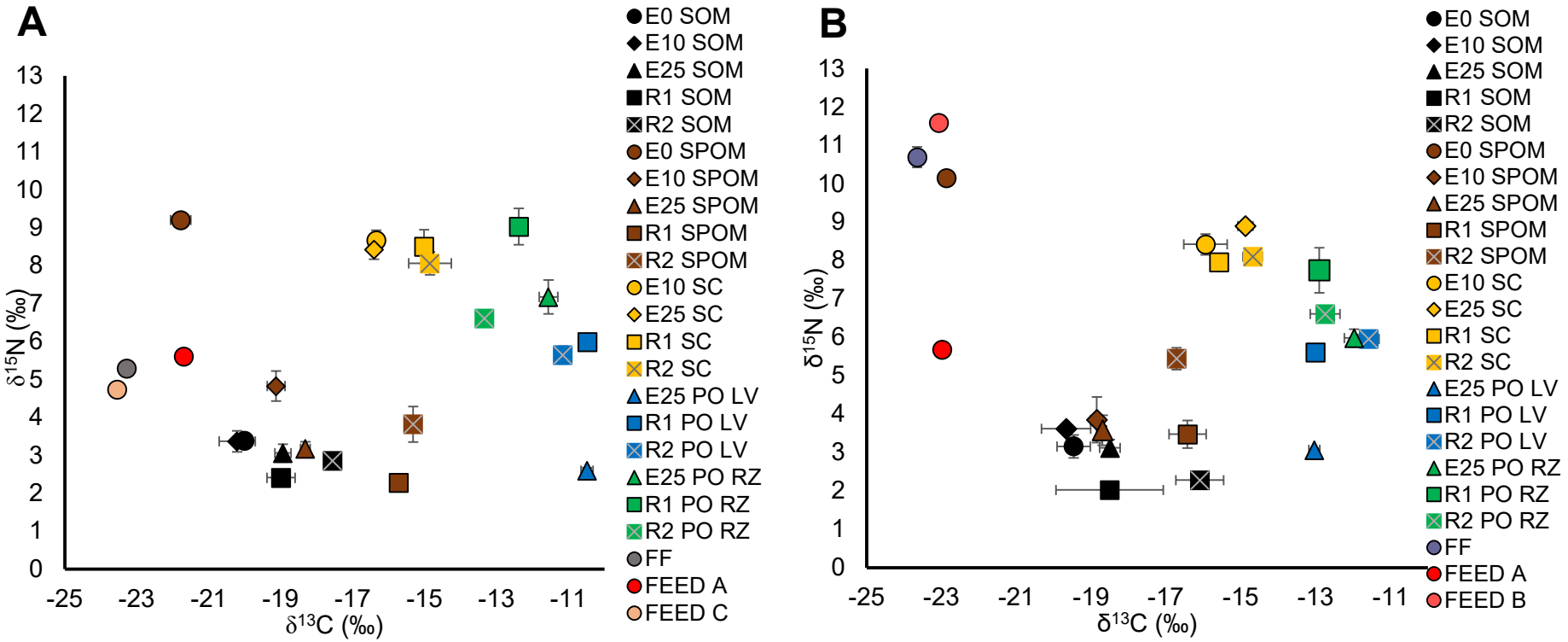
Permutational multivariate analysis of variance (PERMANOVA; PRIMER-e Ltd., UK) was used to test for differences in FA profiling between food sources (fish feeds, fish faeces, *P.*

oceanica leaves and rhizomes, and sediments SPOM and SOM) and the sea cucumber consumer, *H. poli*, between sites. A two-factorial design, with fixed factors 'source' and 'site', was used for each sampling time. PERMANOVA was performed with Monte Carlo corrected *p*-values on FA data previously transformed with arcsine function and then resembled to generate the Euclidean distance matrix. Analysis of percentage similarity (SIMPER) was used on untransformed resembled data to identify the FAs that contributed most to similarities within and between sites. Principal component analysis (PCA) was performed to compare *H. poli* FA data between sites (MVSP 3.22, Kovach Computing Services, Wales). Data were compared with a one-way analysis of variance (ANOVA) followed by Tukey's test for *post hoc* comparisons. A significance value of $p < 0.05$ was applied to all statistical tests.

6.3 Results

6.3.1 Stable isotopes

The significant difference between the isotopic ratios of aquaculture-derived waste (fish feed and faeces), and both *P. oceanica* and sediments from sediments traps and sediment cores ($p < 0.001$), allowed the application of stable isotope mixing models to assess food source contribution to the diet of sea cucumbers in IMTA and at the reference sites. Fish feed and faeces deposited near the fish cages, were the most $\delta^{13}C$-depleted sources, followed overall by sediments from sediments traps and sediment cores, and *P. oceanica* leaves and rhizomes (Fig. 6.2). The different administered feeds throughout the study varied in pellet size and isotopic composition ($p < 0.001$). Fish faeces were generally similar in isotopic composition to the fish feeds ($p > 0.05$). The sampled fish faeces had a wide range of isotopic signatures over time and heterogeneous composition in September. *P. oceanica* leaves and rhizomes were the most $\delta^{13}C$-enriched sources with significant differences between sampling sites and times ($p < 0.001$), except for $\delta^{15}N$ in leaves across time ($p = 0.117$). SPOM deposited near the cages and at the reference sites showed a wider range of $\delta^{13}C$ and $\delta^{15}N$ values than the SOM. The isotopic composition of both sediment typologies differed significantly across sites ($p < 0.05$) throughout the study. SOM was more $\delta^{13}C$-depleted and $\delta^{15}N$-enriched close to the fish cage with the reference sites more $\delta^{15}N$-depleted than the IMTA sites, E0 and E10 ($p < 0.001$). The isotopic signatures of SPOM varied between sites, with those near fish cages more $\delta^{13}C$-depleted ($p < 0.001$) especially at E0. SPOM varied in $\delta^{15}N$ between sites, where E0 was the most $\delta^{15}N$-enriched site ($p < 0.001$) in February and May.


A
B
δ15N (‰)
δ13C (‰)
E0 SOM
E10 SOM
E25 SOM
R1 SOM
R2 SOM
E0 SPOM
E10 SPOM
E25 SPOM
R1 SPOM
R2 SPOM
E10 SC
E25 SC
R1 SC
R2 SC
E25 PO LV
R1 PO LV
R2 PO LV
E25 PO RZ
R1 PO RZ
R2 PO RZ
FF
FEED A
FEED B
FEED C

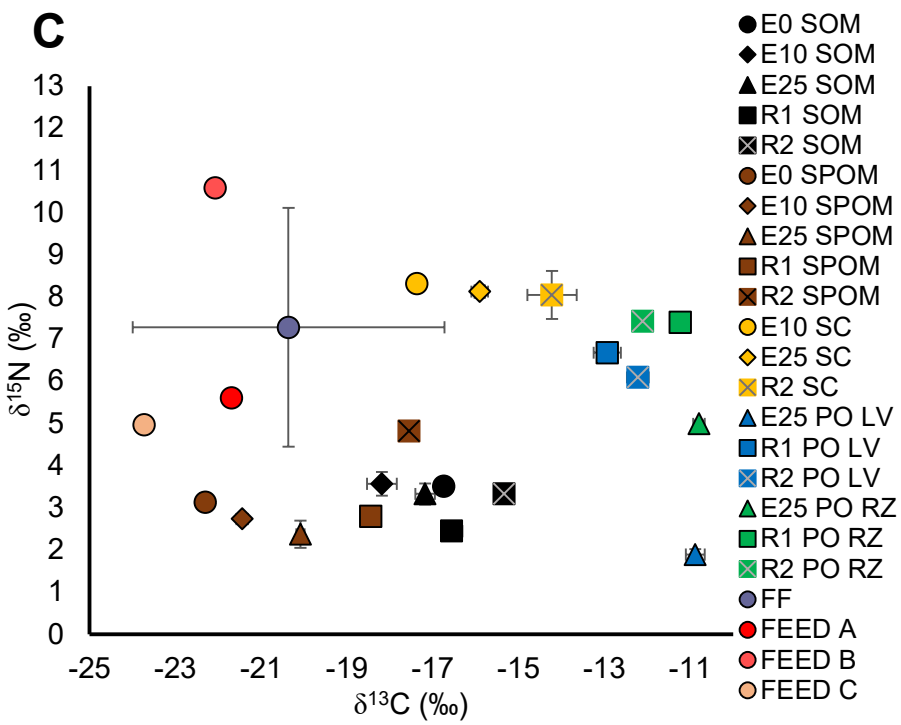
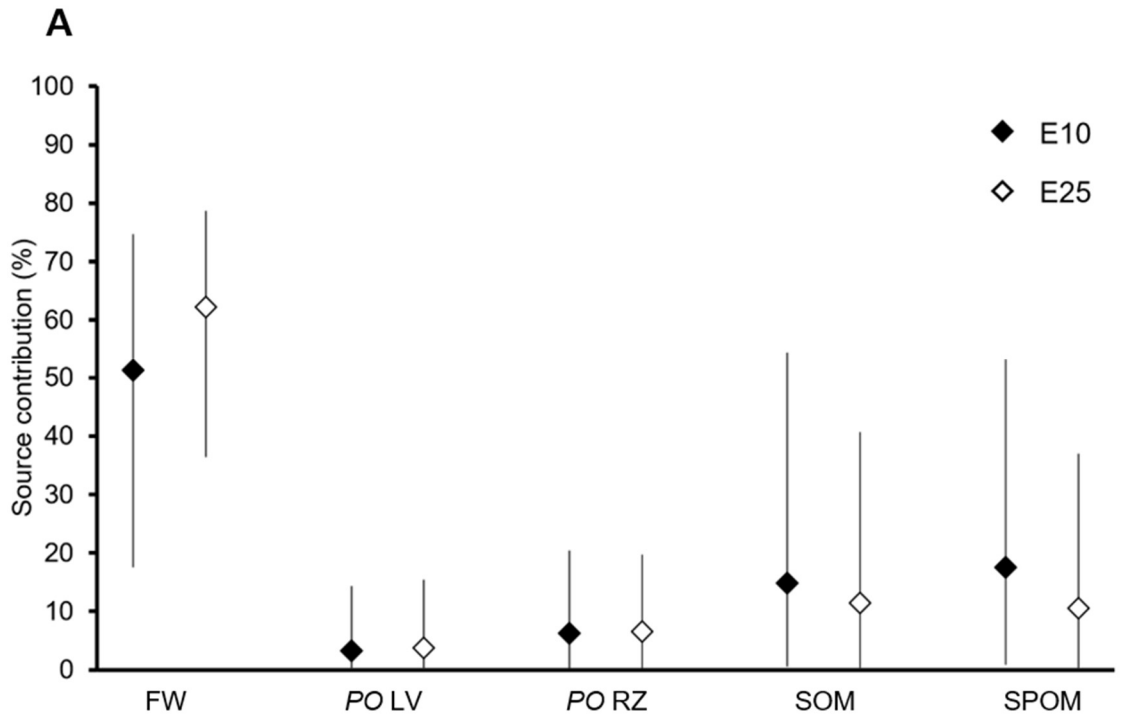
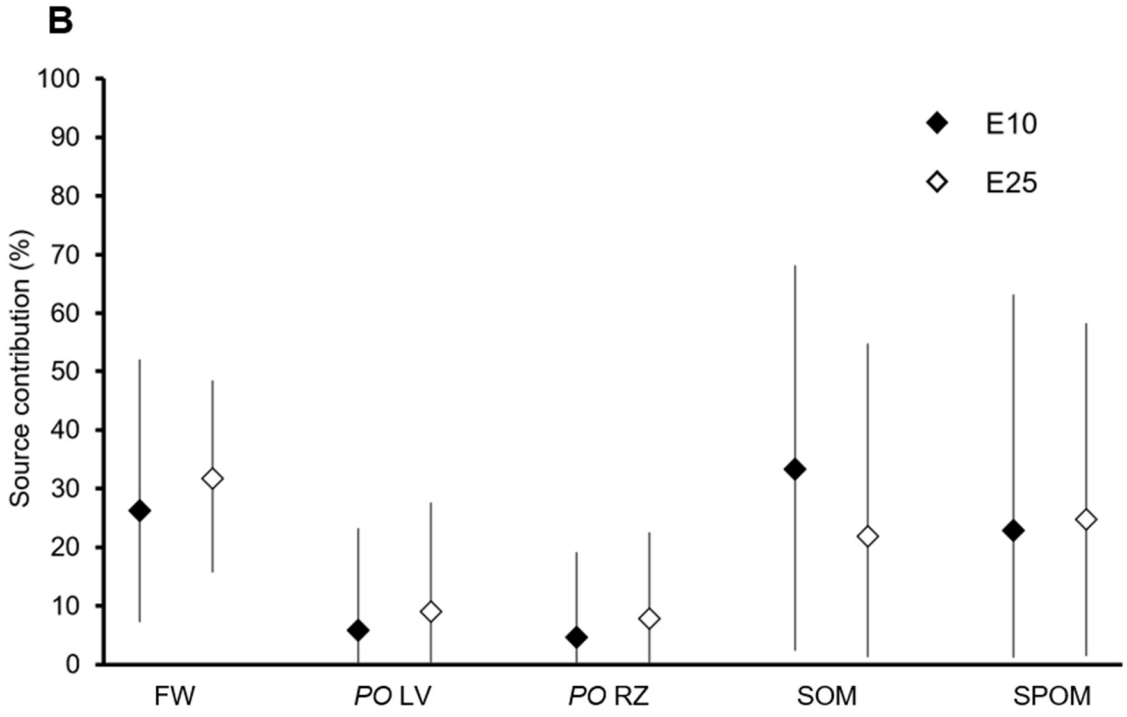


Figure 6.2. Stable isotope biplot indicating the mean $\delta^{13}C$ and $\delta^{15}N$ composition of the different sampled sources and the sea cucumber consumer, *Holothuria poli*, where present at the IMTA sites (E0, E10, and E25) and reference sites (R1 and R2) in A. February, B. May, and C. September 2019. Standard deviation is indicated by error bars. Sample typologies represented by different symbol colour and sites represented by different symbol shape. SOM: sedimentary organic matter, SPOM: suspended particulate organic matter, SC: sea cucumbers, PO LV: *Posidonia oceanica* leaves; PO RZ: *Posidonia oceanica* rhizomes; FF: fish faeces.

Mass mortalities were recorded directly below the fish cage (E0) within the first month of study; therefore, the organic source contribution to *H. poli* diet was not assessed by stable isotope and FA analyses for this site. The isotopic signatures of sea cucumbers varied significantly between the other sites near the fish cages (E10 and 25) and the reference sites ($p < 0.05$) at all sampling times. Conversely, the isotopic signatures of *H. poli* were similar between the individual sites, E10 and E25 ($p > 0.05$), and between the reference sites, R1 and R2 ($p > 0.05$), except in May. The mixing model converged to provide the contribution of different organic sources to the sea cucumber diet at E10 and E25 (Fig. 6.3) (Appendix 6.1 A, B). The isotopic signatures of the sea cucumber consumers at the reference sites did not fall within the mixing polygon of sampled sources (Appendix 6.1 C). Since isotopic mass balance was not established, the signatures of *H. poli* at R1 and R2 could not be explained by the proposed model and consequently they were rejected from subsequent analysis.

Farm-derived waste (fish feed and faeces) was the dominant dietary source of *H. poli* although with varied estimates of contribution to the sea cucumber diet at E10 (26.2% - 63.9%) and E25 (31.7% - 62.2%) during the sampling periods (Figure 6.3). The contribution of farm waste to the diet of *H. poli* decreased from 54.6% to 25.7% at E10 (Figure 6.4 A), and 47.0% to 27.0% at E25 (Figure 6.4 B) between February and May. The contribution then reached peak estimates of 68.5% (Figure 6.4 A) and 52.5% (Figure 6.4 B) at the respective sites by September. The contribution of *P. oceanica* to the diet of *H. poli* was least important at each time (3.0% - 9.0%) and with no apparent differences between the contributions of *P. oceanica* leaves and rhizomes. The contribution of *P. oceanica* slightly increased when moving from E10 to E25, away from the fish cages. The dietary contribution of *P. oceanica* leaves was comparable within the narrow range of 0.9% and 4.7%, whereas that of rhizomes varied between 2.8% and 21.2%, across the different sampling times. The dietary contribution of sediments, SOM and SPOM, to the diet of *H. poli* ranged between 9.0% and 33.3%, with different estimates of contribution between E10 and E25. Peak values of contribution from sediments, SOM and SPOM, were recorded in May at both sites that then decreased in dietary contribution by September.


A
100
90
80
70
60
50
40
30
20
10
0
Source contribution (%)
E10
E25
FW
PO LV
PO RZ
SOM
SPOM


B
100
90
80
70
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50
40
30
20
10
0
Source contribution (%)
E10
E25
FW
PO LV
PO RZ
SOM
SPOM

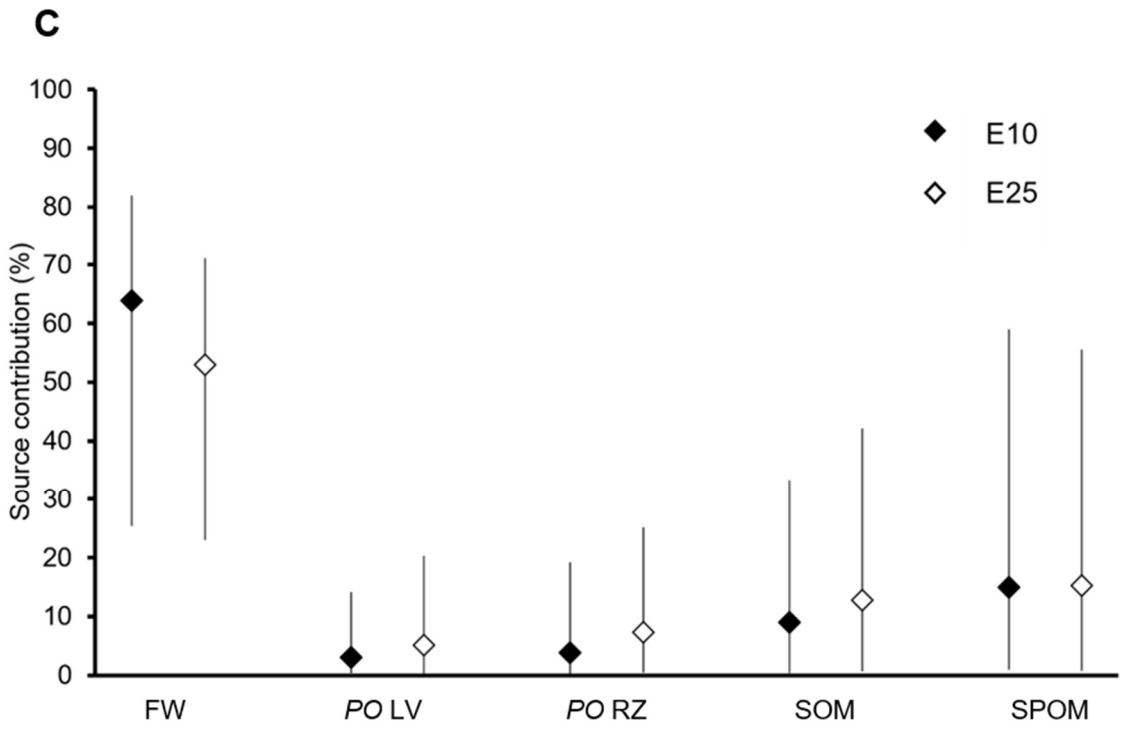

Figure 6.3. Organic source contribution (median ± 95 credible intervals) to *Holothuria poli* diet at E10 and E25 in A. February, B. May, and C. September. FW: farm waste, PO LV: *Posidonia oceanica* leaves; PO RZ: *Posidonia oceanica* rhizomes; SOM: sedimentary organic matter; SPOM: suspended particulate organic matter.

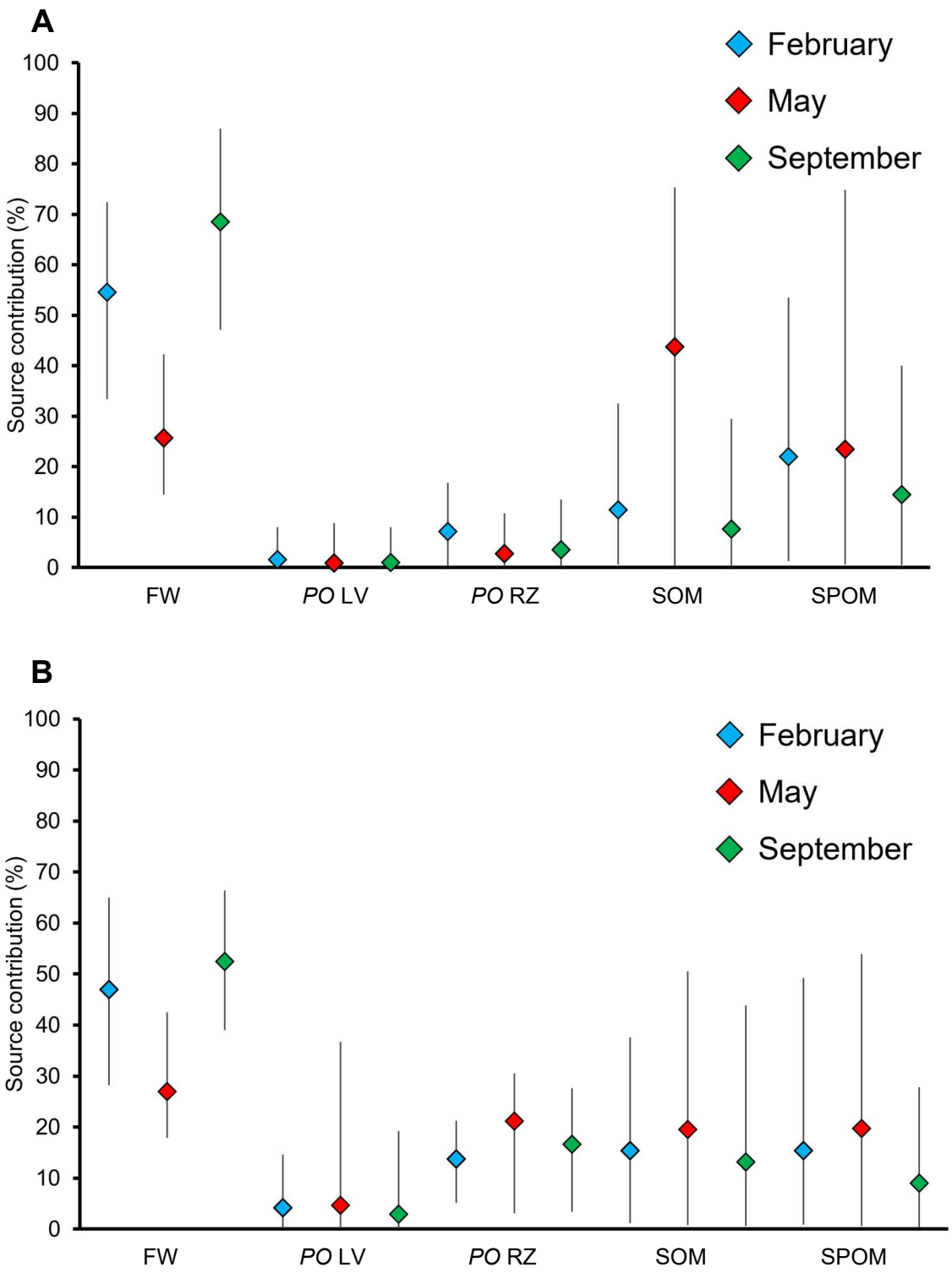

Figure 6.4. Organic source contribution (median ± 95 credible intervals) to *Holothuria poli* diet during the sampling times, February, May, and September, 2019, in A. E10, and B. E25. FW: farm waste, PO LV: *Posidonia oceanica* leaves; PO RZ: *Posidonia oceanica* rhizomes; SOM: sedimentary organic matter; SPOM: suspended particulate organic matter.

6.3.2 Fatty acids

The FA profile of organic sources at each site varied significantly ($p < 0.05$), except for similarities between SPOM and SOM ($p > 0.05$). Fish feeds included high proportions of the FAs, oleic (OA, 18:1*n*-9) (369.6 – 2190.6 mg $100g^{-1}$), linoleic (LA, 18:2*n*-6) (714.0 – 1382.6 mg $100g^{-1}$), and α-linolenic (ALA, 18:3*n*-3) (132.5 – 414.9 mg $100g^{-1}$) to a lesser extent (Appendix 6.2). The feed composition also included saturated fatty acids (SFAs) (621.3 - 1004.2 mg $100g^{-1}$), palmitic (16:0) (405.1 – 642.1 mg $100g^{-1}$) and stearic (18:0) (98.5 – 197.0 mg $100g^{-1}$), and lower abundance of marine *n*-3 PUFAs, particularly eicosapentaenoic acid (EPA, 20:5*n*-3) (109.2 – 296.6 mg $100g^{-1}$) and docosahexaenoic acid (DHA, 22:6*n*-3) (147.5 – 298.0 mg $100g^{-1}$). Fish feeds had higher lipid content (207.3 ± 54.9 mg $100g^{-1}$) than fish faeces (89.4 ± 29.1 mg $100g^{-1}$), which in turn were more heterogeneous in FAs and characterised by varied abundances of OA (18:1*n*-9) (26.4 - 978.1 mg $100g^{-1}$), palmitic acid (16:0) (15.7 – 541.1 mg $100g^{-1}$) and LA (18:2*n*-6) (18.8 - 538.8 mg $100g^{-1}$).

The lipid levels in SPOM close to the fish cage, specifically at E0, were significantly higher than the other sites ($p < 0.001$) (Appendix 6.2). The concentration of lipids in SPOM at the IMTA sites and reference sites varied throughout the year ($p < 0.001$). Sediments (SPOM and SOM) had a high abundance of SFAs particularly at the reference sites whereas OA (18:1*n*-9) and LA (18:2*n*-6) which are associated with the vegetable oils in fish feed were dominant closer to the fish cages. On the other hand, *P. oceanica* leaves and rhizomes comprised ALA (18:3*n*-3), LA (18:2*n*-6) and SFAs, in proximity to fish cages as well as at the reference sites. These distinctly different FA profiles of fish feeds and the natural marine resources were fundamental to assess the dietary relationship between sea cucumbers and food sources.

The analysis of percentage similarity (SIMPER) confirmed these patterns revealing that the IMTA sites were mainly characterised by OA (18:1*n*-9) (9.3% - 30.9%), LA (18:2*n*-6) (7.2% - 23.7%) and SFAs (Appendix 6.3). In particular, an average similarity over 95% was recorded for organic sources in the site directly below the fish cage (E0), which was mainly driven by OA (18:1*n*-9) (21.5% - 30.9%), LA (18:2*n*-6) (13.8% - 21.9%) and 16:0 (13.6% - 19.3%). E10 and E25 were characterised by the same FAs albeit in lower contributions and with higher abundance of the marine *n*-6 PUFA ARA (20:4*n*-6) and *n*-3 PUFAs. Moreover, the *n*-3 PUFA ALA (18:3*n*-3) was particularly more abundant at E25 (6.9% - 15.1%) than at the other IMTA sites across all sampling times. On the other hand, the reference sites showed less homogeneity at R1 (85.51% - 86.71%) and R2 (81.12% - 91.13%), and a greater variation in the relative contribution of FAs between sampling times. These sites

were characterised by *n*-3 PUFAs, primarily ALA (18:3*n*-3) (7.8% - 17.5%) and EPA (2.8% - 9.4%), the *n*-6 PUFAs ARA (20:4*n*-6) (7.6% - 14.3%) and LA (18:2*n*-6) (7.1% - 22.3%), and the ubiquitous SFA 16:0 (10.9% - 21.6%).

The dissimilarity between sites increased with increasing distance from E0, with a 25% range of dissimilarity between E0 and E25. The range of dissimilarity was between 42.45% and 55.16% when comparing E0 and the reference sites, R1 and R2, throughout the study (Appendix 6.3), providing the opportunity to distinguish between these sites. The extent of dissimilarity between the other IMTA sites (E10 and E25) and the reference sites decreased with increasing distance from the fish cages. This was generally driven by the monoenoic FAs OA (18:1*n*-9) (8.2% - 24.2%) and palmitoleic acid (16:1*n*-7) (3.4% - 11.0%), the *n*-6 PUFAs LA (18:2*n*-6) (4.7% - 13.7%) and ARA (20:4*n*-6) (4.6% - 11.2%), and the SFAs 16:0 (3.7% - 10.1%) and 18:0 (3.4% - 15.8%). The contributions of OA (18:1*n*-9) and LA (18:2*n*-6), the primary drivers of these spatial dissimilarities, were lowest in May with an increased contribution from SFAs, 16:0 and 18:0, and monounsaturated fatty acids (MUFAs), 16:1*n*-7 and 18:1*n*-7.

Sea cucumbers from the sites near fish cages (E10 and E25) and those at the reference sites had similar lipid levels ($p = 0.201$) (Appendix 6.4), but showed significant differences between sampling times ($p < 0.05$). The FA profiles of sea cucumbers were similar between E10 and E25, and between R1 and R2, with significant differences between the IMTA and the reference sites ($p < 0.05$). The PCA ordination showed a clear grouping of sea cucumbers where sampled at the sites near fish cages (E10 and E25) in IMTA and the reference sites based on the differences in FA profiles (Fig. 6.5). Along the first principal component (PC1, 70.5% of the total variance) E10 and E25 clustered driven by OA (18:1*n*-9), LA (18:2*n*-6) and eicosenoic acid (20:1*n*-9), and the reference sites driven by ARA (20:4n-6). *Post hoc* analysis ($p < 0.05$) confirmed a higher relative abundance of OA (5.8 – 137.2 mg 100 g^{-1}), LA (18:2*n*-6) (8.5 – 98.2 mg 100 g^{-1}) and eicosenoic acid (20:1*n*-9) (8.8 – 101.1 mg 100 g^{-1}) in the FA profiles of sea cucumbers at the IMTA than the reference sites throughout the study (Appendix 6.4). SFAs 16:0 (1.3 – 60.3 mg 100g^{-1}) and 18:0 (3.1 – 69.0 mg 100g^{-1}) explained the second principal component (PC2), which described 12.7% of total data variance.

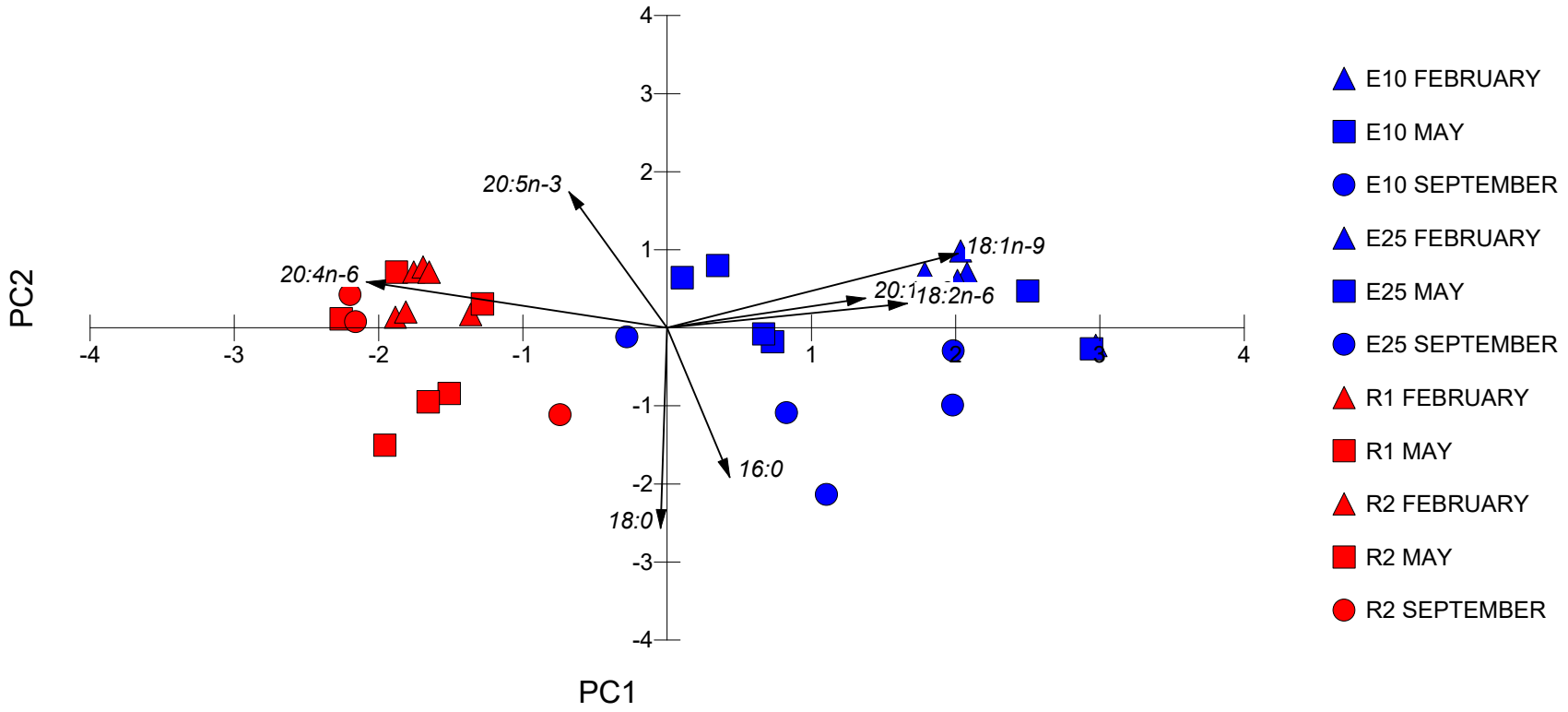

Figure 6.5. Principal component analysis of fatty acid composition of sea cucumbers from the IMTA (E10 and E25), shown in blue, and reference sites (R1 and R2), shown in red, in February, May, and September. Fatty acids driving the dietary differences are represented by vectors. Eigen analysis tolerance set as $1x10^{-7}$.

6.4 Discussion

This study provides complementary evidence for the ability of sea cucumbers to assimilate and mitigate aquaculture-derived organic waste inputs, from a nutritional perspective. The combined stable isotope analysis and FA profiling mutually validated the transfer of aquaculture-derived organic wastes from inshore aquaculture to sea cucumber tissue when other food sources were available.

6.4.1 Stable isotopes

The distinctly different isotopic signatures of fish feed and faeces from other marine resources provided the opportunity to trace nutrients and organic matter from aquaculture-derived organic waste to sea cucumbers co-cultured in IMTA. This approach provided isotopic evidence for the long-term assessment of food utilisation and dietary preferences of *H. poli* in nearshore IMTA. The fish feeds administered to the farmed fish included blends of nitrogen-rich fish oils and vegetable sources. This could explain the more $\delta^{13}C$-depleted and $\delta^{15}N$-enriched sediments near the fish farm, similar to the isotopic composition described for sediments near fish cages in other isotopic studies (Yokoyama et al., 2006; Holmer et al., 2007; Yokoyama, 2013). These organic sources near fish cages could provide food of adequate nutritional value for the value-added production of sea cucumbers in IMTA (Yokoyama, 2013). However, the temporal differences in food availability and quality in this study could reportedly influence the feeding selectivity and the assimilation of food sources in holothurians (Mangion et al., 2004; Mezali and Soualili, 2013), that could add to changes in the biochemical composition of sea cucumbers when constituents (e.g., lipid and protein) are accumulated or when energy substrates are consumed (Peterson and Fry, 1987; Sun et al., 2013).

In stable isotope-based dietary assessments, tracking the flow of organic matter to discern trophic interactions can be limited by the number of stable isotopes available (Peterson and Fry, 1987). Selecting appropriate baselines is crucial, especially considering the spatial and temporal variability in baseline signatures associated with physiological and metabolic changes. In open-water conditions, where control over the potential factors influencing dietary uptake is limited, the representativeness of baselines and their isotopic variability is critical to the true description of dietary contributions from food sources throughout production (Post, 2002). In addition, generally accepted isotopic fractionation values (0 – 1‰ for $\delta^{13}C$ and 2.6 – 4‰ for $\delta^{15}N$) are valid; however, studies show these are tissue- and species-specific and interpretation of dietary contributions should be made with caution,

especially for single trophic transfers (Post, 2002). A trophic enrichment factor of 4.2‰ for the whole-body wall for *Holothuria scabra* (Watanabe et al., 2013) was consistently applied as an enrichment factor for the *H. poli* body wall tissue across all treatment groups in the current study. While this is a valid assumption in the lack of published data on the isotopic fractionation in sea cucumbers, species-specific deviations may affect dietary contribution estimates. To overcome the limitations in this stable isotope approach, the study combined them with fatty acid analysis for higher resolution in discerning trophic interactions.

This study confirms the ability of holothurians to use fish feed and faeces as a nutritional source in isotopic studies (Yokoyama, 2013; Park et al., 2015; Xia et al., 2015). There was mass mortality of sea cucumbers placed directly below the fish cage, due to smothering (see Chapter 5 for further details), but the results also show that sea cucumbers at 10 m and 25 m from the centre of the cage were able to utilise aquaculture waste. Evidently, when setting up commercial scale IMTA systems, there would be a need to consider how waste is dispersed and deposited around the farm. The significance of aquaculture-derived organic matter for sea cucumber production near fish cages were substantiated by findings showing that the nutritional value of sources available at the reference sites, away from the fish farm, could not explain the isotopic signatures of *H. poli* tissue and was not able to sustain sea cucumber production, with low survivability compared to E10 and E25 (Chapter 5). Basis for Bayesian model rejection for the dietary contribution of nutritional sources to *H. poli* at the reference sites could suggest missing food samples and may be indicative of a wider diet strategy and preferences for other organic matter sources, consistent with Belbachir and Mezali (2018, 2020). Evidently, sea cucumber production in coastal areas in the Mediterranean could be limited without aquaculture-derived organic inputs.

6.4.2 Fatty acids

Alternative dietary lipid sources have been adopted as replacement for marine-derived oils in artificial feeds (Turchini et al., 2009, Betancor et al., 2016; Sprague et al., 2016). Addition of vegetable oils in commercial feeds influences the biochemical composition of marine organisms (White et al., 2019). The vegetable oils that supplemented dietary fish oils in feeds are characterised by abundant proportions of monounsaturated fatty acids (MUFAs), primarily OA (18:1*n*-9), LA (18:2*n*-6) and ALA (18:3*n*-3), in decreasing order of proportion (Turchini and Mailer, 2010). However, LA and ALA are also the dominant FAs in *P. oceanica* (Viso et al., 1993; Kelly and Scheibling, 2012; Signa et al., 2017), which verifies the importance of the complementary tracer approach taken in this study. For these reasons, OA and LA were considered important FA biomarkers, particularly when a higher

abundance and contribution characterised the sites near fish cages relative to the reference sites. This was substantiated by lower proportions of LA and ALA in *H. poli* at the reference sites, even though sea cucumbers inhabit *P. oceanica* habitats and take up seagrass-derived organic matter in the natural environment (Belbachir and Mezali, 2018; 2020).

Previous studies showed that holothurians are mainly characterised by PUFAs (53.0% - 59.1%), primarily arachidonic acid ARA (20:4*n*-6), and lesser relative proportions of SFAs and MUFAs (Aydin et al., 2011; David et al., 2020), as in the results here. Findings revealed the relative dominance of ARA and the significance of marine *n*-3 PUFAs (EPA and DPA) in *H. poli*, corroborating studies which show that holothurians take up higher proportions of highly unsaturated fatty acids (HUFAs) (≥ C_{20}) that are attributed to detrital particles, rich in algae and bacteria (Yu et al., 2016; David et al., 2020). In the natural environment, benthic diatoms are the dominant food source of *H. poli* (Belbachir and Mezali, 2020); however, since diatoms are usually poor in ARA and this FA was in low abundance (< 5%) in the organic food sources, the elevated levels of ARA in *H. poli* may be evidence for selective uptake of HUFAs or the capacity of FA biosynthesis as revealed for other echinoids (Carboni et al., 2012; 2013). The high abundance of plant-derived FAs and low relative proportions of ARA in sea cucumbers near fish cages (E10 and E25) confirms the nutritional benefits of IMTA for sea cucumber production. Moreover, the elevated DHA in sea cucumbers at E10 and E25 reveal the supplement use of *n*-3 PUFA for better growth in IMTA, reported in Chapter 5. On the other hand, the low dietary contribution from dead *P. oceanica* leaves and rhizomes substantiates findings of Belbachir and Mezali (2018; 2020) for *H. poli* in the natural environment. Aquaculture-derived organic inputs could support production of sea cucumbers where the natural marine resources are not able to sustain survival and growth under culture conditions in these coastal environments.

6.5 Conclusion

This study validates the trophic connectivity in fish-sea cucumber IMTA in the nearshore environment. It demonstrates the dietary significance of aquaculture-derived organic matter for the production of *H. poli* when cultured near fish cages, as opposed to being placed directly below the fish cages and at the reference sites, away from this source of organic deposits. *H. poli* relied on organic fish farm inputs to grow effectively showing the viability of growing sea cucumbers near fish farms.

Still, fish-sea cucumber IMTA development requires better understanding of the market opportunity and the social acceptability towards these sustainable seafood products,

primarily in terms of food safety and quality. Moreover, IMTA production and development requires that the expected effects that changes in fish farm production would have on food availability and quality be resolved. Temporal variation in sedimentary organic matter would have substantial implications for the transfer of dietary organic matter between farmed fish and sea cucumbers, the uptake and removal of organic wastes, sea cucumber production, and other practices (e.g. optimum period of harvest operation) that need to be considered for IMTA development. These fluctuations in waste output and uptake in fish-sea cucumber IMTA need to be appreciated in predictive modelling and then in practice, especially if producers are to invest in IMTA and scale-up towards commercial production.

Chapter 7. Heavy metal contamination of sea cucumbers cultured in Integrated Multi-Trophic Aquaculture in an industrial bay

Abstract

The accumulation of heavy metals in the edible tissue of *Holothuria poli* revealed the transfer of metal contaminants to sea cucumbers when produced below fish cages in a Mediterranean port area. Sea cucumbers were cultured on the seafloor directly below a fish cage at 0 m, then at 10 m and at 25 m away from the cage, as part of an open-water integrated multi-trophic aquaculture (IMTA) system, and then at a reference site over 1 km from the fish farm, over a one-year period. At the end of the study, in September 2019, sea cucumbers and seafloor sediments were sampled from the IMTA sites near the fish cages, except at 0 m due to mass sea cucumber mortalities within the first month of the study, and again at the reference site. Localised enrichment from marine aquaculture could explain the significant concentration of metals in sediments below fish cages that are typically ascribed to their use in aquaculture. *H. poli* can regulate essential metals like iron (Fe) and zinc (Zn) that characterised the edible tissue of the sea cucumbers. The concentrations of non-essential metals like mercury (Hg) and cadmium (Cd) were the lowest in the sea cucumber body wall. However, the bioaccumulation of toxic metals, Hg and arsenic (As), reveal the bioavailability of these contaminants in sediments and the propensity of bottom-dwelling sea cucumbers to bioconcentrate these metals, when cultured under a commercial fish cage in IMTA and elsewhere in natural sediments in this industrial environment. This bioaccumulation reveals the need to account for the potential effects of farm-level variability throughout longer production cycles and bay-wide dynamics on sediment contamination and bioaccumulation in sea cucumbers until harvest. Site-specific complexities in ports, whether natural or anthropogenic, can be expected to influence bioaccumulation of metal contaminants and therefore require long-term and fine resolution monitoring for better representation in open-water IMTA production.

7.1 Introduction

The potential of the Mediterranean sea cucumber species, *Holothuria poli*, to uptake organic wastes in integrated multi-trophic aquaculture (IMTA) has been validated through growth (Chapter 5) and dietary assimilation of fish farm organic wastes from inshore cage aquaculture (Chapter 6). However, few studies have been published from a contaminants' perspective on sea cucumbers (Sicuro et al., 2012; González-Wangüemert, 2018b; Montero et al., 2021; Marrugo-Negrete et al., 2021). As Mediterranean sea cucumber species (e.g. *H. poli* and *Holothuria tubulosa*) become increasingly popular in IMTA research (Tolon et al., 2017; Neofitou et al., 2019; Grosso et al., 2021; Sadoul et al., 2022), knowledge about the bioaccumulation of contaminants in sea cucumbers under fish cages becomes increasingly relevant. Consequently, contamination from complementary integrated aquaculture systems and wider sources need to be addressed (Rosa et al., 2020).

Among the various contaminants, metals are important elements that can change chemical form yet persist in the environment without degradation and be transferred and bioaccumulated along the food chain. Heavy metal exposure has been linked with fish deformities (Sfakianakis et al., 2015) and known to influence the metabolic and physiological behaviour of crustaceans (Barbieri and Paes, 2011). Nevertheless, heavy metals, such as copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn), are essential micronutrients for many aquatic organisms (Sfakianakis et al., 2015; Li et al., 2016) and play crucial roles in biological processes like enzyme activation, electron transfer, and structural stability. These metals are required in trace amounts for proper growth, development, and overall physiological functioning, and while essential in small quantities, these can become toxic at elevated concentrations (Sfakianakis et al., 2015). Toxic effects are often a consequence of interference with essential biological processes, displacement of essential metals from binding sites, or the generation of reactive oxygen species leading to oxidative stress. Among these metals, certain concentrations of metals like lead (Pb), mercury (Hg), and cadmium (Cd) can cause tissue and organ damage, and impairment of reproductive and immune functions (Telahigue et al., 2018; Lee et al., 2019; Rabeh et al., 2019). Metals are taken up from the environment and diet through direct absorption from water and ingested sediments, and metals accumulated in food sources (Ahlf et al., 2009; Bjerragaard et al., 2015). At cellular levels, aquatic organisms can regulate metal concentration, such as through binding with proteins or sequestration, to prevent their toxicity (Storelli et al., 2001).

Understanding the dynamic interplay between metal availability and exposure, the essentiality and toxicity of these elements, and uptake and regulatory mechanisms in organisms, is key in developing effective strategies for managing the effects of heavy metal exposure in aquatic organisms. Little data is available on the acute and chronic effects of metal exposure on deposit-feeding sea cucumbers that spend a lifetime reworking and feeding in seafloor sediments. This is despite sea cucumbers having greater bioaccumulation capacity for metals than most marine organisms (Parra-Luna et al., 2020; Marrugo-Negrete et al., 2021; Montero et al., 2021) and being considered efficient bioindicators of these contaminants in sediments (Aydın et al., 2017). Holothurians tend to accumulate different metals in separate tissues with muscle having a high affinity for Fe and nickel (Ni), whereas Cd, Cu, Zn and Pb tend to accumulate in the mucopolysaccharide-rich body wall tissue (Warnau et al., 2006). The toxic nature of metals can have chronic effects on growth and activity of the sea cucumbers (Li et al., 2016), and this can affect their potential production (economic potential) and bioremediation or bioturbation of excess sediment nutrients (ecosystem services), two of the key potential benefits of IMTA. Studies have revealed different physiological responses to metals with Wang et al. (2015) substantiating the greater burden of Pb bioaccumulation in the body wall when sea cucumbers were fed Pb-supplemented diets under controlled conditions. Pb bioaccumulation did not influence growth but the antioxidant capacities decreased after metal exposure (Wang et al., 2015). In other studies, the metals Hg (Telahigue et al., 2018; Rabeh et al., 2019) and Zn, Cu, and Cd (Li et al., 2016) induced genotoxicity, oxidative damage and histopathological injuries in various tissues (respiratory tree, intestine, and muscle) of sea cucumbers.

Most of these metals are commonly found in aquatic environments and have been associated with coastal and maritime activities, including shipping and aquaculture (Sutherland et al., 2007; Basaran et al., 2010). In the Mediterranean, surficial sediments directly below and close to fish cages are enriched by metals and trace elements (e.g. Cd and Zn) found as constituents in fish feed and faeces that settle to the seafloor (Kalantzi et al., 2013). Sediment enrichment in proximity to fish cages has also been ascribed to anthropogenic inputs of Cd, Pb, Fe and Zn (Kalantzi et al., 2013) that are associated with extensive use of antifouling by shipping activities and industrial effluent discharges on a wider scale (Sutherland et al. 2007; Basaran et al., 2010). Where aquaculture exists in urban port areas, socio-ecosystems notoriously known for a myriad of pollutants from terrestrial effluents and anthropogenic inputs (Andral et al. 2004; Benali et al. 2015; Lafabrie et al. 2008), contaminants in sea cucumbers placed under fish cages need to be assessed

even if holothurians did not show higher abundance of metal contaminants when in these industrialised spaces in the Mediterranean (Pillet et al., 2023).

This study compares the total concentration of a range of heavy metals in seafloor sediments and in sea cucumber tissues near and away from commercial fish cages in a busy Mediterranean port area. This discusses potentially important implications for the production of sea cucumbers in open-water IMTA in heavily industrialised coastal spaces, from a contaminant's perspective.

7.2 Materials and methods

7.2.1 Sampling and heavy metal analysis

In October 2019, after 12 months of being cultured on the bottom, at increasing distances from a commercial fish cage as part of the IMTA setup described in Chapter 5, sea cucumbers were sampled and analysed together with seafloor sediments for the transfer and accumulation of heavy metals in IMTA.

Sediment corers of 5 cm diameter were used to collect ten seafloor sediment samples near the sea cucumber cages at the IMTA site and another ten from the reference sites described in Chapter 5. At the laboratory, the top 3 cm layer of sediment core samples was extracted, dried at 60 ^{o}C to constant weight, and stored. In addition, ten sea cucumbers were sampled from cages deployed on the seabed at E10 and E25. Similarly, sea cucumbers were sampled from natural populations at a reference site (35°50′2.20″N, 14°32′54.09″E), over 1 km from the fish farm facilities. Samples were transported to the laboratory in a cool box. Sea cucumbers were washed, weighed and processed to extract the body wall tissue, specifically composed of connective tissue and muscle tissue together. Wet body weights of *H. poli* samples were 50.1 ± 3.7 g at the IMTA site and 58.1 ± 12.3 g at the reference site. Processed sea cucumber samples were frozen at -20 ^{o}C. Prior to metal analysis, the dried sediment samples were ground to fine powder using pestle and mortar whereas all sea cucumber samples were freeze-dried (ALPHA 1-4 LDplus, Martin-Christ) and ground to a fine powder using a ball mill (MM 200 Retsch).

The sediment concentrations of arsenic (As), Cd, Cr, Cu, Fe, Hg, Ni, Pb and Zn, measured as total metal content, were determined using inductively coupled plasma mass spectrometry (ICP-MS) (ThermoFisher ICAQ RQ). Sediments and blanks were acid-digested (5 ml HNO_3 69%) in the Microwave Digestion System (MarsXpress, CEM).

Digested samples were treated with MilliQ deionized water, 200 µl gold solution (10 ppm) for Hg determination and diluted further with HNO_3 before analysis. Multi-element standard solutions were used to prepare calibration curves and these were accepted at $R^2 > 0.999$ for concentration calculation. Samples were assessed using an internal quality approach and validated when criteria for quality assurance were met. The analytical procedure was tested using the CRM recovery, which ranged from 85% to 99%; at a significance level of 0.05. All samples were analysed in triplicates to avoid batch-specific errors.

Sea cucumber tissue samples, blanks and Certified Reference Material (CRM) were acid-digested (5 mL HNO_3 67–70%, 1 mL H_2O_2 30% and 4 mL MilliQ deionized water) in a microwave system (MARS 5, CEM), after which the total content of As, Cd, Cu, Fe, Hg, Pb and Zn was determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 8000, PerkinElmer) coupled with a hydride-generation system for Hg determination. The analytical procedure was tested using the CRM recovery, which ranged from 88 to 98%; at significance level of 0.05.

The concentrations were given in mg kg^{-1} dry weight (DW) sediment.

7.2.2 Bioconcentration

To assess bioaccumulation of heavy metals in sea cucumbers from sediment, the bioconcentration factor (BCF) sea cucumbers – sediment was expressed as the ratio of metal concentration in the body wall of *H. poli* to the mean concentration in sediments, separately for the IMTA and reference sites. The BCF was estimated for metal concentration data of sediments and sea cucumber body wall tissue according to Aydin-Onen et al. (2015). The bioconcentration of metals by *H. poli* occurs when BCF > 1 (Aydin-Onen et al., 2015; Islam et al., 2017).

7.2.3 Data analysis

Metal concentration in sea cucumber tissue was from specimens sampled from different sea cucumber cages within the IMTA site. Data for each sediment sample was expressed as the mean of replicates taken. Assumptions of normality and homogeneity of variances were assessed using Shapiro-Wilk and Levene's tests respectively. Box plots were used to identify outliers for each individual heavy metal for sediment and sea cucumber samples. An independent samples *t*-test was used to assess differences in mean metal concentrations between different sites for both sediment and sea cucumbers. The non-parametric test, Mann-Whitney *U*, was used when assumptions of normality were violated,

particularly when outliers were identified. Statistical analysis was performed using SPSS v26.0 for Windows (SPSS Inc., Chicago, USA). Statistical significance criterion was set at $p < 0.05$ level.

7.3 Results

7.3.1 Heavy metal concentration in sediments

The assessment of heavy metal concentrations in sediments near the fish cages and natural sediments, elsewhere in this industrialised bay and port area, reveals differences in the abundance of metals that are presumably linked with inputs from different anthropogenic activities, albeit with consideration for the origin and natural occurrence of these elements in these environments. The concentrations of Cd, Cu, Zn, Ni ($p < 0.001$), and Cr ($p = 0.018$) were significantly higher near fish cages than the reference site (Fig. 7.1). Conversely, no spatial variation was recorded between the IMTA site and reference site for levels of Pb, Hg, As, and Fe in sediments.

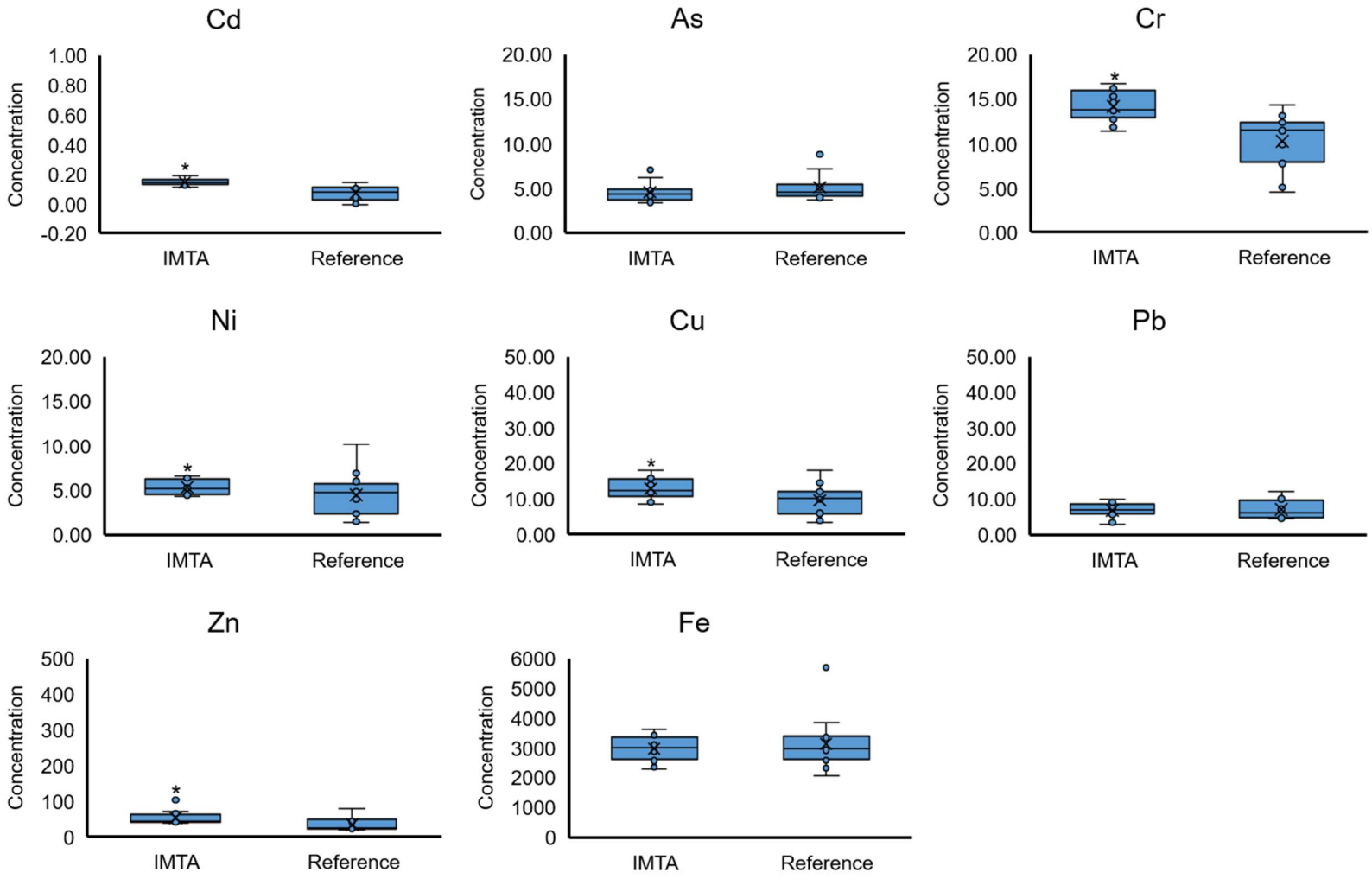

Figure 7.1. Heavy metal concentrations (mg kg^{-1} dry weight) in sediments at the integrated-multi trophic aquaculture site and the reference site, at the end of the study (October 2019). *denotes statistically significant difference ($p < 0.05$) between data for sites.

7.3.2 Heavy metal levels in sea cucumbers

The body wall tissue of the sea cucumber, *H. poli*, revealed high concentrations of the essential metals Fe and Zn at both sites (Fig. 7.2). In sea cucumber tissue, significantly higher mean concentrations of Fe ($p = 0.023$) and Zn ($p = 0.023$) were recorded at the IMTA site when compared to those at the reference site. The Zn levels in *H. poli* in IMTA reflect the higher concentrations of the metal in sediments near fish cages. Conversely, the Pb levels in sea cucumbers cultured in IMTA were significantly lower than those recorded at the reference site ($p < 0.001$) in this industrialised bay that supports a variety of anthropogenic activities. Hg concentration in sea cucumbers cultured in IMTA was higher ($p = 0.004$) than the reference site whereas no significant spatial differences ($p > 0.05$) were recorded for As, Cd and Cu.

The bioconcentration ratios recorded in sea cucumbers over sediments for Hg at the IMTA site (9.12 ± 3.73) and at the reference site (4.78 ± 1.97) were the highest among the metals, followed by As in sea cucumbers near fish cages (3.58 ± 1.77) and at the reference site (2.26 ± 0.81) (Table 7.1). Generally, the BCF values for Hg and As indicated bioaccumulation in *H. poli* in the bay to confirm the high affinity for these metals in sediments. Average BCF values for Fe, Zn, Cd, Cu, and Pb in *H. poli* revealed that bioaccumulation did not occur in the body wall tissue of sea cucumbers over the one-year period of the study.

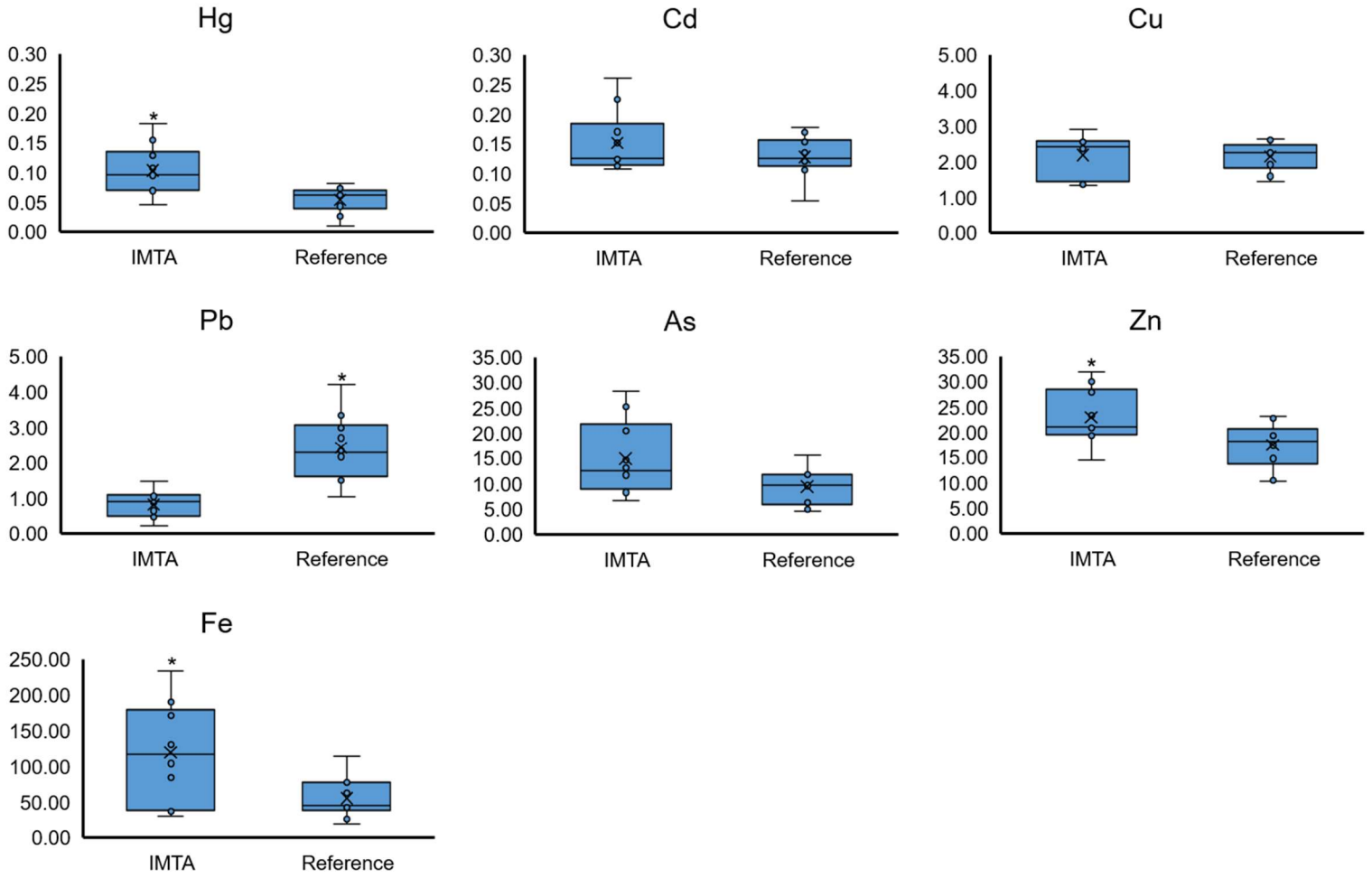

Figure 7.2. Heavy metal concentrations (mg kg^{-1} dry weight) in the body wall tissue of *Holothuria poli* cultured at the integrated-multi trophic aquaculture (IMTA) site and the reference site, at the end of the study (October 2019). *denotes statistically significant difference ($p < 0.05$) between data for sites.

Table 7.1. Mean (± standard deviation) and range of bioconcentration factors for metals at the integrated multi-trophic aquaculture and reference sites.

		Pb		Hg		Cd		As		Fe		Cu		Zn	
IMTA site	Mean (SD)	0.11 (0.05)		9.12 (3.73)		0.99 (0.34)		3.58 (1.77)		0.04 (0.02)		0.16 (0.04)		0.41 (0.01)	
	Range	0.03	0.20	4.07	16.21	0.70	1.70	1.58	6.77	0.01	0.07	0.10	0.21	0.25	0.56
Reference site	Mean (SD)	0.32 (0.12)		4.78 (1.97)		0.84 (0.23)		2.26 (0.82)		0.02 (0.01)		0.16 (0.03)		0.31 (0.08)	
	Range	0.14	0.56	0.89	7.26	0.35	1.16	1.07	3.71	0.01	0.04	0.10	0.19	0.18	0.41

7.4 Discussion

Evidence for elevated levels of metals near fish cages and bioaccumulation of toxic metals in sea cucumbers near fish cages and in the natural sediment, elsewhere in the bay, reveals the significance of understanding the natural and anthropogenic processes that influence bioavailability of metals in industrialised bays and port areas. This study shows that fish farm operations and site-specific characteristics can influence the uptake of contaminants by sea cucumbers and possibly influence their survival, growth response and waste mitigation efficiency throughout production in IMTA.

7.4.1 Heavy metal contamination of surface sediments

Given the proximity of the fish cages, elevated concentrations of metals near fish cages could be due to localised enrichment from marine aquaculture. Higher concentrations of Cd, Cu and Zn in sediments below fish cages have been ascribed to their use in aquaculture feeds and antifouling net coatings (Belias et al., 2003; Dean et al., 2007; Sutherland et al. 2007; Basaran et al., 2010). The variability and origin of heavy metals in coastal sediments, and whether these are naturally occurring or derived from anthropogenic activities particularly in heavily industrialised environments, influence the distribution and availability of metals in sediments and need to be considered.

Non-essential metals (Pb, Hg, As) present below fish cages and at the reference sites are not associated with commercial fish diets. Unlike Fe, these are less likely to accumulate underneath fish cages due to the settlement of particulate wastes to the seafloor. Instead, they can be linked with important anthropogenic inputs associated with industrial activities on a wider bay level that include domestic and industrial effluent discharge, and antifoulants from shipping activities and cage aquaculture (Sutherland et al. 2007; Basaran et al., 2010). On the other hand, studies have reported elevated concentrations of Fe in surface sediments close to fish cages due to fish feed inputs that settle to the seafloor (Belias et al., 2003; Sutherland et al., 2007). Notwithstanding, the high natural background levels in this study can affect accumulation below fish cages when the levels of Fe in commercial sea bass and sea bream diets can be relatively low (160.13 - 249.03 mg kg^{-1} DW) (Kalantzi et al., 2016).

From an environmental quality perspective, the levels of metals near fish cages and elsewhere in the bay are within maximum concentration limits set for good environmental status of contaminants in sediments listed as priority substances that present significant risks to the aquatic environment (Directive 2013/39/EU of the European Parliament and of

the Council of 12 August 2013). Metal levels in sediments at the IMTA and reference sites were below reference values for Cd (0.3 mg kg^{-1} DW), Hg (0.3 mg kg^{-1} DW), and Pb (30 mg kg^{-1} DW) but not for Ni (0.03 mg kg^{-1} DW) in non-industrial marine sediments (ERA, 2020). However, reference values and quality standards for sediments in industrial areas would be more comparable and applicable especially since exceedances in metal concentration in sediments have been reported in the port area in this study (ERA, 2013). Still, threshold values for contaminant levels are not always available for nearshore industrial environments and consideration for the applicability of thresholds that have been adopted is recommended. Notwithstanding, background levels of contaminants in surface sediments in the bay and levels near fish cages could present opportunities for open-water IMTA where aquaculture exists in urban ports.

7.4.2 Metal contamination and bioaccumulation in sea cucumbers

In the Mediterranean region, the body wall of *H. poli* in the natural environment reportedly contains high levels of Fe (19.4 – 40.6 mg kg^{-1}) and Zn (8.9 – 14.9 mg kg^{-1}) that vary according to the spatial distribution of metals in sediments and the distinct geographical origins (Sicuro et al., 2012; González-Wangüemert et al., 2018b, Montero et al., 2021). Essential metals, Fe and Zn, have important physiological functions as reported for *Holothuria floridana* (Marrugo-Negrete et al., 2021) and expectedly have the highest concentrations in sea cucumber body wall tissue. The higher Zn levels in *H. poli* in IMTA reflect the higher concentrations of the metal in sediments near fish cages. These spatial differences in metal accumulation would not only be influenced by a variety of environmental factors but also the physiological traits of the sea cucumber (Warnau et al., 2006). The Pb levels in sea cucumbers cultured in IMTA were comparable with those in *H. poli* collected elsewhere in the Mediterranean (Storelli et al., 2001; Montero et al., 2021) and significantly lower than those recorded at the reference site in this study ($p < 0.001$). Elsewhere, González-Wangüemert et al. (2018b) reported higher levels of Pb in *H. poli* tissue that have their origin in historic anthropogenic sources at the study site. The Hg levels in sea cucumbers near fish cages and at the reference sites in the bay were lower than those reported along the Southern Adriatic coast (0.96 mg kg^{-1} DW) (Storelli et al., 2001), presumably considered a relatively contaminated area, but higher than that in the Gulf of Cagliari in Sardinia (0.023 mg kg^{-1}) (Montero et al., 2021). Elevated Hg concentrations in tissue when compared to surface sediments have been attributed to the inability of *H. poli* to regulate this metal (Storelli et al., 2001). The concentrations recorded for the non-essential metal, Cd, in sea cucumbers in IMTA and the reference site in this industrial bay were higher than those reported by other authors for sediments in contaminated areas

(Storelli et al., 2001; Warnau, et al., 2006; Sicuro, et al., 2012; González-Wangüemert et al., 2018b; Montero et al., 2021). This metal has been attributed to historic anthropogenic activities in González-Wangüemert et al. (2018b) and considering the elevated concentrations of Cd, careful monitoring of anthropogenic influences on the availability of this metal and its effects on the *H. poli* is essential. Conversely, the concentrations of As and Cu in the body tissue of *H. poli* were comparable with those reported for this species (Sicuro et al., 2012; Montero et al., 2021). The As levels reported in sea cucumbers in this study are comparable to concentrations in seafood consumed across the Mediterranean (Ferrante et al., 2019).

As bottom-dwellers, a close association between the metal concentrations of holothurians and sediments would be expected, through tegument absorption from the surrounding environment or from dietary uptake and ingested sediments (Ahlf et al., 2009; Bjerragaard et al., 2015), explaining the greater capacity to accumulate metals in specific tissues (Wang et al., 2015). The bioconcentration ratios recorded in sea cucumbers over sediments for Hg at the IMTA site (9.12 ± 3.73) and at the reference site (4.78 ± 1.97) were the highest among the metals, followed by As in sea cucumbers near fish cages (3.58 ± 1.77) and at the reference site (2.26 ± 0.81) (Table 7.1). Generally, the BCF values for Hg and As indicated bioaccumulation in *H. poli* in the bay to confirm the high affinity for these metals in sediments. Albeit the most abundant metals in sediments and the sea cucumber tissue, the lower concentrations reported for Fe and Zn in *H. poli*, when compared to levels in sediment reveal that these metals are regulated without bioaccumulation beyond metabolic and physiological needs, corroborating Storelli et al. (2001). Average BCF values for Fe, Zn, Cd, Cu, and Pb in *H. poli* revealed that bioaccumulation did not occur in the body wall tissue of sea cucumbers over the one-year period of the study.

Essential metals like Zn and Cu can still pose a threat to sea cucumbers when exceeding normal thresholds, exhibiting toxicity and impairing crucial processes such as metabolism and growth (JunFeng et al., 2015; Li et al., 2016). *Apostichopus japonicus* revealed greater sensitivity to Cu at 96 h LC50 (concentration of toxicant causing 50% mortality of test sea cucumbers) of 0.133 mg L^{-1} than the non-essential Cd (1.574 mg L^{-1}) during a 15-day trial at 17 ^{o}C (Li et al., 2016). This emphasizes the importance of considering not only toxic metals but also essential elements with a higher tendency to accumulate near fish cages. Acute exposure to these metals resulted in severe mortality in while chronic exposure inhibited growth and elevated metal concentrations reduced growth rates (Li et al., 2016). Similarly, exposure to 0.5 mg L^{-1} Zn and 0.05 mg L^{-1} Cu reduced growth rates and survival in *A. japonicus* juveniles during a 75-day trial at 10 - 13 ^{o}C (JunFeng et al., 2015). Exposure of *Holothuria forskali* to 0.04 mg L^{-1} Hg at 18 ^{o}C induced genotoxicity evidenced by

alterations in the antioxidant system and enzyme activity, and DNA degradation, potentially affecting the survival of this sea cucumber (Telahigue et al., 2018). While toxicity is species-dependent and sea cucumbers can have different physiological adaptations under heavy metal stress (Li et al., 2016), the potential adverse effects on the performance of *H. poli* in IMTA require an understanding of the safety concentrations of metals in the culture of this species and the toxicity mechanisms to heavy metal exposure. Then, it becomes crucial to monitor metal concentrations within the surrounding environment throughout IMTA production of sea cucumbers.

Despite the increasing demand for Mediterranean sea cucumbers, commercial aquaculture production of *H. poli* has yet to be launched and presently, work is still limited to research efforts (González-Wangüemert and Domínguez-Godino, 2016; González-Wangüemert et al., 2018a; Rakaj et al., 2019). For this reason, production data for *H. poli* during grow-out especially as part of open-water IMTA is not available. However, additional evidence from a complete harvest cycle would provide valuable complementary information on the bioaccumulation of contaminants in the body wall tissue of *H. poli* and the implications for fish-sea cucumber IMTA. Considering that *H. poli* doubled from an initial average weight of 24 g at an approximate growth rate of 0.2% day^{-1} during a 12-month experimental period (Chapter 4), typically stocking with smaller juveniles and harvesting at a market size of 70 – 110 g would suggest an extended grow-out period that would have probable effects on metal bioaccumulation throughout production to consider.

7.4.3 Implications of metal contamination

After one year of open-water culture, sea cucumbers are evidently vulnerable to contamination when placed in sediments near commercial fish cages. Bioaccumulation of As and Hg reveals greater propensity of *H. poli* to bioconcentrate toxic contaminants in tissue when closer to fish cages. Measured metal concentrations and bioconcentration in sea cucumber tissue reflect the bioavailability of contaminants in natural sediments in this industrial environment and under fish cages were exposed to waste deposition. This has implications for the performance of extractive species in open-water IMTA and reveals the need to monitor and understand how the distribution of particulate wastes below and near fish cages changes as a function of cage production.

This study reveals a snapshot of metal contamination during production of sea cucumbers in open-water IMTA. It shows that the promising role of sea cucumbers in IMTA can be threatened by exposure to contaminants under fish cages. However, if this system is to be scaled up to be an efficient solution for benthic waste management and value-added

production it is important to understand how patterns of waste distribution around fish cages influence metal bioaccumulation in sea cucumber tissue over representative production periods. Throughout production, farm-level practices can add to local site-specific complexities and lead to variable waste distribution patterns and sedimentary conditions around commercial fish cages (Chapter 3). This temporal variation in food availability and quality affects the transfer of organic material in fish-sea cucumber IMTA (Chapter 5) and similarly, irregular trends of waste deposition can possibly influence the bioavailability of metals and the exposure of sea cucumbers to these contaminants. Since this potentially affects the biomitigation and production efficiency of extractive organisms in IMTA, producers need to be able to capture this variability in the bioavailability of metals in sediments and bioconcentration in sea cucumber tissue. This requires detailed and finer resolution monitoring over a longer time scale for a more representative account of e.g. complete production cycles of the fish farm, different feeding regimes, and local hydrographic variabilities. Moreover, this requires a shift from monospecific considerations for cage production towards a better appreciation of the implications that monoculture activities could have on the physiological activities of sea cucumbers, and their growth and biomitigative performance in IMTA. In an integrated system, viability and profitability may depend on the efficient recapture of feed and energy and therefore, the environmental and economic benefits of sea cucumbers need to be understood and reassuringly consistent.

From a food safety perspective, the consumer willingness to eat IMTA-farmed products augurs well for the potential of IMTA as a food production strategy (Barrington et al., 2010). However, food safety concerns towards these products (Barrington et al., 2010; Alexander et al., 2016) highlight the need for a more thorough monitoring and management of the array of important contaminants that can accumulate in extractive organisms and their human health implications. The viability and performance of IMTA systems are contingent upon the relationship between anthropogenic and environment dynamics specific to the site and the impact on IMTA production. As farm management practices and anthropogenic activities on a wider bay level change, these are expected to influence sediment contamination over time, possibly affecting accumulation and having implications for food safety. Furthermore, to recognise sea cucumbers as potential IMTA products, consistent monitoring of metal levels and the implementation of timely harvesting measures and other management practices, with careful consideration for the various sources of contamination within the fish farm and the bay, are essential.

7.5 Conclusion

Sea cucumbers cultured under near commercial fish cages offer the possibility to extract organic waste associated with intensive aquaculture however, the bioconcentration of toxic metals demand careful monitoring over entire production cycles and with consideration for farm and site complexities that could influence the bioavailability of contaminants in sediments. Research is needed to understand the growth response and waste mitigation efficiency of these extractive organisms when exposed to different levels of contaminants and the implications for the scalability and viability of fish-sea cucumber IMTA in these environments.

Chapter 8. Discussion and conclusions

8.1 Discussion

8.1.1 Complexities of farm-scale waste dispersion modelling in inshore coastal areas

Marine aquaculture will continue to grow (FAO, 2022) and inshore areas will remain important sheltered coastal spaces for production. These are multi-use environments where environmental conditions can be suitable for aquaculture and where this industry can co-exist with other industries and possibly grow. Nonetheless, these are complex areas where multiple anthropogenic activities can be sources of inputs that can influence marine aquaculture. These coastal dynamics need to be understood, especially where anthropogenic activities change and expand over time, if aquaculture is to persist and grow where it exists in these environments. Then, impacts can be managed and mitigated effectively through long-term and high-resolution monitoring and appropriate use of decision-support tools that can maximise uptake of resources and optimise use of space through emerging strategies like integrated multi-trophic aquaculture (IMTA).

Marine fish farming is dynamic and can be complicated by different natural and anthropogenic processes. These include bay-scale processes and farm-level practices that can influence cage production and waste dispersion. This thesis presents some of these complexities as considerations for the development of decision-support tools for IMTA and advances towards commercial-scale IMTA. This research work is set in a multiple-use coastal environment where space is highly contested and where navigation and dredging operations near aquaculture facilities in this area presumably contribute to intermittent hydrodynamic disturbances that were identified. These can be qualitatively distinct and with different implications for marine aquaculture (Klebert et al., 2013; Johansson et al., 2014; Kjelland et al., 2015; Faltinsen and Shen, 2018). The identification of under-predicted currents, potentially linked to ship traffic, shows some of the physical forces contributing to sea current disturbances, revealing the complicated dynamics of near-surface currents in busy port areas. The impact of both natural and human-induced forces on water movement around inshore marine fish farms, emphasise the significance of understanding the potential distinct effects on the environmental conditions surrounding fish cages, fish behaviour, and health and welfare, in the development of marine aquaculture, especially in heavily industrialised coastal spaces. The insights provided by this study shed light on the need for models to consider site-specific conditions in aquaculture planning. Moreover, decision-making tools for coastal management and development need to be refined for the

complexities of multiple-use marine spaces to be represented and addressed more effectively.

Site-specific complexities revealed around the fish farm at the study site in this thesis can influence waste dispersion near cages. Models used to predict waste deposition around fish farms generally use short-term or averaged data that could present limitations to resolving the complexities of these coastal dynamics. Environmental data and modelling would need detailed information to represent hydrodynamic disturbances associated with maritime activity, beyond oversimplistic scenarios. This research shows that consideration of farm-specific variables and cage level dynamics is necessary if farm-scale models are to be representative decision-support tools for effective management. An overarching recommendation emerges for the utilization of waste dispersion models in licensing and environmental regulation contexts. The ability of IMTA models to predict deposition footprint magnitude and the influence of cage management practices provides regulatory bodies with finer assessment tools to allow for improved precision in the management of benthic impacts, especially where complicated by coastal anthropogenic activities. This marks a step beyond one-time or one-point environmental impact assessments, aligning with the continuous variability inherent in cage production and the pressing necessity to incorporate real-world complexities into management strategies.

Mitigating benthic impacts through IMTA with deposit-feeding organisms such as sea cucumbers, demands the detailed mapping of seafloor sediment organic waste during cage production. Considering that the feasibility of IMTA hinges on the well-informed decisions of IMTA practitioners, the thorough representation of particulate organic waste availability and quality through the use of farm-scale models as portrayed in Chapters 4 and 5 in this research, under commercial conditions, is an important pillar for the development of open-water benthic IMTA. Bottom-dwelling extractive species in an IMTA system depend on these organic waste particulates in seafloor sediments. When waste dispersion models are applied to set up IMTA systems these variabilities need to be represented for the removal of organic wastes under fish cages and mitigation of benthic impact to be efficient. Consequently, this allows better use of space and resources, and under these circumstances, optimisation depends on detailed and fine-scale environmental assessments. For aquaculture to survive in co-existence with coastal industries that might be growing around it, and then to grow sustainably itself in areas where limitations of space can be a substantial barrier, it is important to know and understand the variability in the local surroundings and the environmental and economic implications. Decision-support models developed or applied to IMTA need to provide a flexible modelling approach that caters for

these site-specific complexities, as well as farm-level considerations, and therefore convey realistic descriptions of the local environment.

Farm-level practices intended to maximise the available space for production where marine fish farming has spatial restrictions can lead to complicated cage management practices described in Chapter 4 and elsewhere (Magill et al., 2006; Ferreira et al., 2008; Cromey et al., 2012). These farm-level practices can add a layer of complexity to describing waste deposition. Real-world cage management practices have been described for Mediterranean (Magill et al., 2006) and Asian regions (Ferreira et al., 2008), and now farm-level complexities presented in this thesis, reveal new challenges for waste dispersion models to predict deposition footprints for IMTA application. Models have a key role in addressing significant issues at different scales of the aquaculture industry including IMTA challenges and bottlenecks associated with effective mitigation of benthic impacts under fish cages. Further development of decision-support models benefits the translation of the principles of the ecosystem approach to aquaculture into tangible actions for sustainable development (Soto et al., 2008, Ferreira et al., 2012, Byron and Costa-Pierce, 2013). Applying an ecosystem-based approach requires effective management and mitigation of aquaculture impacts and relies on models to represent waste deposition, even where this is complicated in real complex systems.

Advances in model development have improved the accuracy of waste dispersion simulations but there can still be limitations to farm-scale modelling of real-world cage management complications. Models that provide the flexibility to adapt to farm management complexities (e.g. allow combinations of cage sizes and layouts) are more representative of complex systems (Cromey et al., 2012; Chang et al., 2014). The application of waste dispersion models in field-based open-water IMTA research to-date has still been limited. Contrastingly the key function of the waste dispersion model CAPOT in the IMTA research presented in this thesis denotes the profound importance of farm-scale models for effective planning and management in IMTA. The farm-scale waste dispersion model was adapted for proper siting of extractive species and waste mitigation in IMTA (Chapters 4 and 5). The placement of deposit-feeding sea cucumbers to match the availability and quality of food is seafloor sediments with the metabolic needs of the extractive species has yet to be widely based on waste dispersion simulations in open-water IMTA research. This thesis presents an approach where planning and management in open-water IMTA were based on the distribution and quality of organic wastes modelled at a finer spatial resolution and over a longer period to account for farm management complications. In benthic IMTA application, particle-tracking models need to be realistic and resolve farm-level complexities so production is within carrying capacity limit and organic waste recycling is efficient in seafloor

sediments. Resolving the variability in waste deposition under these circumstances can possibly address concerns that seasonal mismatch in nutrient budgets can impede the commercialisation of IMTA (Christensen, 2020).

Some of the important challenges for IMTA development, especially for sea-based installations, include legal considerations and regulatory gaps that have yet to be addressed for pilot-scale IMTA to be commercialised (Alexander et al., 2015; Kleitou et al., 2018; Cavallo et al., 2020; Falconer et al., 2023). While particulate waste dispersion models are widely applied in aquaculture licensing and planning processes (Cromey et al., 2002; Corner et al., 2006), the lack of suitable decision-support tools and regulatory frameworks for application in IMTA will impede the transition from pilot phase to commercial-scale production. The implications of farm-level processes and site-specific complications for waste deposition present opportunities for particle-dispersion models to evaluate waste distribution variations at a finer scale. Models that represent real-world complexities and variability can contribute towards improved carrying capacity assessments of IMTA systems, guidelines for production threshold limits for a given area, and environmental standards (e.g. allowable nutrient and organic waste inputs, benthic habitat requirements) to monitor the effectiveness of IMTA. These contributions are needed for the development of effective regulation and licensing processes especially where these are needed to push barriers in the growth of sustainable aquaculture. This needs to be driven by increased research efforts into the development of waste dispersion models and comprehensive decision-support approaches for IMTA to provide the evidence and stimulus for the development of policies and regulatory frameworks for commercial-scale IMTA.

8.1.2 Waste dispersion model application in IMTA

The pivotal role of waste dispersion models in IMTA emerges from concrete empirical findings in this research. Firstly, Chapter 4 laid bare the variability encapsulated within deposition footprints, revealing the significance of cage management intricacies that wield influence over the quantity and fate of waste distribution near fish cages. Then, *in situ* studies evaluating the growth and survivability of extractive species in IMTA (Chapter 5) funnel tangible real-world data into models, grounding predictions in empirical realities and fine-tuning IMTA systems by maximizing organic matter uptake throughout production. An illustrative example emerges from observations of sea cucumbers placed directly beneath commercial fish cages, which, due to excessive waste deposition, showed the dire need for more accurate deposition footprint predictions and judicious placement of deposit-feeding organisms within an IMTA setup. Sedimentation directly below the fish cage smothered sea cucumbers, where deposition rates varied between 125 gC m^{-2} $month^{-1}$ and 2438.3 gC m^{-2}

month^{-1} between October 2018 and July 2019. Settlement of 1 gC m^{-2} day^{-1} has been considered as the threshold for possible impacts measured as benthic change (Hargrave, 1994; Cromey et al., 2009; Telfer et al., 2023). When properly placed along a gradient of decreasing sedimentary organic carbon levels within metres from the fish cages, sea cucumbers survived and grew better and validated the central role waste dispersion models should have in open-water IMTA development (Chapter 4). This is a key consideration for IMTA where the balanced relationship between fed and extractive organisms hinges on understanding these waste dynamics. Moreover, this is particularly relevant in scenarios where the optimal utilization of space and resources surrounding fish cages emerges as a crucial solution for steering sustainable aquaculture expansion in the face of spatial constraints.

Secondly, the refinement of farm-scale models to mirror these farm-specific complexities directly translates into enhanced predictive representativeness. Accurate inputs, detailing actual food contributions and cage biomass fluctuations, alongside the incorporation of cage management practices, stand as imperative prerequisites for the advancement of IMTA. Farm-scale dispersion models need to be developed and used with site and bay-scale models, as part of more complex decision-support tools and IMTA models, for planning and management of processes in IMTA. Dispersion models need to be user-friendly and flexible scoping tools that help producers plan and manage IMTA under different real-world production scenarios. Then, considering the variability in waste deposition, the efficiency of an established IMTA system depends on how producers address changes in deposition and the organic footprint over time. The flux of waste in an IMTA system is dynamic and management practices in these integrated setups should be based on data and models that reflect this variability for extractive species to maximise the uptake of nutrients and organic matter and producers to optimise their environmental and economic benefits. The performance and feasibility of IMTA depends on synchronous nutrient loading and uptake dynamics and suitable siting of extractive species relative to the distribution of waste streams (Reid et al. 2020; Chary et al., 2019). However, challenges of coastal and open-water IMTA are not only limited to nutrient-use efficiency, and contradictory findings (Mazzola and Sarà, 2001; Cheshuk et al., 2003; Handå et al., 2012; Irisarri et al., 2013, 2014; Jiang et al., 2013; Giangrande et al., 2020) revealed the need to understand the feasibility and efficiency of the system in terms of transfer of nutrients in wastes in IMTA (Falconer et al., 2023), among other biological and operational considerations discussed in further detail in Chapters 5 and 6.

8.1.3 Open-water IMTA in multiple-use coastal areas

From an operational perspective, challenges in practicability of open-water IMTA is still among the bottlenecks of IMTA development (Kleitou et al., 2018). Observations made over one year of production in Chapter 5 revealed some of the routine and seasonal practicalities of IMTA under commercial conditions that could be less evident during short-term studies. Field observations substantiate concerns of stakeholders that technological investment is needed to improve the technology and infrastructure (Kleitou et al., 2018) to address environmental considerations operational issues identified in Chapter 5. These lessons learnt need to drive research and development of higher technology and better infrastructures to be implemented using appropriate designs and configurations of IMTA to resolve bottlenecks in the commercialisation of IMTA that have been ascribed to experimental and pilot scales of research.

In open-water conditions, nutrient and organic matter transfer and uptake are still among the biological concerns (Chopin et al., 2012; Handå et al., 2012; Kleitou et al., 2018) that obscure the development of IMTA. This thesis, specifically Chapter 6, offers a crucial insight that should guide the establishment of effective IMTA systems. This research established a critical link between the fish waste and sea cucumbers confirming that there was waste uptake by the extractive organisms in IMTA. Stable isotope and fatty acid analysis provided complementary evidence for the trophic link in IMTA and validated the capacity of sea cucumbers to recycle organic matter in aquaculture-derived wastes and to contribute towards mitigation of benthic impact. This finding underscores the indispensable recommendation for IMTA practitioners to emphasize and validate trophic connectivity in open-water environments, ensuring the system is truly an integrated one. It amplifies the mitigation of benthic impact but also strengthens the foundational underpinning of IMTA's potential in facilitating more resource-efficient practices.

From a nutritional perspective, a validated fish-sea cucumber IMTA reveals the potential of this strategy to help sustainable aquaculture grow in heavily contested coastal spaces through better use of seafloor space around fish cages and the uptake organic waste resources that have been linked with improved sea cucumber growth in this study as seen in Chapter 5. Notwithstanding, producers need to be cognizant of the possible effects that changes in fish farm practices have on waste deposition and the availability and quality of food in seafloor sediments for extractive species in IMTA. The temporal variation revealed in predicted sedimentary organic carbon would influence the transfer of dietary organic matter between farmed fish and sea cucumbers, the uptake and removal of organic wastes, sea cucumber production, and other farm management practices (e.g. optimum period of

harvest operation) that need to be considered for a successful IMTA operation. This would have implications for any environmental and economic benefits that the producer could expect. From a business perspective, these fluctuations in waste output and uptake in fish-sea cucumber IMTA should be resolved for better predictions of feasibility and performance of the system to lower the investment risks of commercialisation for producers. The validated uptake of aquaculture-derived organic waste and the increasing preferences for eco-labelled aquaculture products (van Osch et al., 2017; Xuan, 2021) present economic opportunities for producers that might be interested to adopt environmentally friendlier technologies in this Mediterranean region. These findings are encouraging for product diversification through IMTA especially considering the willingness of consumers to pay a price premium for sustainably farmed seafood (van Osch et al., 2017; Knowler et al., 2020). The nutritional benefits of sea cucumbers produced near fish cages could create economic value for producers to invest in sustainably farmed products.

Despite the nutritional gain of sea cucumbers in IMTA, contaminants under fish cages can lessen the expected benefits of fish-sea cucumber IMTA development. Bioconcentration of toxic metals in sea cucumbers reveal the need to understand how the bioavailability of contaminants in sediments changes throughout the production cycle of sea cucumbers under commercial conditions and the effects on growth response and waste mitigation efficiency in fish-sea cucumber IMTA. This variability presents additional challenges to the environmental and economic benefits that sea cucumbers can provide through IMTA. However, thorough and finer-resolution monitoring helps producers account for the effects of changes in waste deposition and environmental conditions. The dynamics that influence the quantity and distribution patterns of organic wastes are similar for metals and various contaminants. Moreover, the relationship between organic matter and contaminants in wastes within sediments can influence the bioavailability of metals. Therefore, representative data becomes necessary to understand these mechanisms and to refine models and improve predictions for the fate and transport of heavy metals and other contaminants in sediments, along with their uptake. Essentially, dispersion models may play a crucial role in informing producers about the potential uptake of contaminants by sea cucumbers.

In the broader perspective, the possibilities and challenges for IMTA development are multifaceted and require further research. In terms of contamination, multiple stressors can influence the contamination of sea cucumber tissues and the performance of extractive organisms in IMTA. The performance of extractive species feasibility of IMTA requires an understanding of single and combined effects of contaminants, and environmental and anthropogenic complexities, especially in industrialised areas. Tank-based trials should

provide additional evidence for the transfer of other important contaminants in sediments (e.g., organometallic compounds, aromatic organics, and halogenated hydrocarbons) and the effects of exposure over representative production timescales. Essentially, this is a complex system and unless knowledge gaps and uncertainties are addressed in consideration of real-world aquaculture processes, scaling up of IMTA will remain a challenge.

8.2 Conclusions

The slower pace at which IMTA is developing at lower latitudes in Europe (Kleitou et al., 2018) substantiates the need for IMTA research in the Mediterranean and unprecedented coastal areas where the local environment and aquaculture conditions might seem unfamiliar but actually, are realities that exist in this region and other parts of the world. Research from this thesis provides a stepwise approach to predict the deposition footprint under complicated aquaculture settings using a flexible spreadsheet dispersion model and applied to fish-sea cucumber IMTA. As coastal areas become increasingly crowded and contested, this research contributes towards a better understanding of farm-level biological and operational challenges of open-water IMTA under commercial aquaculture conditions to overcome some of the knowledge gaps that reportedly serve as barriers for IMTA in this region of Europe (Kleitou et al., 2018).

This thesis presents important conclusions:

- Sustainable aquaculture development is complicated by bay-wide and farm-level processes in contested coastal environments.
- Nearshore aquaculture faces variable and anthropogenically influenced environmental conditions in multi-use coastal spaces.
- Farm-level management practices can complicate the uptake of organic wastes released from fish cages through IMTA.
- Efficient waste recycling under fish cages requires long-term data and representative predictions of waste distribution that account for real-world farm complexities.
- Farm-scale models can represent complex environmental and farm dynamics beyond oversimplistic generalisations to inform effective waste uptake in IMTA.
- In the open environment, *H. poli* grew better near fish cages and the suitability of these extractive organisms was validated for open-water IMTA through stable isotope and fatty acid analysis.

- Variable waste deposition patterns influence food availability and quality, and contaminants in seafloor sediments, and possibly, the feasibility of fish-sea cucumber IMTA at commercial scale.
- Toxic metals bioconcentrate in sea cucumbers in IMTA demanding careful monitoring over entire production cycles.

From the perspectives of this thesis, IMTA has the potential of being the solution for better use of space and resources where these are a limitation for the development of sustainable aquaculture. However, there are still site-specific challenges and universal bottlenecks that are only to-date being appreciated at pilot scale which further underpins the rate of sustainable aquaculture development and that need to be addressed for the industry and authorities to take IMTA to the commercial phase. Then, planning and legislation for IMTA need to account for the specific requirements of the system to account for its full complexity and therefore, these processes may need to be distinctly different from existing aquaculture legislations that rely on assessments as a function of amount of farm production expected.

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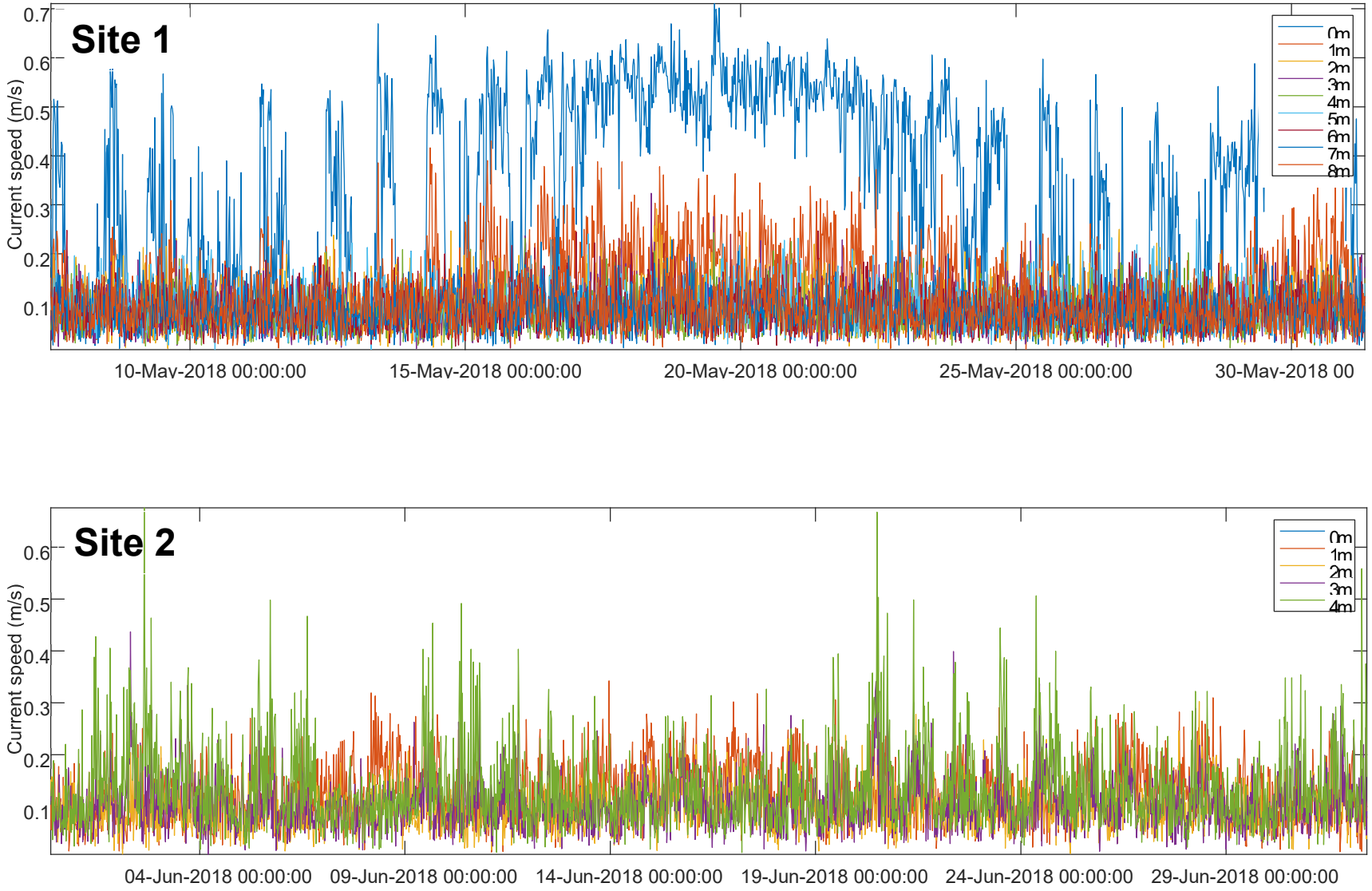
Appendix

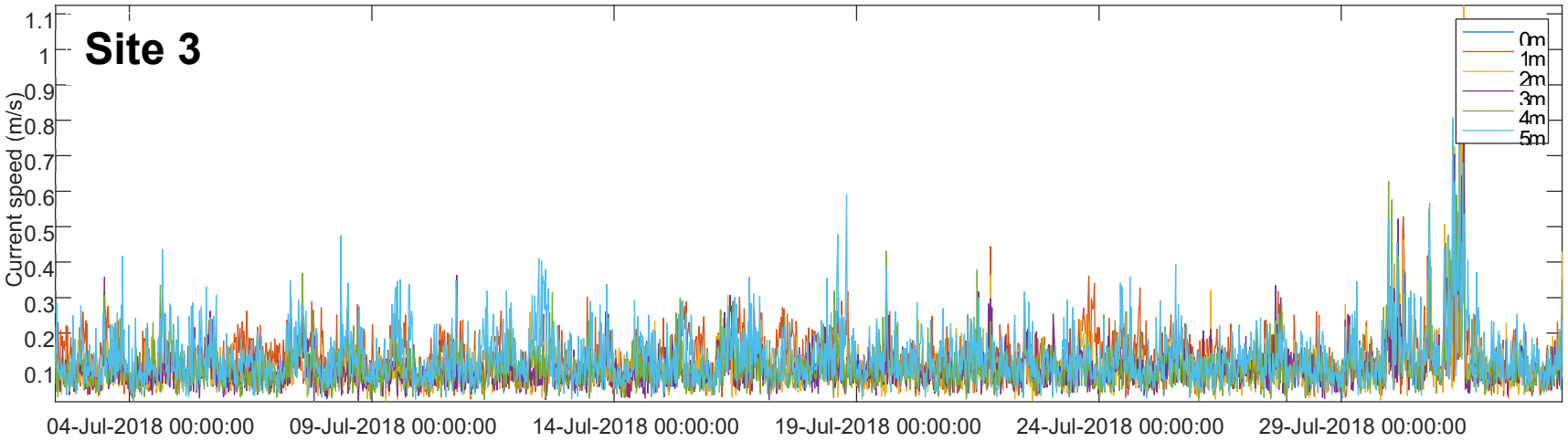
Appendix 3.1. Current magnitudes measured at different sites and depths around a nearshore fish farm in Marsaxlokk Bay between May 2018 and August 2019. Current magnitude measurements were taken at 20-minute intervals with a 400 kHz acoustic Doppler current profiler.

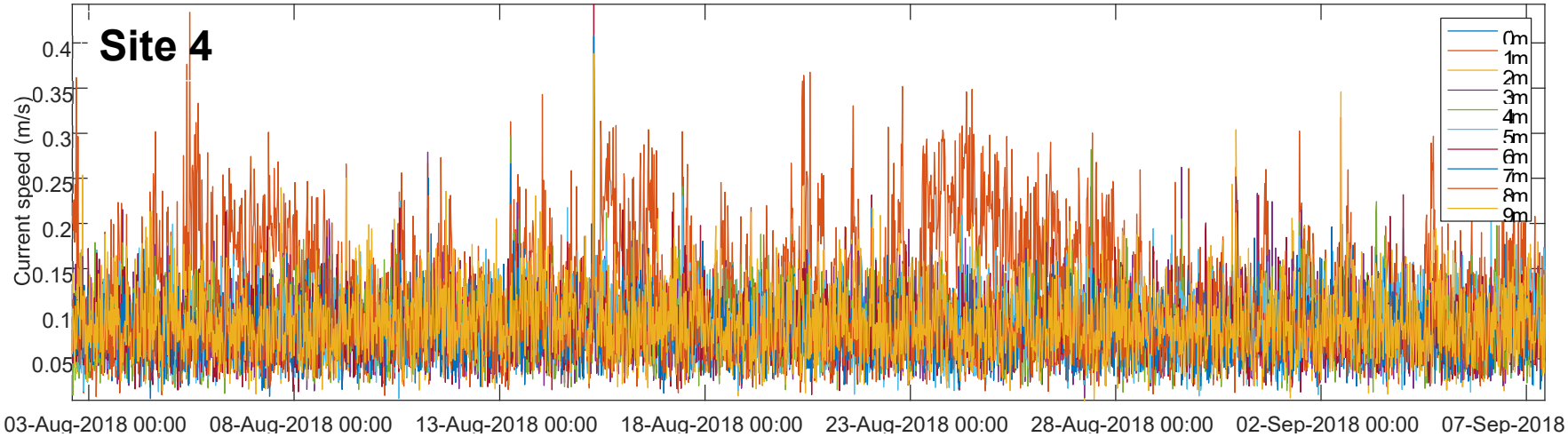
Site 1	Depth (m)	-0.16	0.84	1.84	2.84	3.84	4.84	5.84	6.84	7.84	8.84
	Min. magnitude (m s^{-1})	0.002	0.003	0.000	0.002	0.001	0.002	0.001	0.002	0.000	0.001
	Max. magnitude (m s^{-1})	0.796	0.504	0.326	0.357	0.284	0.292	0.290	0.286	0.299	0.324
	Mean magnitude (m s^{-1})	0.365	0.136	0.096	0.085	0.078	0.095	0.087	0.085	0.089	0.091
	Standard deviation (m s^{-1})	0.226	0.088	0.052	0.046	0.042	0.051	0.047	0.044	0.048	0.051
Site 2	Depth (m)	0.44	1.44	2.44	3.44	4.44					
	Min. magnitude (m s^{-1})	0.001	0.000	0.000	0.001	0.000					
	Max. magnitude (m s^{-1})	0.546	0.324	0.370	0.573	1.101					
	Mean magnitude (m s^{-1})	0.158	0.096	0.093	0.114	0.149					
	Standard deviation (m s^{-1})	0.094	0.052	0.054	0.077	0.112					
Site 3	Depth (m)	0.34	1.34	2.34	3.34	4.34	5.34				
	Min. magnitude (m s^{-1})	0.000	0.001	0.000	0.000	0.002	0.001				
	Max. magnitude (m s^{-1})	0.660	1.126	1.389	0.850	0.797	1.030				
	Mean magnitude (m s^{-1})	0.168	0.102	0.099	0.096	0.111	0.141				
	Standard deviation (m s^{-1})	0.121	0.076	0.081	0.070	0.080	0.104				
Site 4	Depth (m)	0.44	1.44	2.44	3.44	4.44	5.44	6.44	7.44	8.44	9.44
	Min. magnitude (m s^{-1})	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.000	0.000	0.000
	Max. magnitude (m s^{-1})	0.746	0.374	0.334	0.429	0.310	0.426	0.456	0.369	0.486	0.346
	Mean magnitude (m s^{-1})	0.183	0.090	0.085	0.080	0.085	0.082	0.082	0.078	0.086	0.091
	Standard deviation (m s^{-1})	0.129	0.051	0.046	0.045	0.047	0.045	0.045	0.042	0.049	0.049
Site 5	Depth (m)	0.34	1.34	2.34	3.34	4.34	5.34	6.34	7.34	8.34	
	Min. magnitude (m s^{-1})	0.000	0.002	0.001	0.001	0.004	0.001	0.003	0.000	0.002	
	Max. magnitude (m s^{-1})	0.702	0.404	0.314	1.165	0.334	1.095	0.342	0.632	0.724	
	Mean magnitude (m s^{-1})	0.217	0.101	0.083	0.184	0.080	0.157	0.086	0.093	0.106	
	Standard deviation (m s^{-1})	0.174	0.059	0.046	0.145	0.047	0.119	0.047	0.053	0.067	
Site 6	Depth (m)	0.94	1.94	2.94	3.94	4.94					
	Min. magnitude (m s^{-1})	0.004	0.002	0.000	0.000	0.002					
	Max. magnitude (m s^{-1})	0.466	0.432	0.622	0.441	0.587					
	Mean magnitude (m s^{-1})	0.122	0.100	0.092	0.104	0.108					
	Standard deviation (m s^{-1})	0.070	0.056	0.054	0.062	0.063					

Site 7	Depth (m)	0.24	1.24	2.24	3.24	4.24	5.24	6.24	7.24	8.24	9.24
	Min. magnitude (m s^{-1})	0.001	0.002	0.004	0.001	0.002	0.002	0.004	0.002	0.001	0.000
	Max. magnitude (m s^{-1})	0.816	0.421	0.414	0.398	0.363	0.460	0.349	0.305	0.278	0.695
	Mean magnitude (m s^{-1})	0.346	0.103	0.092	0.090	0.083	0.086	0.085	0.085	0.082	0.088
	Standard deviation (m s^{-1})	0.210	0.059	0.052	0.050	0.047	0.047	0.045	0.046	0.045	0.050
Site 8	Depth (m)	0.74	1.74	2.74	3.74	4.74	5.74	6.74			
	Min. magnitude (m s^{-1})	0.001	0.002	0.002	0.001	0.001	0.000	0.001			
	Max. magnitude (m s^{-1})	0.592	0.429	0.294	0.487	0.289	0.324	0.519			
	Mean magnitude (m s^{-1})	0.207	0.093	0.086	0.090	0.085	0.082	0.101			
	Standard deviation (m s^{-1})	0.124	0.052	0.047	0.054	0.048	0.045	0.064			
Site 9	Depth (m)	0.44	1.44	2.44	3.44	4.44	5.44				
	Min. magnitude (m s^{-1})	0.005	0.003	0.004	0.001	0.001	0.001				
	Max. magnitude (m s^{-1})	0.672	0.432	0.402	0.394	0.550	0.605				
	Mean magnitude (m s^{-1})	0.184	0.094	0.088	0.084	0.101	0.118				
	Standard deviation (m s^{-1})	0.117	0.052	0.049	0.049	0.062	0.085				
Site 10	Depth (m)	0.94	1.94	2.94	3.94	4.94	5.94	6.94	7.94	8.94	9.94
	Min. magnitude (m s^{-1})	0.001	0.001	0.005	0.000	0.002	0.003	0.001	0.001	0.000	0.001
	Max. magnitude (m s^{-1})	0.658	0.334	0.273	0.300	0.416	0.724	0.419	0.397	0.396	0.329
	Mean magnitude (m s^{-1})	0.139	0.089	0.087	0.081	0.082	0.087	0.083	0.083	0.084	0.090
	Standard deviation (m s^{-1})	0.097	0.049	0.046	0.045	0.045	0.052	0.046	0.046	0.047	0.048
Site 11	Depth (m)	0.44	1.44	2.44	3.44	4.44	5.44	6.44	7.44		
	Min. magnitude (m s^{-1})	0.000	0.001	0.001	0.001	0.000	0.000	0.001	0.000		
	Max. magnitude (m s^{-1})	0.547	0.330	0.362	0.385	0.418	0.475	0.301	0.322		
	Mean magnitude (m s^{-1})	0.165	0.087	0.102	0.097	0.099	0.082	0.087	0.092		
	Standard deviation (m s^{-1})	0.111	0.048	0.057	0.052	0.057	0.047	0.049	0.050		
Site 12	Depth (m)	1.14	2.14	3.14	4.14	5.14	6.14	7.14			
	Min. magnitude (m s^{-1})	0.000	0.003	0.002	0.000	0.002	0.000	0.002			
	Max. magnitude (m s^{-1})	0.421	0.298	0.456	0.393	0.411	0.462	0.725			
	Mean magnitude (m s^{-1})	0.105	0.086	0.089	0.090	0.105	0.093	0.102			
	Standard deviation (m s^{-1})	0.060	0.048	0.051	0.056	0.061	0.056	0.064			
Site 13	Depth (m)	1.80	2.80	3.80	4.80	5.80	6.80	7.80	8.80	9.80	10.80
	Min. magnitude (m s^{-1})	0.001	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000
	Max. magnitude (m s^{-1})	0.726	0.433	0.483	0.974	1.207	0.768	0.507	0.639	0.661	1.063
	Mean magnitude (m s^{-1})	0.203	0.101	0.092	0.088	0.092	0.091	0.087	0.084	0.087	0.093
	Standard deviation (m s^{-1})	0.141	0.056	0.052	0.055	0.058	0.058	0.048	0.050	0.052	0.063

Appendix 3.2. Time series of water current magnitude normalised for water depth at the different deployment positions of the acoustic Doppler current profiler, at sites 1 to 13 in order of placement around the fish farm between May 2018 and August 2019.




Site 3
Current speed (m/s)
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0m
1m
2m
3m
4m
5m
04-Jul-2018 00:00:00
09-Jul-2018 00:00:00
14-Jul-2018 00:00:00
19-Jul-2018 00:00:00
24-Jul-2018 00:00:00
29-Jul-2018 00:00:00


Site 4
Current speed (m/s)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0m
1m
2m
3m
4m
5m
6m
7m
8m
9m
03-Aug-2018 00:00
08-Aug-2018 00:00
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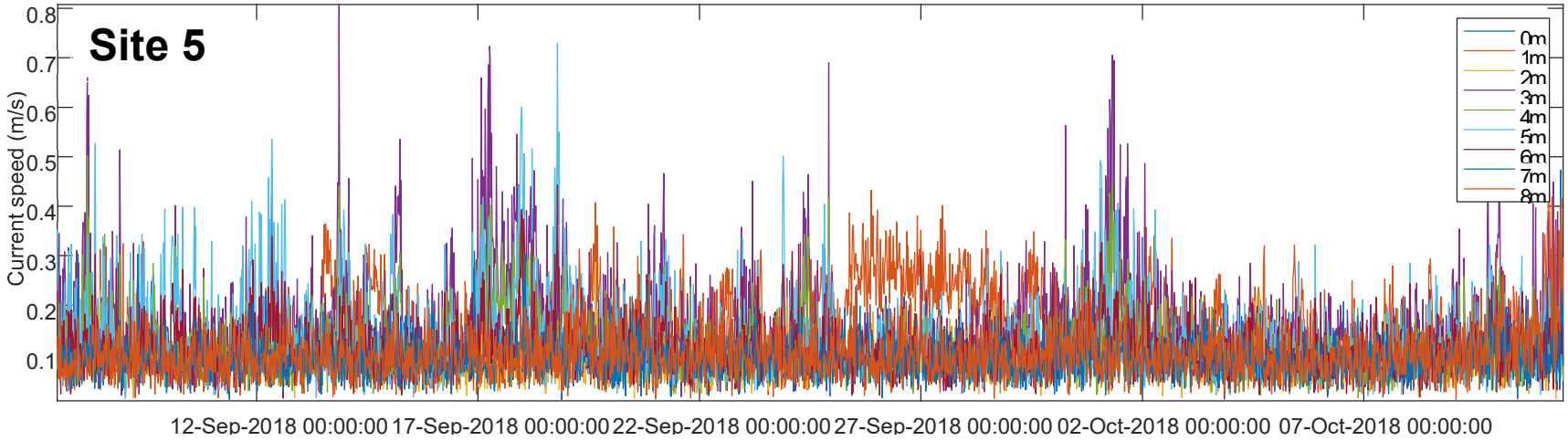
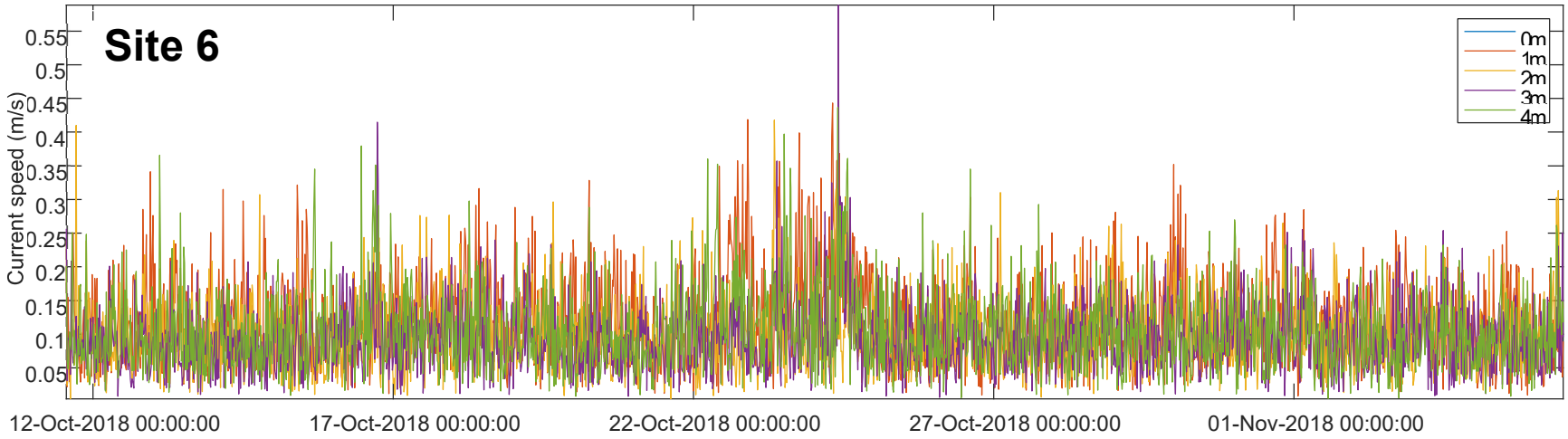
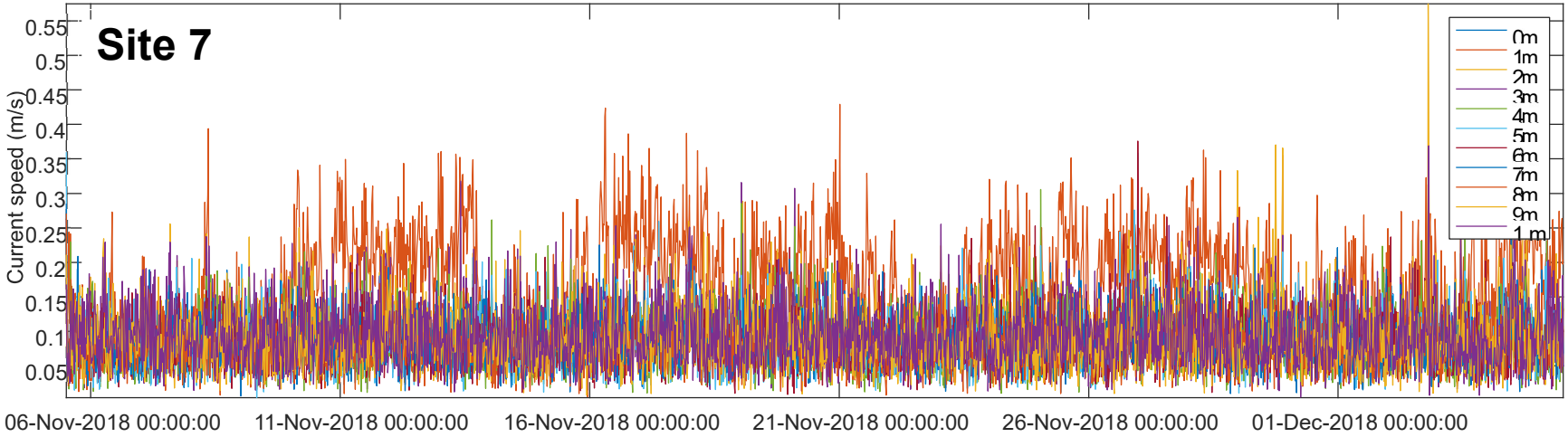
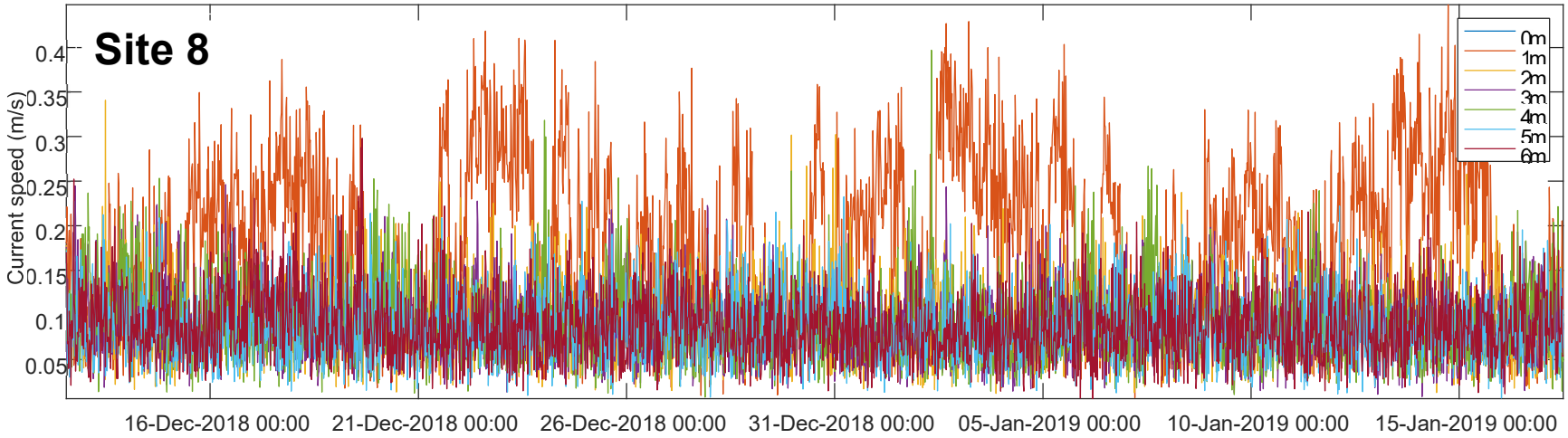
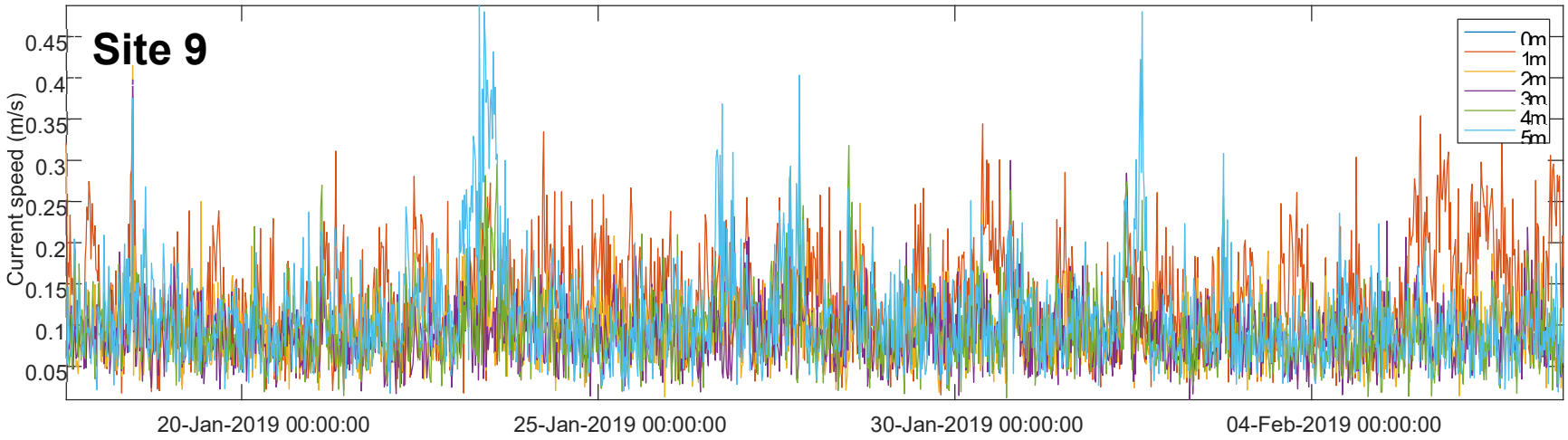
18-Aug-2018 00:00
23-Aug-2018 00:00
28-Aug-2018 00:00
02-Sep-2018 00:00
07-Sep-2018

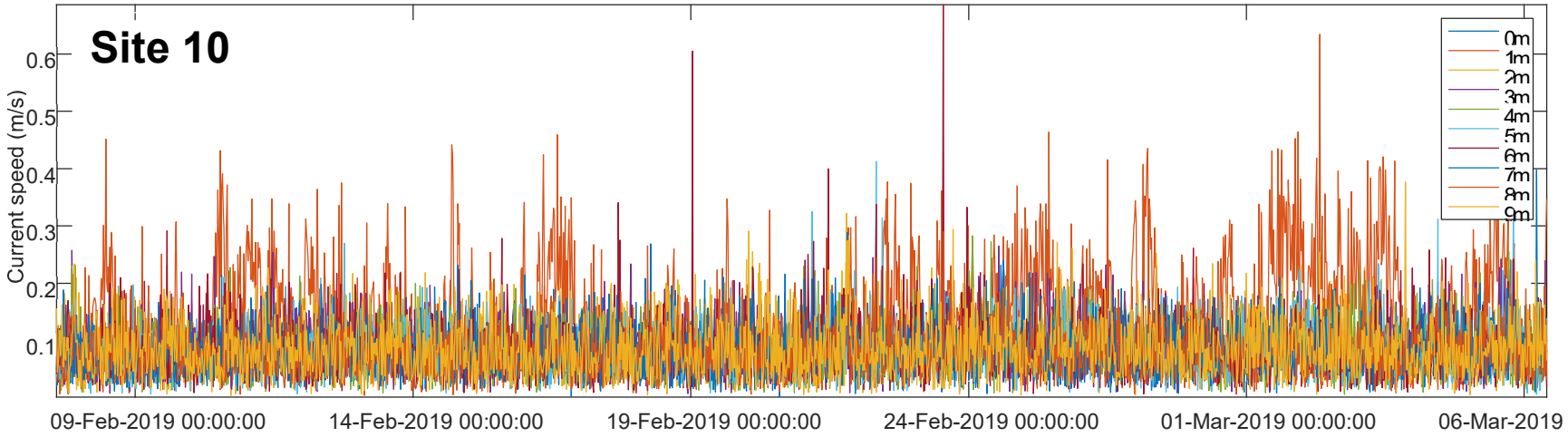

Site 5
Current speed (m/s)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0m
1m
2m
3m
4m
5m
6m
7m
8m
12-Sep-2018 00:00:00
17-Sep-2018 00:00:00
22-Sep-2018 00:00:00
27-Sep-2018 00:00:00
02-Oct-2018 00:00:00
07-Oct-2018 00:00:00


Site 6
Current speed (m/s)
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0m
1m
2m
3m
4m
12-Oct-2018 00:00:00
17-Oct-2018 00:00:00
22-Oct-2018 00:00:00
27-Oct-2018 00:00:00
01-Nov-2018 00:00:00


Site 7
Current speed (m/s)
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0m
1m
2m
3m
4m
5m
6m
7m
8m
9m
10m
06-Nov-2018 00:00:00
11-Nov-2018 00:00:00
16-Nov-2018 00:00:00
21-Nov-2018 00:00:00
26-Nov-2018 00:00:00
01-Dec-2018 00:00:00


Site 8
Current speed (m/s)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0m
1m
2m
3m
4m
5m
6m
16-Dec-2018 00:00
21-Dec-2018 00:00
26-Dec-2018 00:00
31-Dec-2018 00:00
05-Jan-2019 00:00
10-Jan-2019 00:00
15-Jan-2019 00:00


Site 9
Current speed (m/s)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
20-Jan-2019 00:00:00
25-Jan-2019 00:00:00
30-Jan-2019 00:00:00
04-Feb-2019 00:00:00
0m
1m
2m
3m
4m
5m


Site 10
Current speed (m/s)
0.6
0.5
0.4
0.3
0.2
0.1
09-Feb-2019 00:00:00
14-Feb-2019 00:00:00
19-Feb-2019 00:00:00
24-Feb-2019 00:00:00
01-Mar-2019 00:00:00
06-Mar-2019
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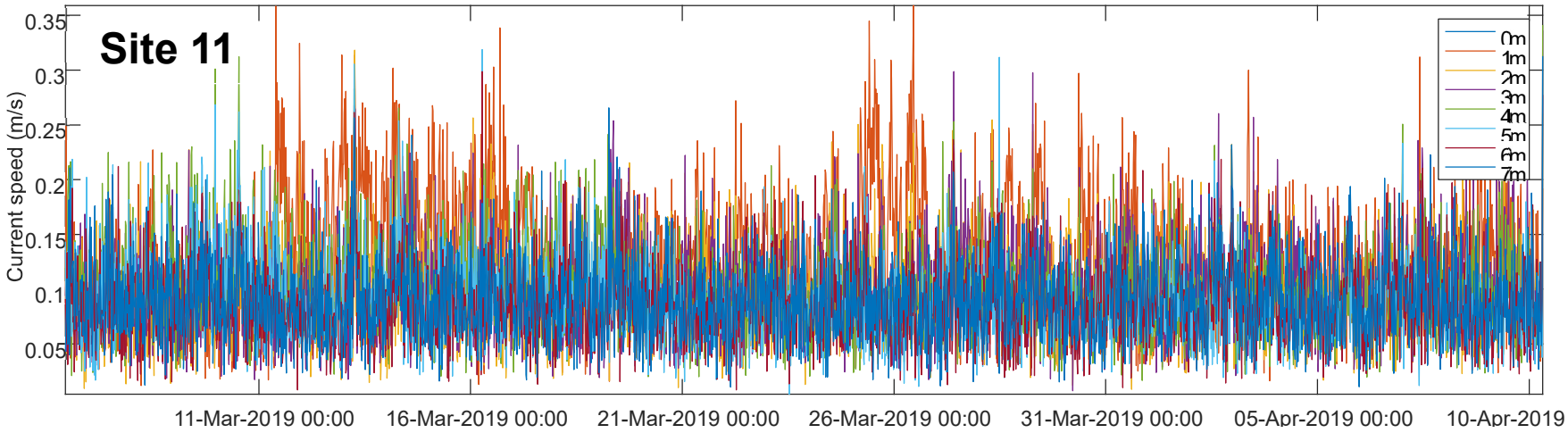
3m
4m
5m
6m
7m
8m
9m


Site 11
Current speed (m/s)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0m
1m
2m
3m
4m
5m
6m
7m
11-Mar-2019 00:00
16-Mar-2019 00:00
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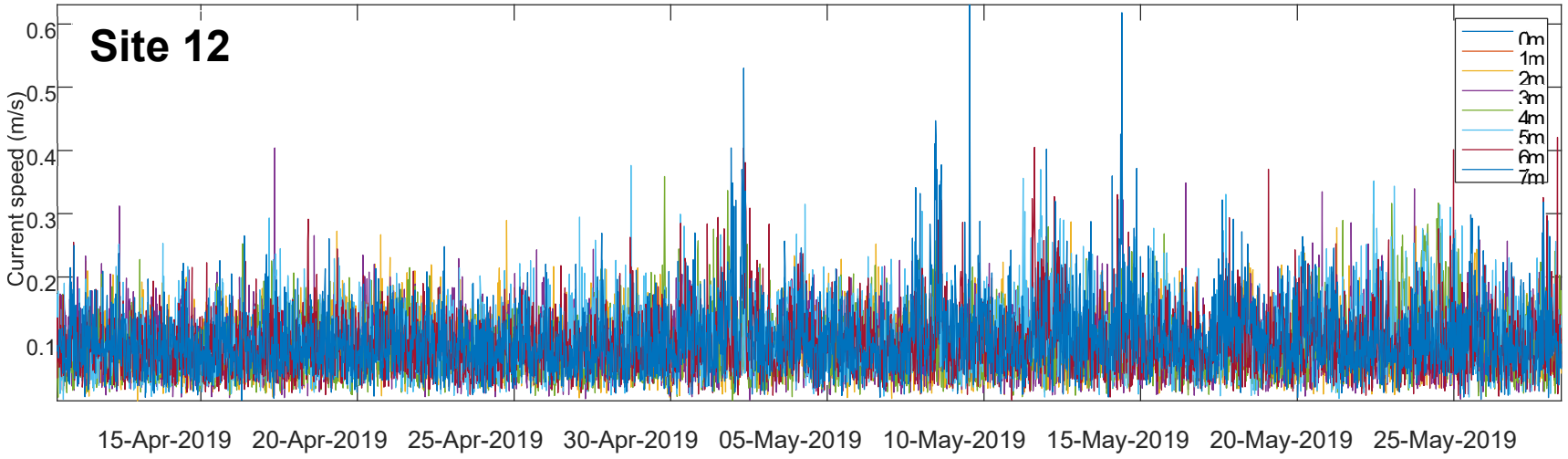
31-Mar-2019 00:00
05-Apr-2019 00:00
10-Apr-2019


Site 12
Current speed (m/s)
0.6
0.5
0.4
0.3
0.2
0.1
0m
1m
2m
3m
4m
5m
6m
7m
15-Apr-2019
20-Apr-2019
25-Apr-2019
30-Apr-2019
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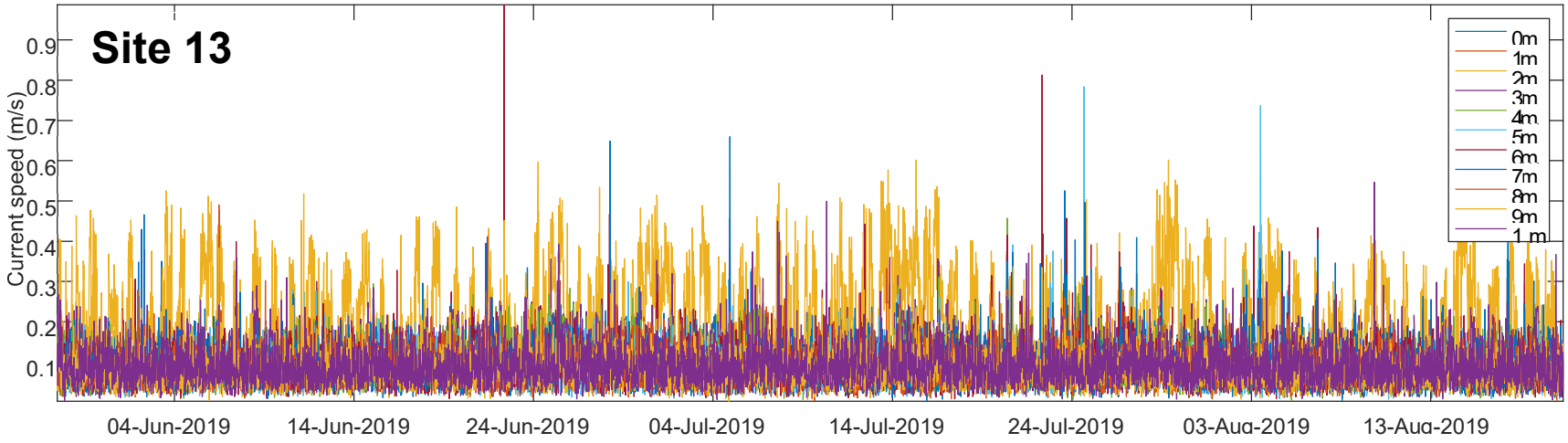
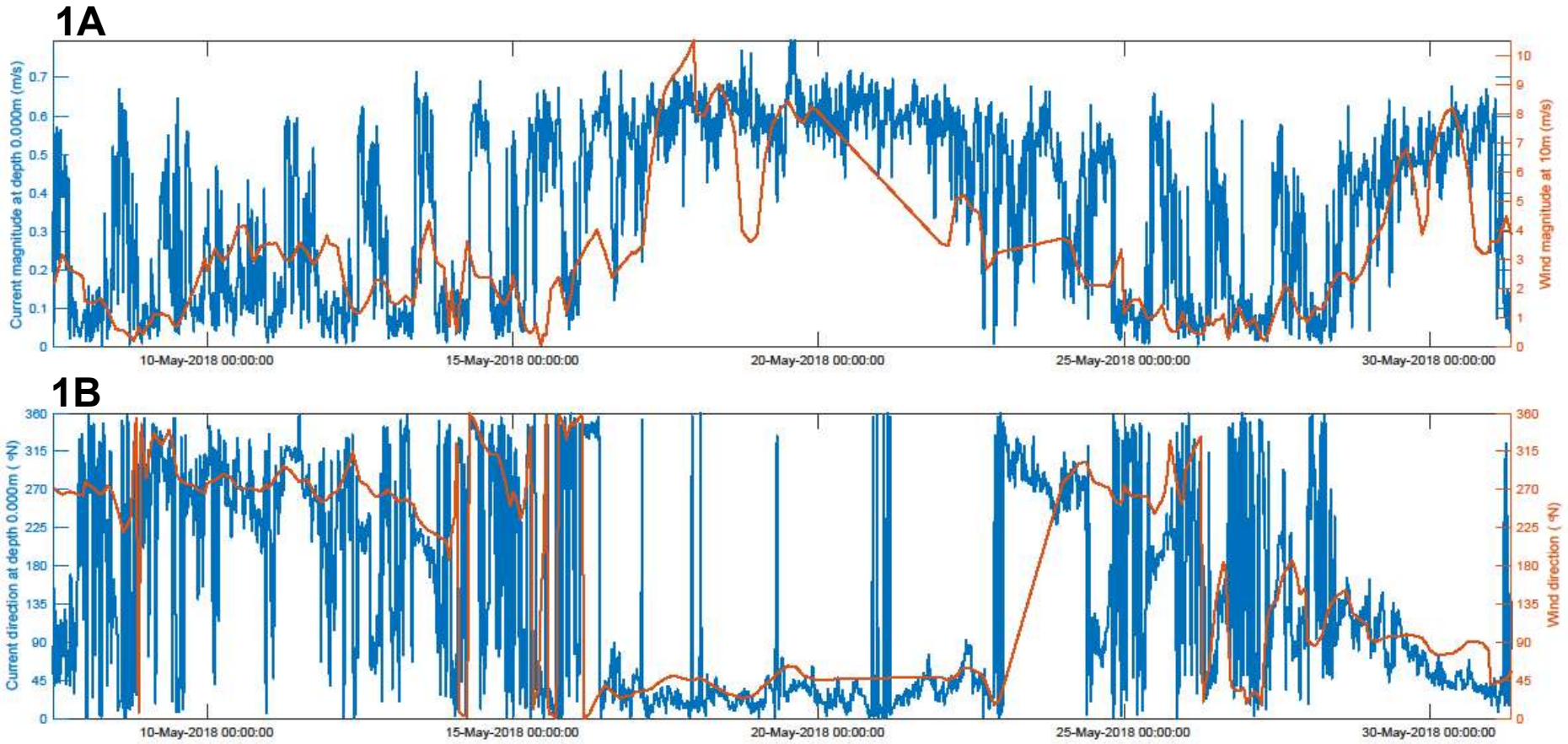
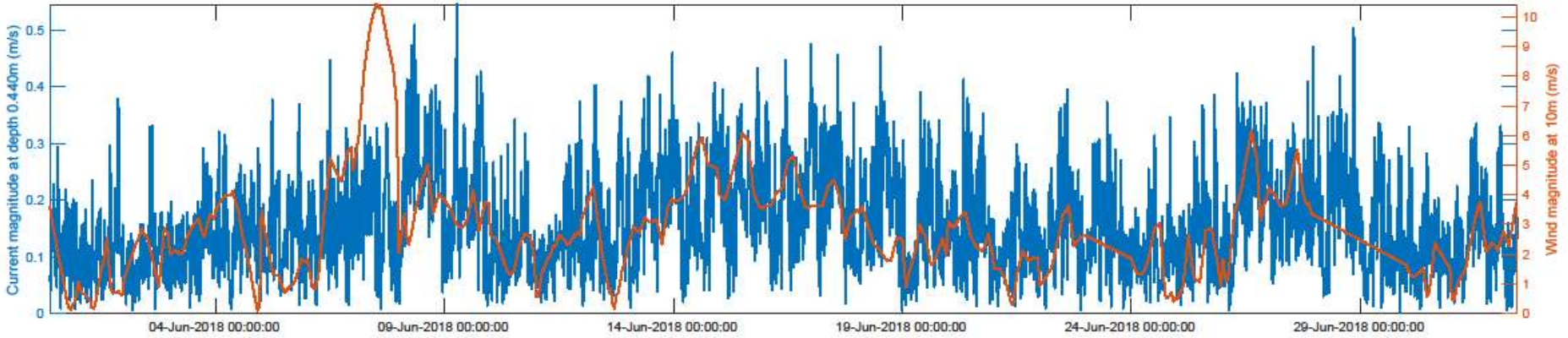
10-May-2019
15-May-2019
20-May-2019
25-May-2019


Site 13
Current speed (m/s)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
04-Jun-2019
14-Jun-2019
24-Jun-2019
04-Jul-2019
14-Jul-2019
24-Jul-2019
03-Aug-2019
13-Aug-2019
0m
1m
2m
3m
4m
5m
6m
7m
8m
9m
10m

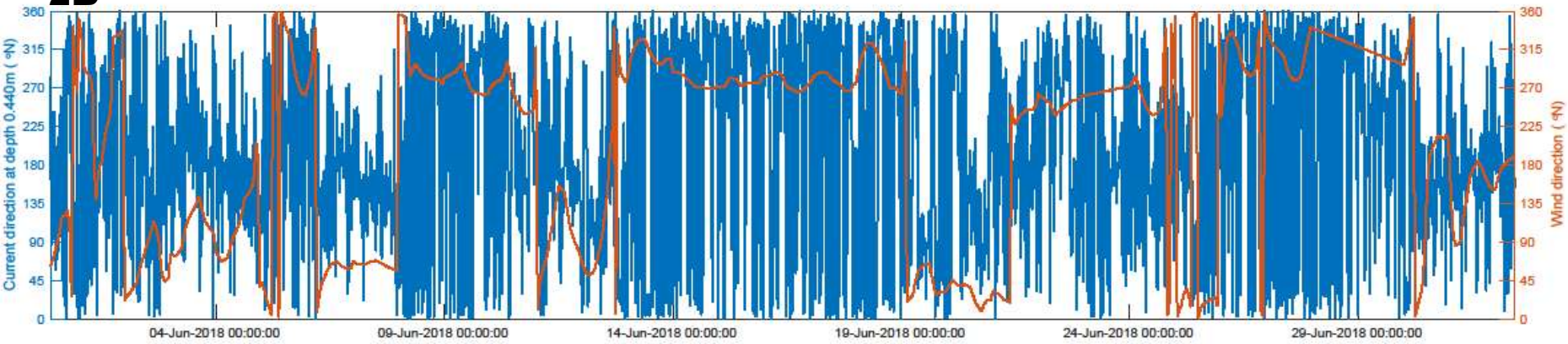
Appendix 3.3. Time series of A. magnitude and B. direction in the uppermost near-surface currents recorded by the acoustic Doppler current profiler (ADCP) at 20-minute intervals and winds at 10 m above sea level forecast by the 'MARIA/Eta' high-resolution atmospheric model at 3 hour temporal resolution for the ADCP deployment sites, 1 to 13, around the fish farm in Marsaxlokk Bay between May 2018 and August 2019



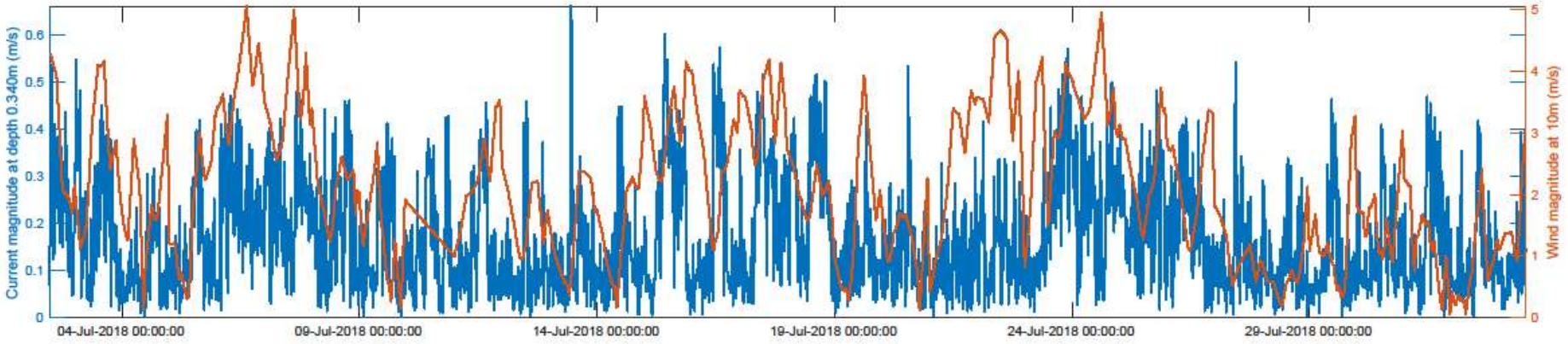
2A


Current magnitude at depth 0.440m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0.5
0
1
2
3
4
5
6
7
8
9
10
04-Jun-2018 00:00:00
09-Jun-2018 00:00:00
14-Jun-2018 00:00:00
19-Jun-2018 00:00:00
24-Jun-2018 00:00:00
29-Jun-2018 00:00:00

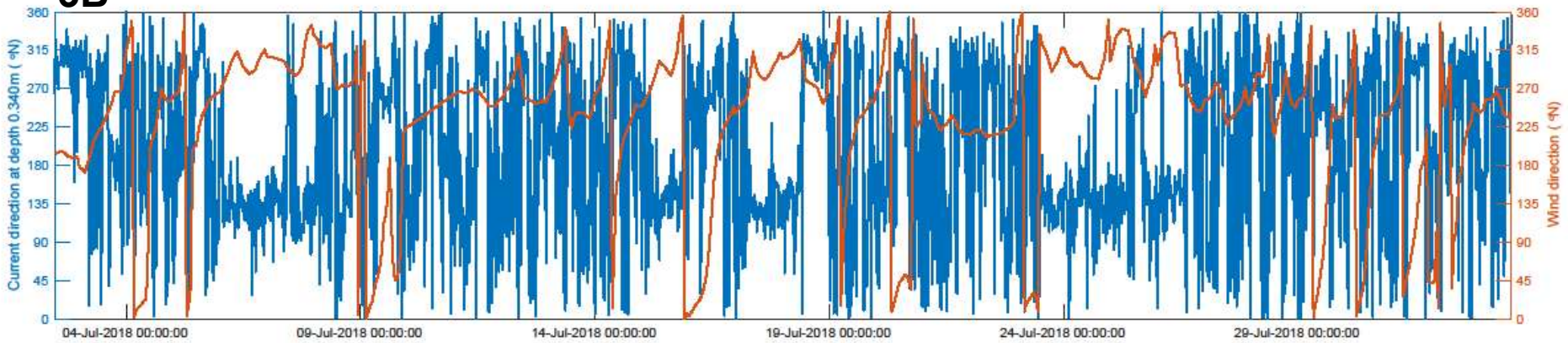
2B


Current direction at depth 0.440m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
04-Jun-2018 00:00:00
09-Jun-2018 00:00:00
14-Jun-2018 00:00:00
19-Jun-2018 00:00:00
24-Jun-2018 00:00:00
29-Jun-2018 00:00:00

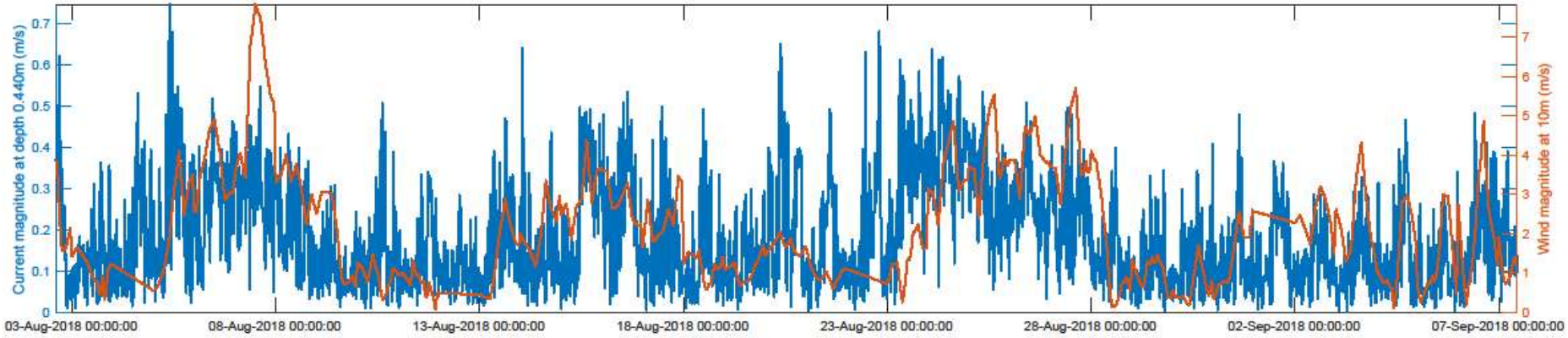
3A


Current magnitude at depth 0.340m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0.5
0.6
0
1
2
3
4
5
04-Jul-2018 00:00:00
09-Jul-2018 00:00:00
14-Jul-2018 00:00:00
19-Jul-2018 00:00:00
24-Jul-2018 00:00:00
29-Jul-2018 00:00:00

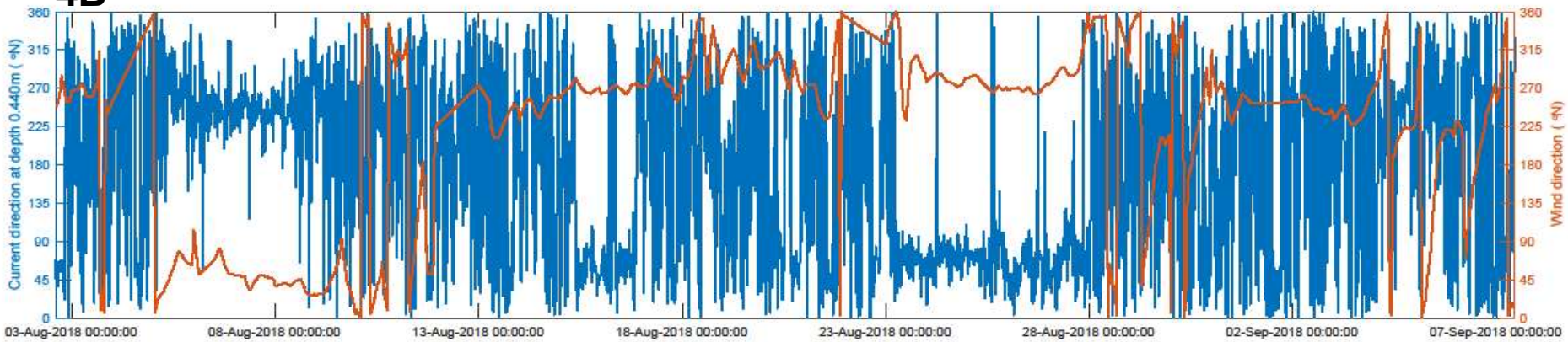
3B


Current direction at depth 0.340m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
04-Jul-2018 00:00:00
09-Jul-2018 00:00:00
14-Jul-2018 00:00:00
19-Jul-2018 00:00:00
24-Jul-2018 00:00:00
29-Jul-2018 00:00:00

4A


Current magnitude at depth 0.440m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
1
2
3
4
5
6
7
03-Aug-2018 00:00:00
08-Aug-2018 00:00:00
13-Aug-2018 00:00:00
18-Aug-2018 00:00:00
23-Aug-2018 00:00:00
28-Aug-2018 00:00:00
02-Sep-2018 00:00:00
07-Sep-2018 00:00:00

4B


Current direction at depth 0.440m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
03-Aug-2018 00:00:00
08-Aug-2018 00:00:00
13-Aug-2018 00:00:00
18-Aug-2018 00:00:00
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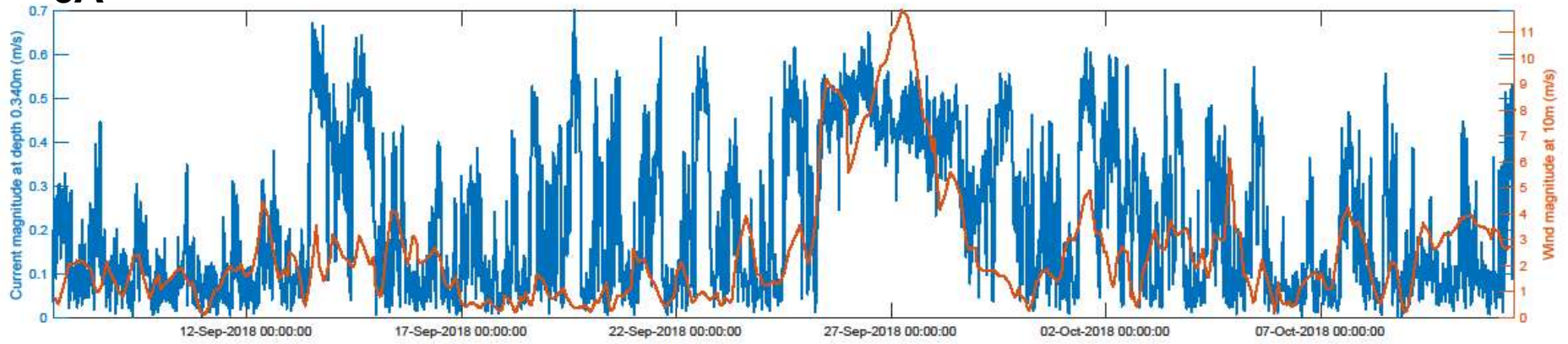
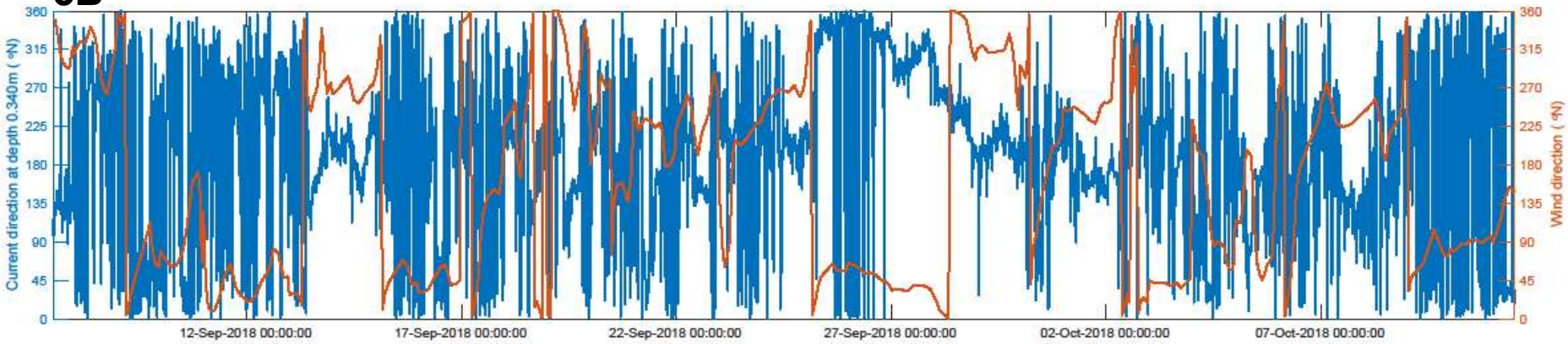
28-Aug-2018 00:00:00
02-Sep-2018 00:00:00
07-Sep-2018 00:00:00

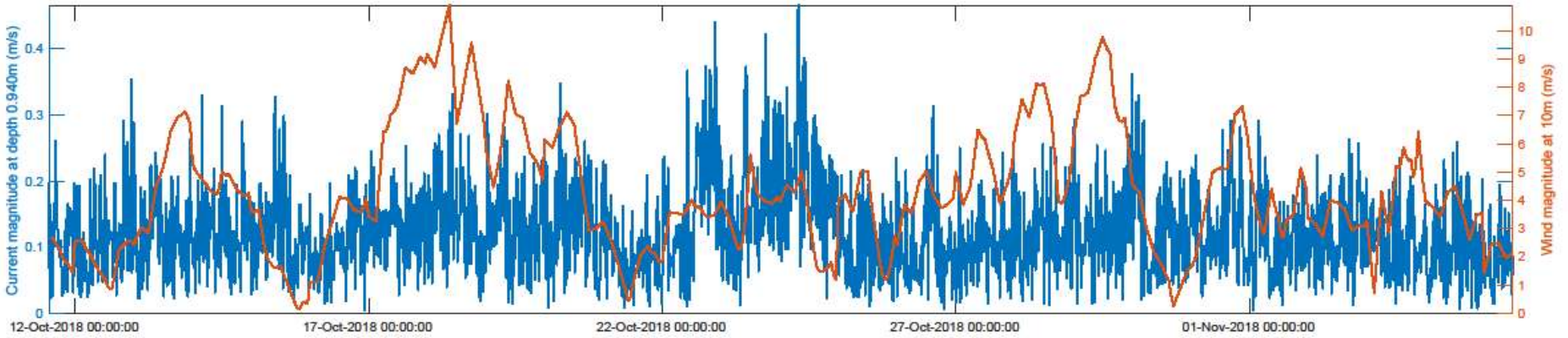
5A


Current magnitude at depth 0.340m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
1
2
3
4
5
6
7
8
9
10
11
12-Sep-2018 00:00:00
17-Sep-2018 00:00:00
22-Sep-2018 00:00:00
27-Sep-2018 00:00:00
02-Oct-2018 00:00:00
07-Oct-2018 00:00:00

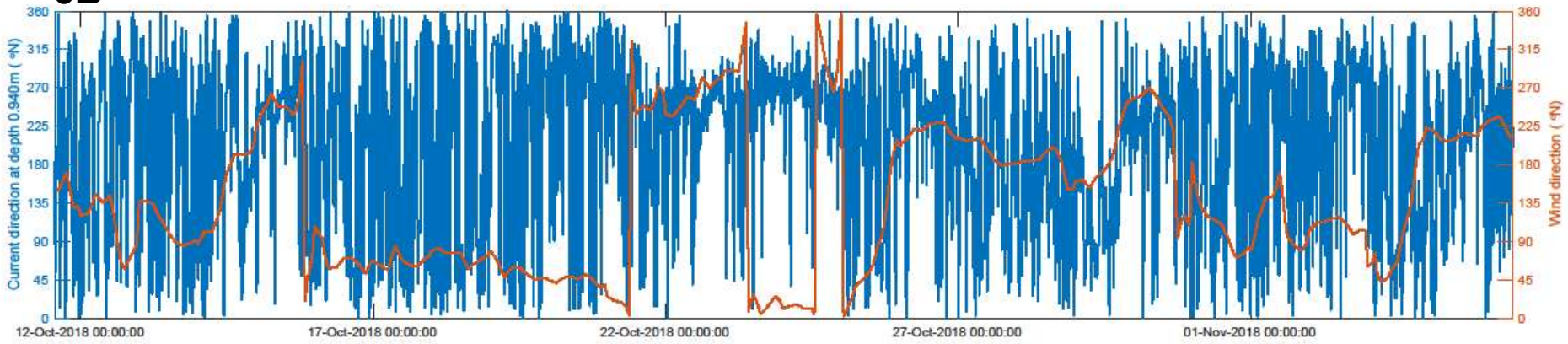
5B


Current direction at depth 0.340m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
12-Sep-2018 00:00:00
17-Sep-2018 00:00:00
22-Sep-2018 00:00:00
27-Sep-2018 00:00:00
02-Oct-2018 00:00:00
07-Oct-2018 00:00:00

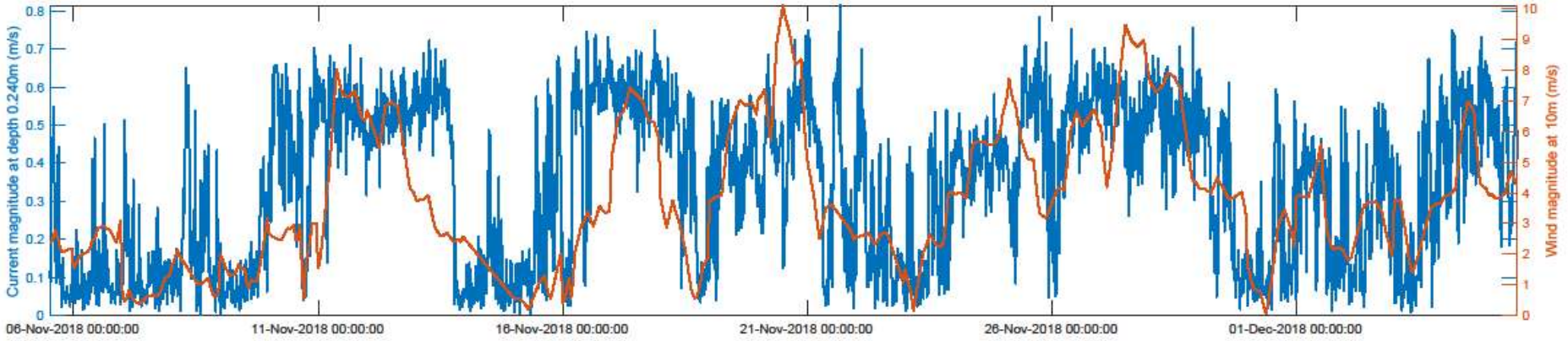
6A


Current magnitude at depth 0.940m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0
1
2
3
4
5
6
7
8
9
10
12-Oct-2018 00:00:00
17-Oct-2018 00:00:00
22-Oct-2018 00:00:00
27-Oct-2018 00:00:00
01-Nov-2018 00:00:00

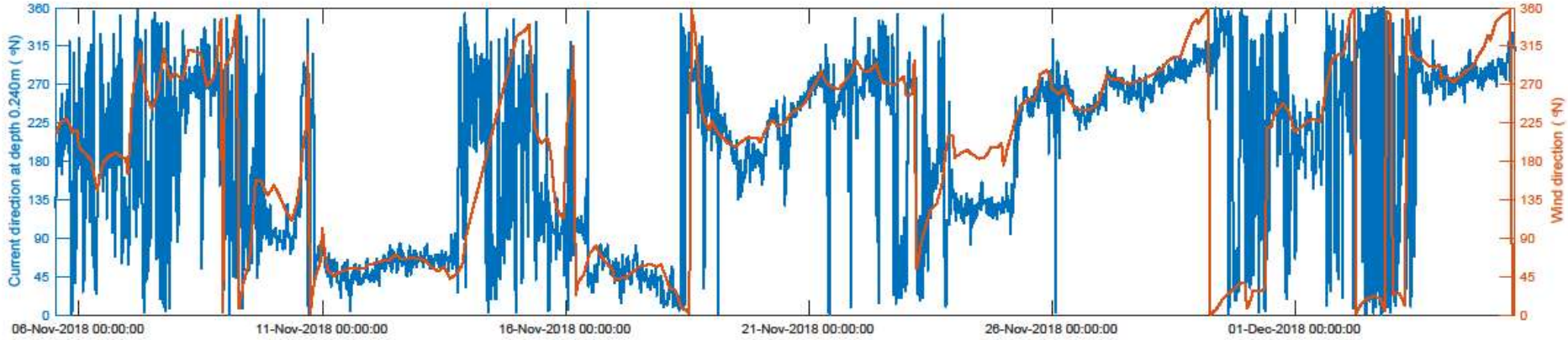
6B


Current direction at depth 0.940m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
12-Oct-2018 00:00:00
17-Oct-2018 00:00:00
22-Oct-2018 00:00:00
27-Oct-2018 00:00:00
01-Nov-2018 00:00:00

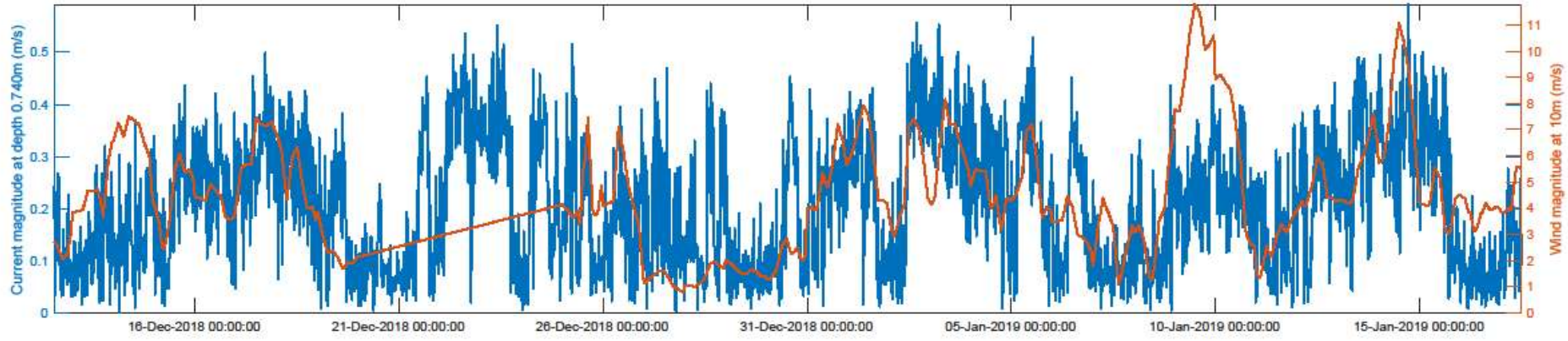
7A


Current magnitude at depth 0.240m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
1
2
3
4
5
6
7
8
9
10
06-Nov-2018 00:00:00
11-Nov-2018 00:00:00
16-Nov-2018 00:00:00
21-Nov-2018 00:00:00
26-Nov-2018 00:00:00
01-Dec-2018 00:00:00

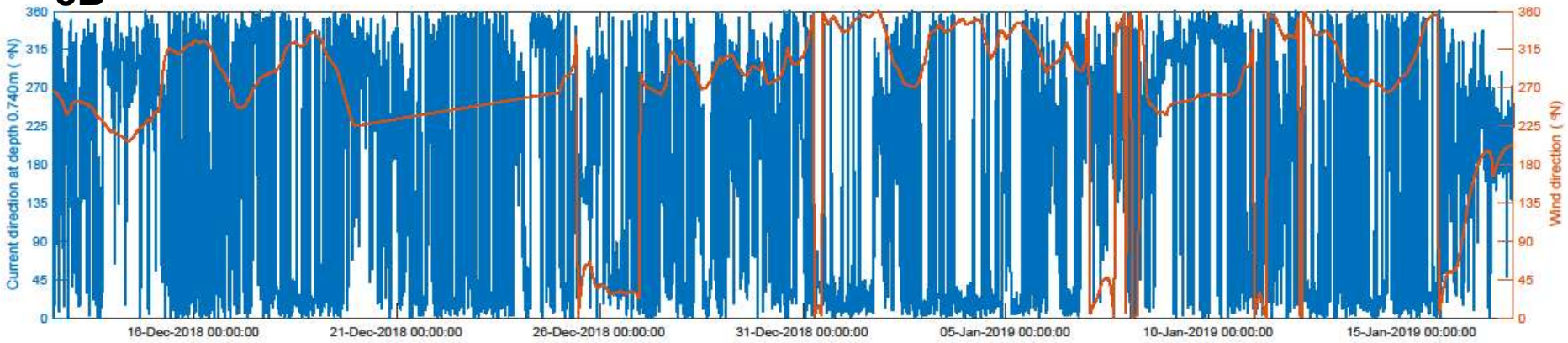
7B


Current direction at depth 0.240m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
06-Nov-2018 00:00:00
11-Nov-2018 00:00:00
16-Nov-2018 00:00:00
21-Nov-2018 00:00:00
26-Nov-2018 00:00:00
01-Dec-2018 00:00:00

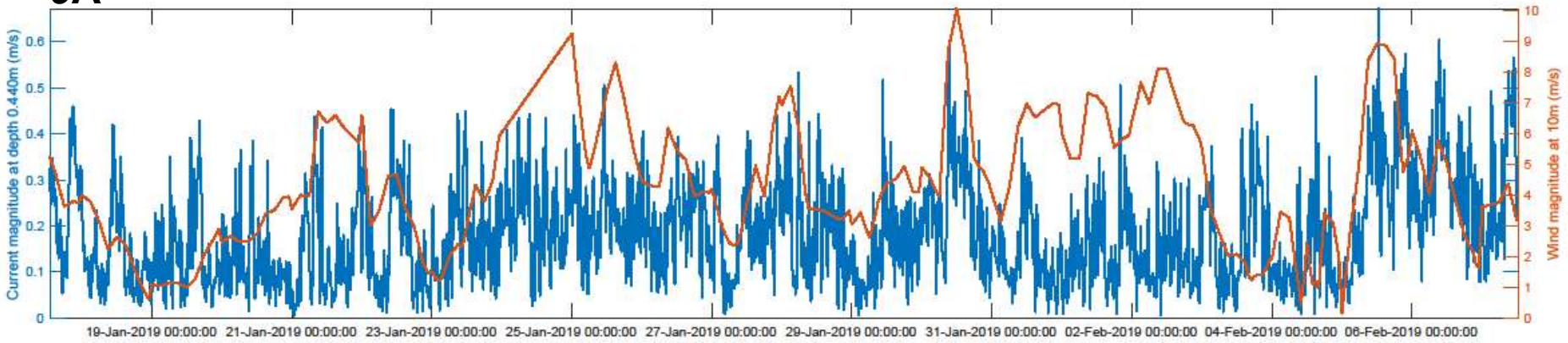
8A


Current magnitude at depth 0.740m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0.5
0
1
2
3
4
5
6
7
8
9
10
11
16-Dec-2018 00:00:00
21-Dec-2018 00:00:00
26-Dec-2018 00:00:00
31-Dec-2018 00:00:00
05-Jan-2019 00:00:00
10-Jan-2019 00:00:00
15-Jan-2019 00:00:00

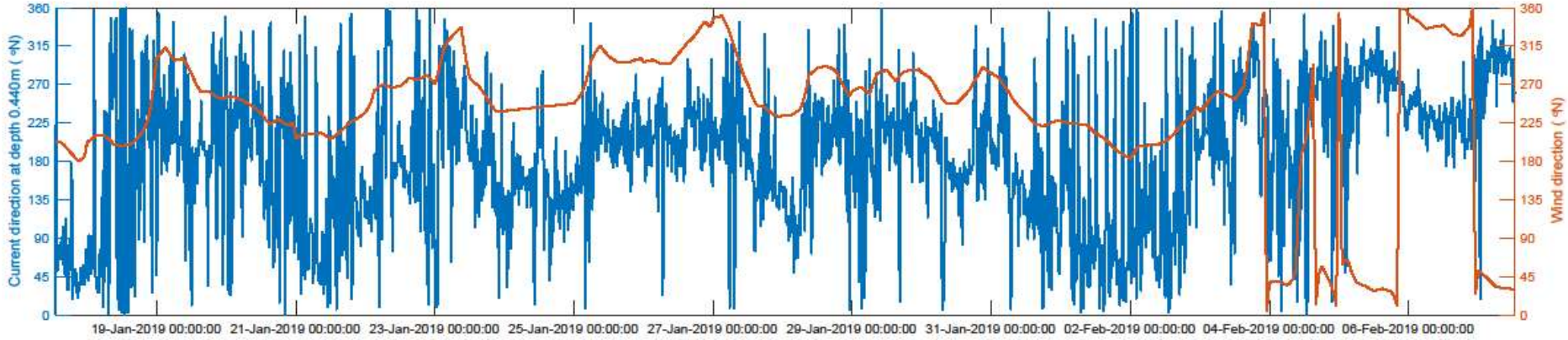
8B


Current direction at depth 0.740m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
16-Dec-2018 00:00:00
21-Dec-2018 00:00:00
26-Dec-2018 00:00:00
31-Dec-2018 00:00:00
05-Jan-2019 00:00:00
10-Jan-2019 00:00:00
15-Jan-2019 00:00:00

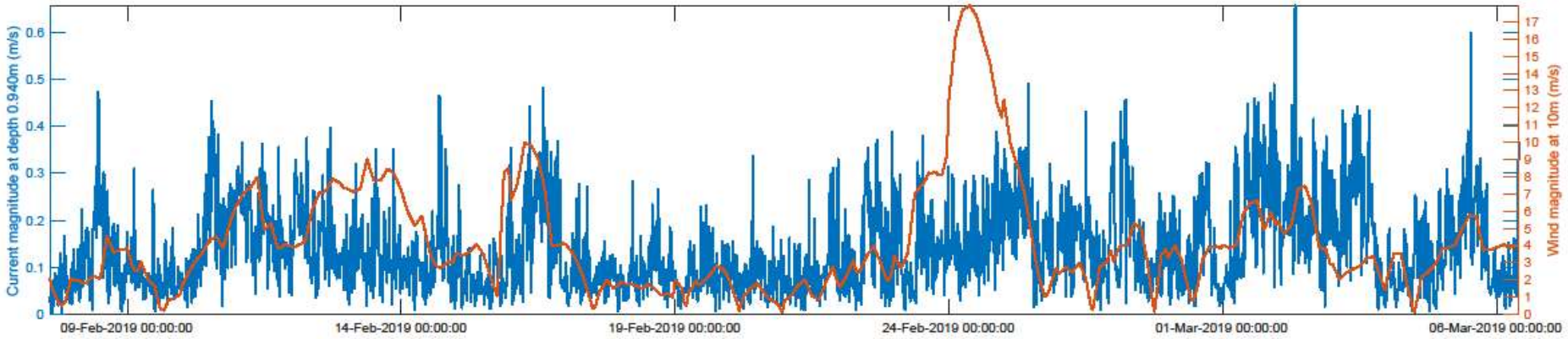
9A


Current magnitude at depth 0.440m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0.5
0.6
0
1
2
3
4
5
6
7
8
9
10
19-Jan-2019 00:00:00
21-Jan-2019 00:00:00
23-Jan-2019 00:00:00
25-Jan-2019 00:00:00
27-Jan-2019 00:00:00
29-Jan-2019 00:00:00
31-Jan-2019 00:00:00
02-Feb-2019 00:00:00
04-Feb-2019 00:00:00
06-Feb-2019 00:00:00

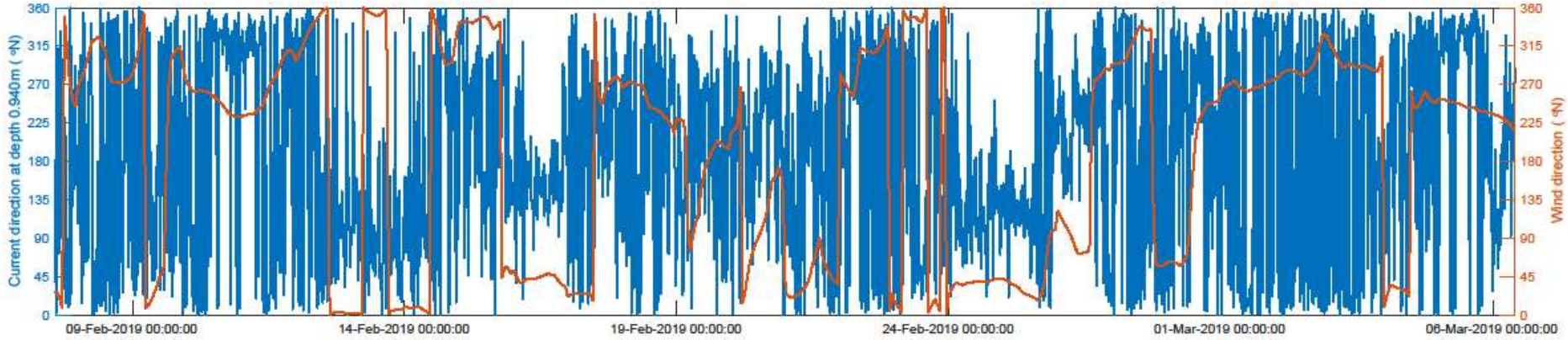
9B


Current direction at depth 0.440m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
19-Jan-2019 00:00:00
21-Jan-2019 00:00:00
23-Jan-2019 00:00:00
25-Jan-2019 00:00:00
27-Jan-2019 00:00:00
29-Jan-2019 00:00:00
31-Jan-2019 00:00:00
02-Feb-2019 00:00:00
04-Feb-2019 00:00:00
06-Feb-2019 00:00:00

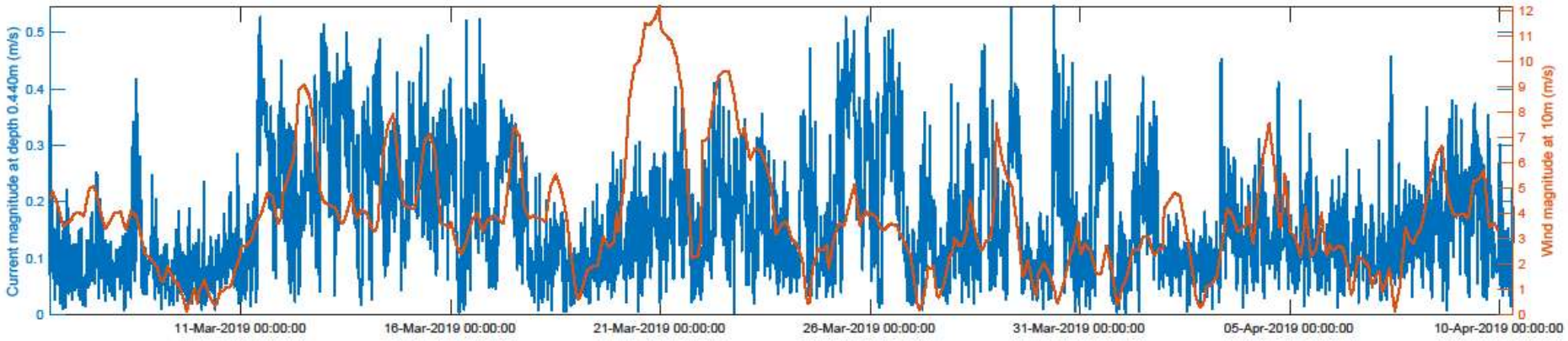
10A


Current magnitude at depth 0.940m (m/s)
Wind magnitude at 10m (m/s)
09-Feb-2019 00:00:00
14-Feb-2019 00:00:00
19-Feb-2019 00:00:00
24-Feb-2019 00:00:00
01-Mar-2019 00:00:00
06-Mar-2019 00:00:00

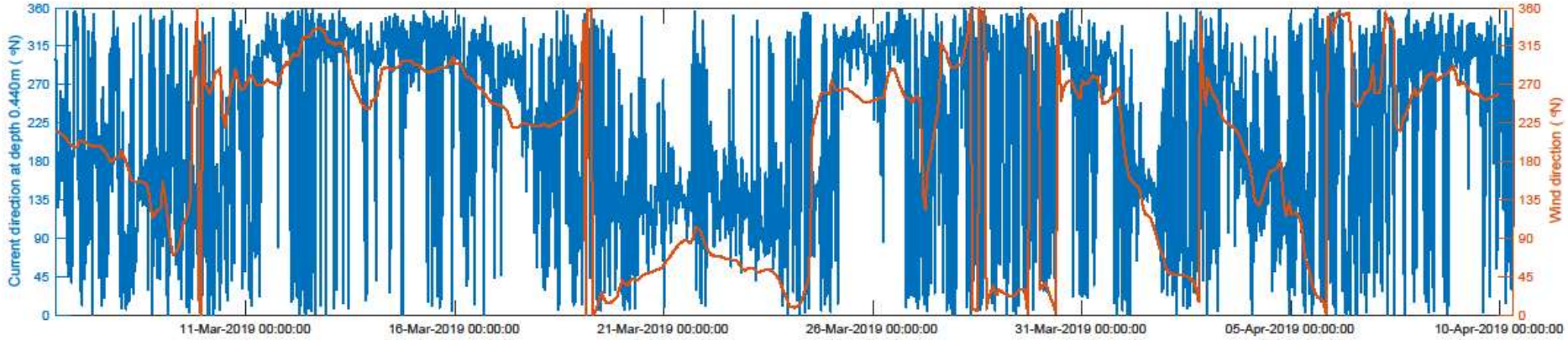
10B


Current direction at depth 0.940m (°N)
Wind direction (°N)
09-Feb-2019 00:00:00
14-Feb-2019 00:00:00
19-Feb-2019 00:00:00
24-Feb-2019 00:00:00
01-Mar-2019 00:00:00
06-Mar-2019 00:00:00

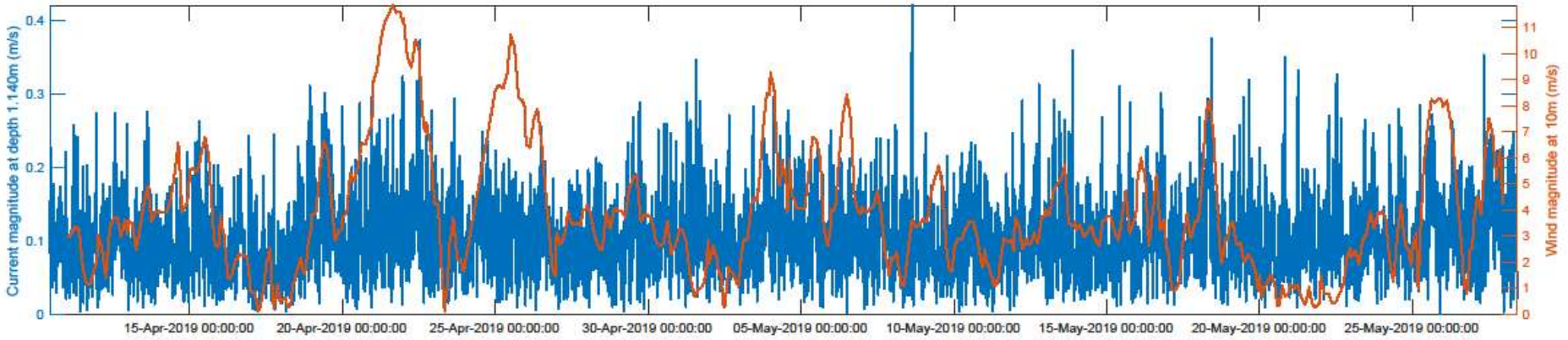
11A


Current magnitude at depth 0.440m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0.5
0
1
2
3
4
5
6
7
8
9
10
11
12
11-Mar-2019 00:00:00
16-Mar-2019 00:00:00
21-Mar-2019 00:00:00
26-Mar-2019 00:00:00
31-Mar-2019 00:00:00
05-Apr-2019 00:00:00
10-Apr-2019 00:00:00

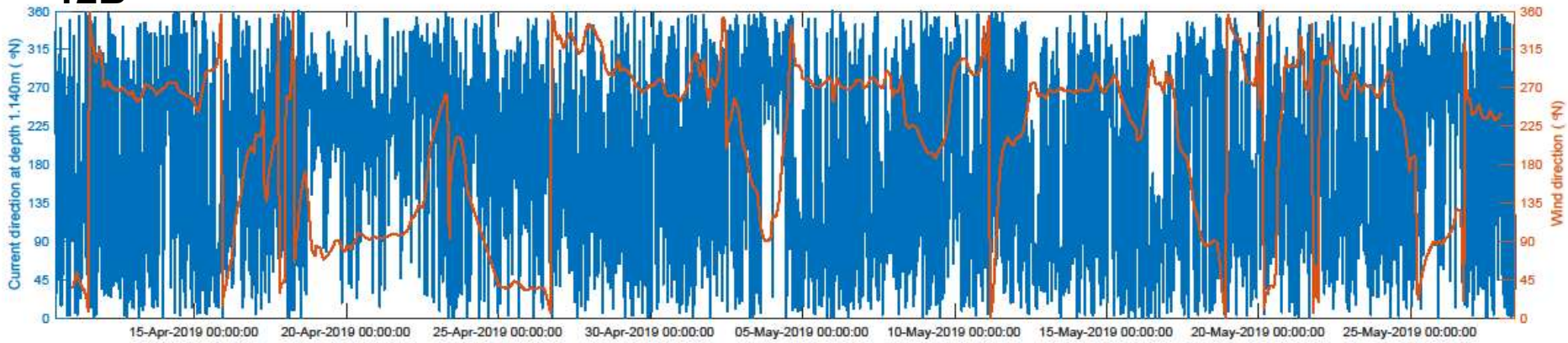
11B


Current direction at depth 0.440m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
11-Mar-2019 00:00:00
16-Mar-2019 00:00:00
21-Mar-2019 00:00:00
26-Mar-2019 00:00:00
31-Mar-2019 00:00:00
05-Apr-2019 00:00:00
10-Apr-2019 00:00:00

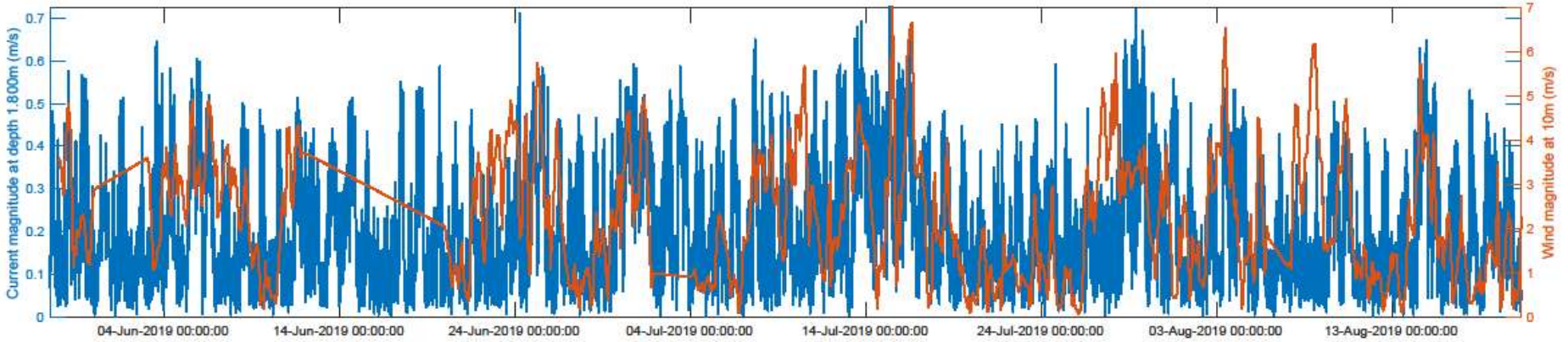
12A


Current magnitude at depth 1.140m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0
1
2
3
4
5
6
7
8
9
10
11
15-Apr-2019 00:00:00
20-Apr-2019 00:00:00
25-Apr-2019 00:00:00
30-Apr-2019 00:00:00
05-May-2019 00:00:00
10-May-2019 00:00:00
15-May-2019 00:00:00
20-May-2019 00:00:00
25-May-2019 00:00:00

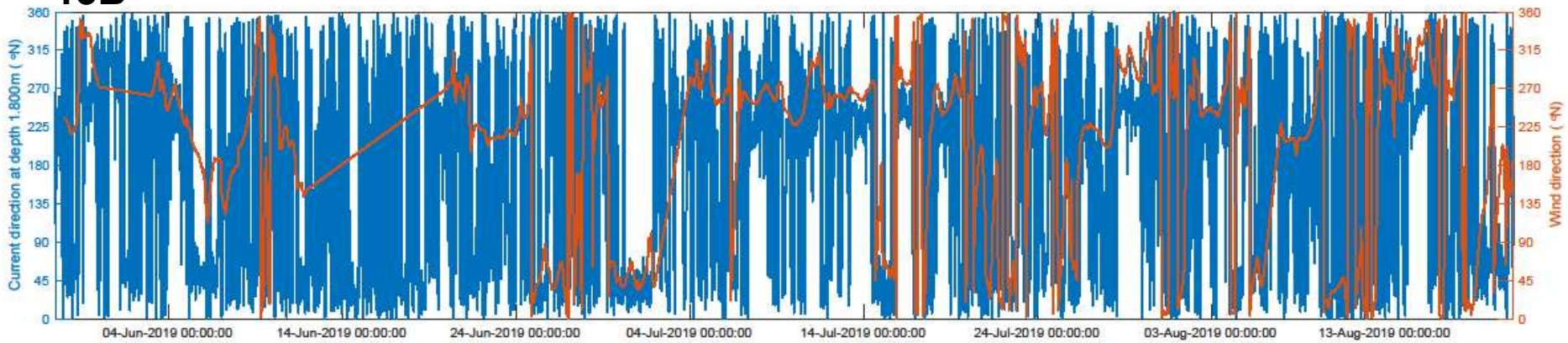
12B


Current direction at depth 1.140m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
15-Apr-2019 00:00:00
20-Apr-2019 00:00:00
25-Apr-2019 00:00:00
30-Apr-2019 00:00:00
05-May-2019 00:00:00
10-May-2019 00:00:00
15-May-2019 00:00:00
20-May-2019 00:00:00
25-May-2019 00:00:00

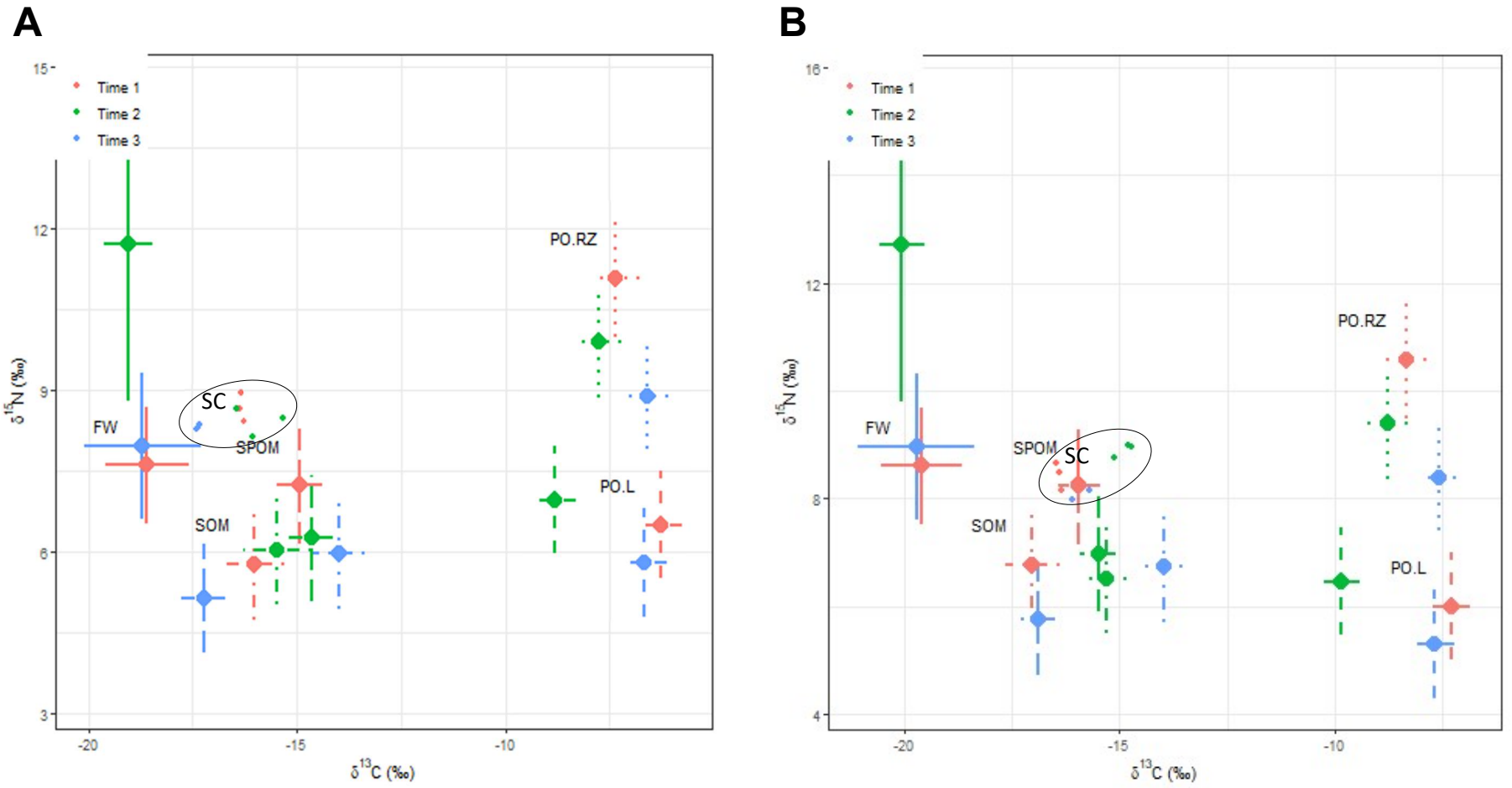
13A


Current magnitude at depth 1.800m (m/s)
Wind magnitude at 10m (m/s)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
1
2
3
4
5
6
7
04-Jun-2019 00:00:00
14-Jun-2019 00:00:00
24-Jun-2019 00:00:00
04-Jul-2019 00:00:00
14-Jul-2019 00:00:00
24-Jul-2019 00:00:00
03-Aug-2019 00:00:00
13-Aug-2019 00:00:00

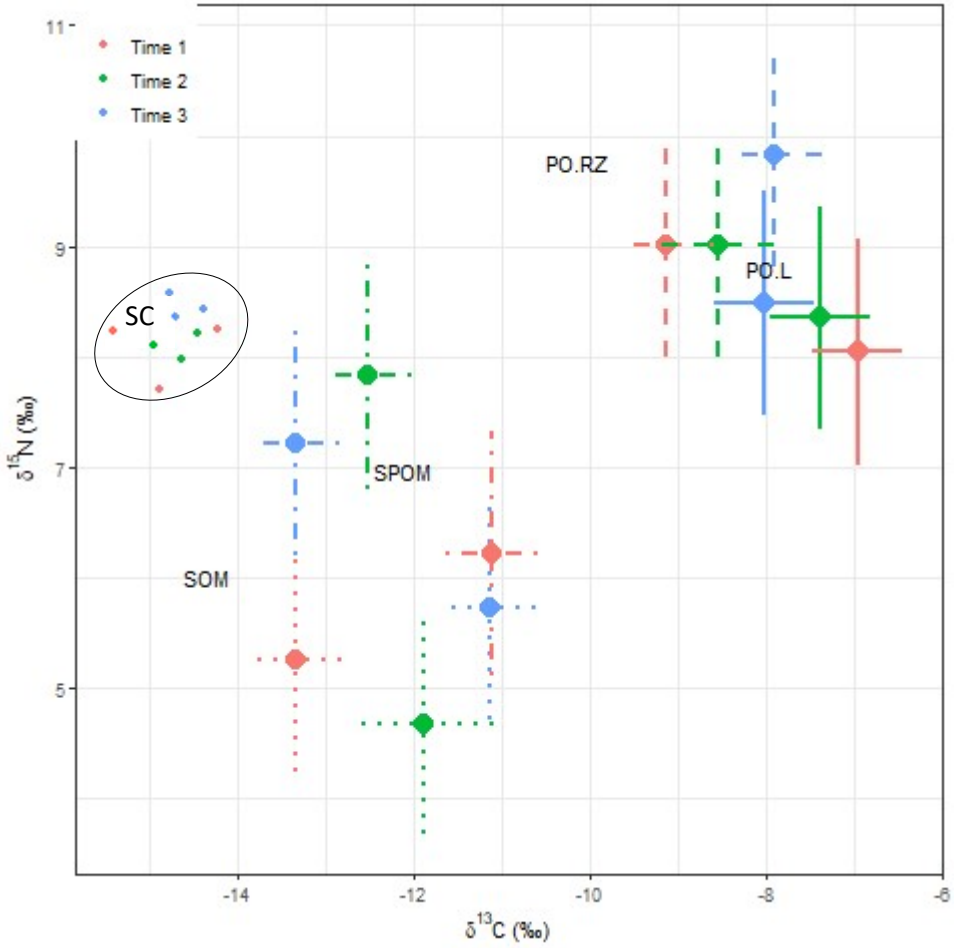
13B


Current direction at depth 1.800m (°N)
Wind direction (°N)
0
45
90
135
180
225
270
315
360
04-Jun-2019 00:00:00
14-Jun-2019 00:00:00
24-Jun-2019 00:00:00
04-Jul-2019 00:00:00
14-Jul-2019 00:00:00
24-Jul-2019 00:00:00
03-Aug-2019 00:00:00
13-Aug-2019 00:00:00

Appendix 6.1. Isospace plots for mean isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ (mean ± SD) of the different sampled sources and the sea cucumber consumer, *Holothuria poli*, where present in February (Time 1), May (Time 2), and September (Time 3), 2019, in A. E10, B. E25, and C. R2. in A. February, B. May, and C. September 2019. Standard deviation is indicated by error bars. Sample typologies represented by different symbol colours. SOM: sedimentary organic matter, SPOM: suspended particulate organic matter, SC: sea cucumbers, PO LV: *Posidonia oceanica* leaves; PO RZ: *Posidonia oceanica* rhizomes; FW: farm waste (fish faeces and uneaten fish feed).



C


Time 1
Time 2
Time 3
PO.RZ
PO.L
SC
SPOM
SOM
11
9
7
5
-14
-12
-10
-8
-6
δ15N (‰)
δ13C (‰)

Appendix 6.2. Lipid content and fatty acid composition of organic source groups in IMTA (E10 and E25) and at reference sites (R1 and R2), in February, May and September.

Suspended organic matter (SPOM)															
							February								
	E0			**E10**			**E25**			**R1**			**R2**		
n	3			3			3			3			3		
Lipid (mg 100g^{-1})	89.9	±	31.7	4.4	±	2.4	3.1	±	0.3	2.0	±	0.7	0.9	±	0.5
FA content (mg 100g^{-1})															
10:0	7.6	±	1.6	2.1	±	0.9	2.1	±	0.0	2.1	±	0.9	2.5	±	1.3
14:0	318.8	±	53.2	66.5	±	22.2	23.9	±	2.7	4.3	±	1.0	5.0	±	0.4
16:0	1579.4	±	283.3	368.4	±	64.6	162.9	±	11.7	15.0	±	2.2	18.3	±	1.1
18:0	290.3	±	78.2	122.7	±	8.0	66.3	±	3.8	2.6	±	1.6	4.9	±	3.1
20:0	40.9	±	7.3	15.7	±	1.2	10.0	±	0.1	0.3	±	0.1	0.6	±	0.2
22:0	20.6	±	4.7	9.9	±	0.3	7.1	±	0.2	0.5	±	0.3	0.3	±	0.2
***Total saturated*[1]**	***2425.3***	**±**	***308.3***	***659.9***	**±**	***101.9***	***321.4***	**±**	***20.1***	***46.9***	**±**	***4.0***	***58.9***	**±**	***4.5***
16:1*n*-7	317.5	±	57.3	75.2	±	22.3	32.7	±	1.1	9.4	±	0.2	11.9	±	0.5
18:1*n*-9	1941.1	±	358.2	494.2	±	73.5	221.7	±	17.2	6.7	±	3.5	6.3	±	1.1
18:1*n*-7	209.6	±	19.0	67.7	±	9.3	37.3	±	2.5	8.1	±	0.4	10.0	±	0.5
20:1*n*-9	156.9	±	41.3	35.2	±	6.2	17.2	±	1.3	0.2	±	0.2	0.2	±	0.1
22:1*n*-9	25.4	±	5.9	6.0	±	0.5	3.4	±	0.1	0.2	±	0.1	0.1	±	0.2
Total monoenes	***2650.4***	**±**	***307.9***	***678.2***	**±**	***93.6***	***312.3***	**±**	***22.0***	***24.7***	**±**	***3.3***	***28.5***	**±**	***2.1***
18:2*n*-6	1398.5	±	457.5	147.9	±	20.0	71.2	±	2.5	2.6	±	2.0	2.1	±	0.1
20:2*n*-6	32.1	±	8.8	10.9	±	0.8	5.6	±	0.9	0.0	±	0.0	0.0	±	0.0

20:4*n*-6	46.0	±	9.4	8.6	±	0.5	7.8	±	0.8	1.4	±	0.4	2.7	±	1.2
Total n-6 PUFA	***1476.6***	**±**	***475.0***	***167.3***	**±**	***20.9***	***84.5***	**±**	***2.8***	***4.1***	**±**	***2.0***	***4.8***	**±**	***1.1***
18:3*n*-3	292.1	±	91.7	19.3	±	3.6	12.2	±	1.4	0.3	±	0.4	0.9	±	0.2
20:5*n*-3 (EPA)	495.2	±	81.7	28.2	±	4.8	21.6	±	3.5	5.1	±	2.2	6.6	±	2.8
22:5*n*-3 (DPA)	245.3	±	56.4	31.1	±	3.6	16.0	±	1.1	1.7	±	1.1	0.9	±	0.5
22:6*n*-3 (DHA)	577.4	±	121.0	37.5	±	6.8	22.5	±	3.9	3.7	±	1.3	4.8	±	0.7
Total n-3 PUFA	1610.0	±	328.7	116.1	±	5.1	72.2	±	8.0	10.8	±	4.5	13.2	±	3.1
Total PUFA	***3086.5***	**±**	***785.0***	***283.4***	**±**	***25.4***	***156.8***	**±**	***6.7***	***14.9***	**±**	***6.5***	***18.0***	**±**	***3.8***

Suspended organic matter (SPOM)														
May														
	E0			**E10**			**E25**			**R1**			**R2**	
n	3			3			3			3			3	
Lipid (mg 100g^{-1})	25.8	±	21.1	1.3	±	0.1	1.2	±	0.1	0.7	±	0.1	0.8	±	0.1
FA content (mg 100g^{-1})														
10:0	5.6	±	0.5	4.6	±	1.2	2.2	±	0.4	2.2	±	1.0	2.4	±	0.7
14:0	589.9	±	32.8	62.1	±	13.9	13.4	±	0.9	5.8	±	0.6	6.2	±	0.1
16:0	2260.9	±	161.7	317.2	±	65.7	74.1	±	4.5	23.2	±	2.5	23.0	±	1.2
18:0	600.4	±	39.7	105.7	±	8.1	24.2	±	3.9	3.3	±	0.6	3.6	±	2.8
20:0	110.4	±	10.8	20.8	±	4.1	4.6	±	0.2	0.8	±	0.1	0.7	±	0.1
22:0	50.5	±	5.3	10.2	±	2.2	2.3	±	0.1	0.0	±	0.0	0.0	±	0.0
Total saturated[1]	***3885.1***	**±**	***265.1***	***582.7***	**±**	***105.0***	***153.3***	**±**	***9.5***	***61.6***	**±**	***3.9***	***61.7***	**±**	***3.8***
16:1*n*-7	259.0	±	12.4	43.3	±	6.7	21.4	±	0.8	14.9	±	0.2	16.5	±	0.6
18:1*n*-9	793.9	±	60.4	179.1	±	33.0	67.3	±	2.4	5.6	±	0.9	5.0	±	0.3

18:1*n*-7	205.4	±	8.4	42.0	±	5.4	19.6	±	0.4	10.5	±	0.6	10.9	±	0.3
20:1*n*-9	159.4	±	3.9	20.9	±	4.6	5.9	±	0.5	0.2	±	0.0	0.0	±	0.0
22:1*n*-9	41.0	±	1.7	5.4	±	1.5	0.9	±	0.4	0.2	±	0.1	0.3	±	0.0
Total monoenes	***1458.7***	***±***	***83.9***	***290.7***	***±***	***51.0***	***115.0***	***±***	***2.9***	***31.5***	***±***	***1.5***	***32.7***	***±***	***1.0***
18:2*n*-6	374.6	±	62.3	65.2	±	12.1	29.1	±	10.8	1.4	±	0.2	1.1	±	0.2
20:2*n*-6	17.9	±	1.6	3.9	±	1.0	1.4	±	0.4	0.0	±	0.0	0.0	±	0.0
20:4*n*-6	49.0	±	5.2	7.7	±	2.0	6.3	±	1.1	4.0	±	0.9	4.5	±	0.8
Total n-6 PUFA	***441.4***	***±***	***68.9***	***76.8***	***±***	***14.2***	***36.8***	***±***	***11.7***	***5.4***	***±***	***1.0***	***5.6***	***±***	***0.9***
18:3*n*-3	52.1	±	9.1	11.0	±	2.3	4.1	±	0.8	0.3	±	0.1	0.2	±	0.1
20:5*n*-3 (EPA)	215.3	±	54.7	30.5	±	6.1	18.0	±	2.7	10.5	±	1.7	6.5	±	2.0
22:5*n*-3 (DPA)	343.7	±	11.3	36.2	±	7.2	7.3	±	0.6	0.3	±	0.1	0.3	±	0.1
22:6*n*-3 (DHA)	444.1	±	84.3	42.6	±	10.6	14.6	±	3.6	6.9	±	1.9	5.7	±	0.9
Total n-3 PUFA	***1055.2***	***±***	***150.0***	***120.4***	***±***	***23.1***	***44.0***	***±***	***7.5***	***18.1***	***±***	***3.4***	***12.8***	***±***	***2.9***
Total PUFA	***1496.6***	***±***	***218.7***	***197.2***	***±***	***37.3***	***80.9***	***±***	***17.2***	***23.5***	***±***	***4.4***	***18.4***	***±***	***2.5***

Suspended organic matter (SPOM)															
September															
	E0			**E10**			**E25**			**R1**			**R2**		
n	3			3			3			3			3		
Lipid (mg 100g^{-1})	5.4	±	0.8	1.5	±	0.7	0.9	±	0.5	1.0	±	0.5	1.0	±	0.1
FA content (mg 100g^{-1})															
10:0	0.0	±	0.0	0.1	±	0.0	0.4	±	0.1	5.1	±	3.5	1.9	±	1.6
14:0	4.0	±	0.1	2.7	±	0.1	3.8	±	0.1	5.3	±	0.6	6.4	±	1.7
16:0	25.4	±	0.4	20.0	±	0.6	23.5	±	0.6	20.2	±	5.2	19.3	±	15.7

18:0	8.1	±	0.8	9.6	±	0.1	10.9	±	0.2	16.1	±	8.2	24.5	±	19.6
20:0	1.0	±	0.1	1.5	±	0.0	1.8	±	0.2	0.9	±	0.8	1.3	±	0.6
22:0	1.7	±	0.1	3.7	±	0.1	4.5	±	0.2	0.5	±	0.3	0.0	±	0.1
Total saturated[1]	***44.4***	***±***	***1.7***	***43.2***	***±***	***0.9***	***52.6***	***±***	***0.7***	***61.7***	***±***	***8.3***	***61.7***	***±***	***6.2***
16:1*n*-7	1.9	±	0.1	2.9	±	0.1	4.2	±	0.0	8.2	±	2.5	7.9	±	1.2
18:1*n*-9	27.2	±	1.8	24.3	±	1.5	16.9	±	0.4	4.0	±	0.4	2.5	±	1.2
18:1*n*-7	3.4	±	0.0	3.1	±	0.0	4.1	±	0.1	8.2	±	1.9	13.9	±	1.0
20:1*n*-9	2.8	±	0.1	2.2	±	0.0	1.5	±	0.0	0.2	±	0.0	0.4	±	0.4
22:1*n*-9	0.5	±	0.0	0.5	±	0.0	0.3	±	0.0	0.3	±	0.1	0.0	±	0.0
Total monoenes	***35.9***	***±***	***1.8***	***33.0***	***±***	***1.3***	***26.9***	***±***	***0.5***	***21.0***	***±***	***4.6***	***24.6***	***±***	***0.6***
18:2*n*-6	12.3	±	0.1	14.3	±	0.6	10.3	±	0.1	1.9	±	0.8	1.7	±	0.5
20:2*n*-6	0.5	±	0.1	0.4	±	0.0	0.3	±	0.0	0.1	±	0.1	0.0	±	0.0
20:4*n*-6	0.2	±	0.0	0.4	±	0.0	0.7	±	0.1	1.4	±	0.7	1.6	±	0.7
Total n-6 PUFA	***13.0***	***±***	***0.0***	***15.0***	***±***	***0.6***	***11.3***	***±***	***0.1***	***3.4***	***±***	***0.5***	***3.2***	***±***	***1.2***
18:3*n*-3	1.8	±	0.0	2.0	±	0.1	1.6	±	0.3	1.0	±	1.3	0.0	±	0.0
20:5*n*-3 (EPA)	1.1	±	0.1	1.9	±	0.1	2.2	±	0.2	5.8	±	2.1	2.7	±	2.5
22:5*n*-3 (DPA)	1.9	±	0.0	2.0	±	0.1	2.2	±	0.0	1.4	±	0.6	3.5	±	3.5
22:6*n*-3 (DHA)	1.9	±	0.1	2.9	±	0.1	3.0	±	0.2	5.7	±	2.3	4.2	±	2.1
Total n-3 PUFA	***6.7***	***±***	***0.2***	***8.8***	***±***	***0.3***	***9.1***	***±***	***0.7***	***13.9***	***±***	***3.8***	***10.4***	***±***	***6.4***
Total PUFA	***19.7***	***±***	***0.2***	***23.8***	***±***	***0.9***	***20.4***	***±***	***0.8***	***17.3***	***±***	***3.9***	***13.6***	***±***	***5.6***

Sedimentary organic matter (SOM)					
February					
	E0	**E10**	**E25**	**R1**	**R2**
n	2	2	2	2	2

Lipid (mg $100g^{-1}$)	4.2	±	1.9	4.1	±	2.6	1.1	±	0.1	0.5	±	0.2	1.4	±	1.3
FA content (mg $100g^{-1}$)															
10:0	0.2	±	0.1	0.3	±	0.1	0.1	±	0.1	0.1	±	0.0	0.2	±	0.0
14:0	7.9	±	4.8	13.0	±	12.7	3.5	±	2.0	0.6	±	0.0	8.5	±	11.7
16:0	46.8	±	24.2	94.4	±	97.1	33.1	±	19.4	4.0	±	2.5	4.3	±	1.2
18:0	15.0	±	4.1	33.5	±	28.2	14.5	±	9.8	2.6	±	0.3	7.0	±	4.0
20:0	2.8	±	2.0	3.0	±	4.1	0.8	±	0.4	0.1	±	0.0	1.0	±	0.2
22:0	2.0	±	2.4	2.5	±	3.0	0.7	±	0.3	1.0	±	0.1	2.3	±	2.0
Total saturated[1]	***102.3***	***±***	***49.3***	***176.1***	***±***	***168.1***	***66.4***	***±***	***34.7***	***10.9***	***±***	***2.7***	***47.3***	***±***	***36.2***
16:1*n*-7	16.0	±	4.1	21.7	±	16.4	7.6	±	3.8	2.0	±	0.5	14.9	±	16.1
18:1*n*-9	50.8	±	29.8	209.0	±	259.2	72.8	±	55.9	0.5	±	0.1	102.5	±	144.3
18:1*n*-7	17.3	±	5.9	34.7	±	36.6	13.5	±	6.7	2.3	±	0.2	22.2	±	26.7
20:1*n*-9	4.7	±	3.2	19.8	±	24.8	6.4	±	4.6	0.0	±	0.0	10.2	±	14.4
22:1*n*-9	0.5	±	0.2	2.0	±	2.0	0.4	±	0.1	0.3	±	0.4	0.8	±	1.1
Total monoenes	***89.3***	***±***	***43.1***	***287.2***	***±***	***338.9***	***100.7***	***±***	***70.8***	***5.2***	***±***	***1.2***	***150.5***	***±***	***202.6***
18:2*n*-6	21.7	±	13.4	116.4	±	145.0	43.3	±	33.7	0.4	±	0.1	52.1	±	73.6
20:2*n*-6	3.8	±	3.7	32.5	±	40.7	16.1	±	13.2	0.0	±	0.0	16.8	±	23.3
20:4*n*-6	6.2	±	6.2	26.3	±	30.6	10.7	±	6.9	0.5	±	0.2	13.0	±	16.8
Total n-6 PUFA	***31.7***	***±***	***23.3***	***175.2***	***±***	***216.3***	***70.1***	***±***	***53.8***	***0.8***	***±***	***0.2***	***81.9***	***±***	***113.7***
18:3*n*-3	4.8	±	3.6	21.0	±	26.5	6.4	±	5.3	0.0	±	0.0	10.2	±	13.8
20:5*n*-3 (EPA)	13.7	±	12.4	43.2	±	47.8	18.3	±	11.2	0.6	±	0.7	25.8	±	34.8
22:5*n*-3 (DPA)	8.2	±	6.3	19.7	±	18.8	5.6	±	3.3	0.5	±	0.7	9.2	±	11.9
22:6*n*-3 (DHA)	8.9	±	9.0	36.1	±	45.7	12.3	±	7.5	0.2	±	0.1	14.2	±	19.5
Total n-3 PUFA	***35.6***	***±***	***31.4***	***120.0***	***±***	***138.8***	***42.6***	***±***	***27.4***	***1.3***	***±***	***0.1***	***59.4***	***±***	***80.0***

Total PUFA	***67.3***	***±***	***54.6***	***295.2***	***±***	***355.1***	***112.8***	***±***	***81.2***	***2.2***	***±***	***0.1***	***141.3***	***±***	***193.7***
Sedimentary organic matter (SOM)															
May															
	E0			**E10**			**E25**			**R1**			**R2**		
n	3			3			3			3			3		
Lipid (mg 100g^{-1})	0.6	±	0.2	1.1	±	0.2	1.3	±	0.6	0.6	±	0.3	1.1	±	0.5
FA content (mg 100g^{-1})															
10:0	0.1	±	0.1	0.1	±	0.1	0.1	±	0.0	0.2	±	0.2	0.1	±	0.1
14:0	8.0	±	1.9	6.9	±	1.3	3.2	±	2.5	0.5	±	0.3	1.8	±	0.5
16:0	41.9	±	9.6	43.5	±	11.2	23.0	±	19.8	2.7	±	0.6	8.0	±	1.6
18:0	23.9	±	3.1	18.0	±	11.5	13.4	±	9.5	2.6	±	2.1	1.3	±	0.5
20:0	1.6	±	0.7	1.4	±	0.4	0.4	±	0.1	0.7	±	0.3	0.2	±	0.1
22:0	0.3	±	0.0	0.2	±	0.0	0.5	±	0.1	0.9	±	0.1	0.9	±	0.0
Total saturated[1]	***92.4***	***±***	***17.9***	***89.8***	***±***	***25.9***	***54.0***	***±***	***36.6***	***10.9***	***±***	***3.3***	***22.7***	***±***	***4.4***
16:1*n*-7	9.7	±	1.5	11.4	±	1.2	4.9	±	2.7	0.9	±	0.1	6.2	±	1.2
18:1*n*-9	21.5	±	3.3	49.9	±	15.2	26.5	±	24.5	0.5	±	0.4	1.4	±	0.7
18:1*n*-7	11.6	±	1.5	16.7	±	2.3	9.3	±	6.2	2.3	±	0.1	3.6	±	0.7
20:1*n*-9	2.7	±	0.7	6.7	±	2.4	4.4	±	4.8	0.0	±	0.0	0.0	±	0.0
22:1*n*-9	0.2	±	0.1	0.2	±	0.2	0.3	±	0.1	0.0	±	0.0	0.0	±	0.0
Total monoenes	***45.7***	***±***	***6.8***	***85.0***	***±***	***20.4***	***45.3***	***±***	***38.0***	***3.7***	***±***	***0.3***	***11.2***	***±***	***2.4***
18:2*n*-6	10.0	±	1.0	26.8	±	9.1	12.6	±	10.3	0.1	±	0.1	0.7	±	0.3
20:2*n*-6	1.9	±	0.2	13.1	±	5.1	6.0	±	7.1	0.0	±	0.0	0.0	±	0.0
20:4*n*-6	2.9	±	0.3	12.8	±	4.0	6.9	±	7.3	0.2	±	0.2	1.6	±	1.2

Total n-6 PUFA	***14.8***	***±***	***0.7***		***52.7***	***±***	***18.0***		***25.5***	***±***	***24.6***		***0.3***	***±***	***0.2***		***2.4***	***±***	***1.5***
18:3*n*-3	1.2	±	0.3		3.8	±	1.1		2.1	±	1.5		0.0	±	0.0		0.1	±	0.1
20:5*n*-3 (EPA)	9.3	±	1.4		20.9	±	5.4		9.6	±	8.0		0.2	±	0.1		3.7	±	1.4
22:5*n*-3 (DPA)	5.7	±	1.4		9.2	±	2.7		7.5	±	6.7		0.2	±	0.4		0.4	±	0.7
22:6*n*-3 (DHA)	7.7	±	1.9		15.5	±	5.3		7.4	±	5.6		0.0	±	0.0		0.8	±	0.3
Total n-3 PUFA	***23.9***	***±***	***4.9***		***49.5***	***±***	***13.8***		***26.6***	***±***	***21.6***		***0.4***	***±***	***0.3***		***5.0***	***±***	***2.1***
Total PUFA	***38.8***	***±***	***5.5***		***102.2***	***±***	***31.5***		***52.1***	***±***	***46.1***		***0.7***	***±***	***0.5***		***7.4***	***±***	***3.5***

Sedimentary organic matter (SOM)												
September												
	E25					**R1**				**R2**		
n	2					3				3		
Lipid (mg 100g^{-1})	1.3	±	0.5			0.8	±	0.2		1.3	±	0.6
FA content (mg 100g^{-1})												
10:0	0.2	±	0.0			0.1	±	0.1		0.1	±	0.1
14:0	2.4	±	0.3			1.9	±	0.2		2.2	±	0.8
16:0	16.1	±	0.7			13.4	±	4.2		9.0	±	4.7
18:0	13.5	±	0.6			14.1	±	12.3		3.6	±	2.9
20:0	0.0	±	0.0			0.0	±	0.0		0.0	±	0.0
22:0	0.6	±	0.1			0.0	±	0.0		1.0	±	0.0
Total saturated[1]	***41.6***	***±***	***4.8***			***36.6***	***±***	***15.7***		***29.1***	***±***	***10.3***
16:1*n*-7	4.9	±	0.4			5.5	±	0.9		5.8	±	2.5
18:1*n*-9	6.5	±	2.5			1.1	±	0.3		1.9	±	0.6
18:1*n*-7	6.4	±	0.6			3.5	±	0.7		5.0	±	1.3

20:1*n*-9	0.1	±	0.1	0.0	±	0.0	0.0	± 0.0
22:1*n*-9	0.0	±	0.0	0.0	±	0.0	0.0	± 0.0
Total monoenes	***17.9***	**±**	***1.6***	***10.0***	**±**	***1.1***	***12.8***	**±** ***4.2***
18:2*n*-6	4.1	±	1.9	1.0	±	0.4	0.9	± 0.4
20:2*n*-6	0.1	±	0.1	0.0	±	0.0	0.0	± 0.0
20:4*n*-6	2.3	±	0.7	1.6	±	0.3	2.4	± 0.9
Total n-6 PUFA	***6.5***	**±**	***2.7***	***2.6***	**±**	***0.3***	***3.3***	**±** ***1.2***
18:3*n*-3	1.0	±	0.1	0.0	±	0.0	0.8	± 0.7
20:5*n*-3 (EPA)	2.3	±	1.7	3.7	±	1.1	3.7	± 1.5
22:5*n*-3 (DPA)	0.7	±	1.0	0.0	±	0.0	0.9	± 0.8
22:6*n*-3 (DHA)	1.8	±	0.7	0.3	±	0.2	0.6	± 0.4
Total n-3 PUFA	***5.8***	**±**	***3.2***	***4.0***	**±**	***1.2***	***6.0***	**±** ***3.3***
Total PUFA	***12.3***	**±**	***5.9***	***6.5***	**±**	***1.5***	***9.3***	**±** ***4.5***

	***Posidonia oceanica* leaves**								
	February								
	IMTA			**R1**			**R2**		
n	2			2			2		
Lipid (mg $100g^{-1}$)	77.8	±	37.4	40.7	±	12.0	45.1	±	16.5
FA content (mg $100g^{-1}$)									
10:0	0.5	±	0.2	0.4	±	0.0	2.4	±	2.6
14:0	1.6	±	0.8	1.8	±	0.1	2.3	±	0.1
16:0	92.1	±	24.6	89.4	±	8.8	95.7	±	1.8
18:0	50.5	±	16.7	36.9	±	14.6	38.3	±	8.4

20:0	2.1	±	0.9	1.7	±	0.5	1.7	±	0.3
22:0	3.4	±	1.1	4.1	±	0.1	4.3	±	0.6
Total saturated[1]	***232.8***	***±***	***48.1***	***210.7***	***±***	***24.3***	***226.0***	***±***	***1.8***
16:1*n*-7	10.5	±	3.1	17.3	±	2.3	13.0	±	2.9
18:1*n*-9	7.7	±	0.0	12.8	±	0.5	6.4	±	4.2
18:1*n*-7	2.1	±	0.0	2.5	±	0.0	2.8	±	0.4
20:1*n*-9	0.2	±	0.1	0.1	±	0.1	0.3	±	0.0
22:1*n*-9	0.3	±	0.4	0.5	±	0.0	0.3	±	0.4
Total monoenes	***20.8***	***±***	***3.6***	***33.2***	***±***	***1.8***	***22.7***	***±***	***2.2***
18:2*n*-6	94.2	±	29.4	84.8	±	13.5	79.0	±	12.3
20:2*n*-6	1.0	±	0.7	0.2	±	0.0	0.2	±	0.2
20:4*n*-6	0.2	±	0.3	0.4	±	0.5	0.4	±	0.0
Total n-6 PUFA	***95.4***	***±***	***29.8***	***85.3***	***±***	***13.0***	***79.7***	***±***	***12.1***
18:3*n*-3	441.1	±	224.5	527.8	±	116.6	551.4	±	64.4
20:5*n*-3 (EPA)	0.3	±	0.3	0.5	±	0.3	0.6	±	0.5
22:5*n*-3 (DPA)	0.0	±	0.0	0.6	±	0.3	0.7	±	1.0
22:6*n*-3 (DHA)	0.0	±	0.0	0.5	±	0.4	0.5	±	0.8
Total n-3 PUFA	***441.3***	***±***	***224.2***	***529.4***	***±***	***117.6***	***553.2***	***±***	***62.2***
Total PUFA	***536.7***	***±***	***254.0***	***614.7***	***±***	***130.6***	***632.9***	***±***	***50.0***

	***Posidonia oceanica* leaves**		
	May		
	IMTA	**R1**	**R2**
n	3	3	3
Lipid (mg $100g^{-1}$)	98.4 ± 18.6	89.5 ± 21.6	87.1 ± 11.2

FA content (mg $100g^{-1}$)									
10:0	1.7	±	2.6	1.8	±	2.2	1.0	±	0.3
14:0	19.4	±	1.1	1.8	±	0.5	3.2	±	2.4
16:0	130.9	±	7.0	125.8	±	15.4	115.9	±	10.6
18:0	10.2	±	1.3	6.1	±	1.4	6.2	±	0.8
20:0	1.6	±	0.1	1.3	±	0.2	1.9	±	0.3
22:0	6.0	±	0.5	5.3	±	0.3	6.5	±	0.7
Total saturated[1]	***291.1***	***±***	***2.5***	***257.0***	***±***	***7.6***	***252.0***	***±***	***8.5***
16:1*n*-7	25.0	±	4.7	17.5	±	3.8	20.5	±	2.0
18:1*n*-9	17.3	±	1.6	16.7	±	11.0	9.1	±	0.7
18:1*n*-7	4.2	±	0.8	3.0	±	0.1	4.0	±	0.7
20:1*n*-9	0.2	±	0.1	0.1	±	0.2	0.2	±	0.2
22:1*n*-9	0.2	±	0.3	0.3	±	0.3	0.4	±	0.3
Total monoenes	***46.8***	***±***	***7.2***	***37.6***	***±***	***8.9***	***34.2***	***±***	***3.1***
18:2*n*-6	180.1	±	11.0	165.3	±	12.1	131.0	±	21.7
20:2*n*-6	0.4	±	0.5	0.1	±	0.1	0.2	±	0.1
20:4*n*-6	0.2	±	0.2	0.3	±	0.3	0.2	±	0.2
Total n-6 PUFA	***180.7***	***±***	***11.1***	***165.8***	***±***	***12.0***	***131.4***	***±***	***21.6***
18:3*n*-3	830.5	±	93.8	1039.9	±	121.9	818.6	±	34.0
20:5*n*-3 (EPA)	0.5	±	0.4	0.2	±	0.2	1.6	±	1.5
22:5*n*-3 (DPA)	12.7	±	1.2	0.2	±	0.3	0.3	±	0.1
22:6*n*-3 (DHA)	0.2	±	0.3	0.4	±	0.2	0.2	±	0.3
Total n-3 PUFA	***843.9***	***±***	***93.2***	***1040.7***	***±***	***121.8***	***820.7***	***±***	***34.7***
Total PUFA	***1024.6***	***±***	***104.0***	***1206.4***	***±***	***123.3***	***952.1***	***±***	***21.2***

Posidonia oceanica leaves									
September									
	IMTA			**R1**			**R2**		
n	3			3			3		
Lipid (mg $100g^{-1}$)	43.1	±	14.0	45.0	±	0.8	46.1	±	4.7
FA content (mg $100g^{-1}$)									
10:0	0.1	±	0.0	0.1	±	0.0	0.0	±	0.0
14:0	4.3	±	0.4	2.9	±	0.8	2.6	±	0.7
16:0	123.0	±	13.3	101.3	±	5.9	103.0	±	3.9
18:0	39.8	±	17.9	23.6	±	10.8	22.3	±	15.0
20:0	2.9	±	0.2	1.6	±	0.4	1.8	±	0.9
22:0	1.3	±	0.3	0.4	±	0.2	0.4	±	0.3
***Total saturated*[1]**	***242.1***	**±**	***21.5***	***194.2***	**±**	***14.7***	***199.3***	**±**	***16.4***
16:1*n*-7	9.1	±	2.8	5.6	±	0.3	6.2	±	1.0
18:1*n*-9	20.0	±	5.3	16.9	±	3.3	17.8	±	1.8
18:1*n*-7	4.1	±	0.5	4.0	±	0.6	3.9	±	0.2
20:1*n*-9	0.0	±	0.0	0.0	±	0.0	0.1	±	0.1
22:1*n*-9	0.1	±	0.1	0.3	±	0.3	0.4	±	0.1
Total monoenes	***33.3***	**±**	***4.8***	***26.9***	**±**	***3.7***	***28.5***	**±**	***1.7***
18:2*n*-6	137.0	±	18.3	153.6	±	19.4	155.9	±	17.4
20:2*n*-6	0.5	±	0.2	0.1	±	0.0	0.1	±	0.1
20:4*n*-6	0.1	±	0.1	0.3	±	0.2	0.2	±	0.4
Total n-6 PUFA	***137.6***	**±**	***18.2***	***154.0***	**±**	***19.2***	***156.2***	**±**	***17.0***

18:3*n*-3	194.9	±	36.6	281.7	±	51.4	230.9	±	27.1
20:5*n*-3 (EPA)	0.6	±	0.2	0.1	±	0.1	0.1	±	0.1
22:5*n*-3 (DPA)	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0
22:6*n*-3 (DHA)	0.0	±	0.0	0.1	±	0.1	0.0	±	0.0
Total n-3 PUFA	***195.5***	**±**	***36.6***	***281.8***	**±**	***51.3***	***231.0***	**±**	***27.0***
Total PUFA	***333.1***	**±**	***52.8***	***435.8***	**±**	***70.5***	***387.2***	**±**	***38.1***

					***Posidonia oceanica* rhizomes**				
					February				
			IMTA			**R1**			**R2**
n			2			2			2
Lipid (mg 100g^{-1})	39.9	±	23.6	41.1	±	23.9	35.0	±	34.8
FA content (mg 100g^{-1})									
10:0	0.4	±	0.2	0.6	±	0.1	0.4	±	0.0
14:0	0.6	±	0.4	1.3	±	0.5	0.7	±	0.1
16:0	24.8	±	3.6	33.3	±	2.7	28.7	±	7.7
18:0	20.3	±	6.3	20.5	±	5.9	11.6	±	3.0
20:0	0.0	±	0.0	0.3	±	0.4	0.0	±	0.0
22:0	0.5	±	0.1	0.2	±	0.0	0.0	±	0.0
Total saturated[1]	***56.9***	**±**	***10.2***	***68.5***	**±**	***13.6***	***53.7***	**±**	***6.9***
16:1*n*-7	0.2	±	0.2	0.4	±	0.1	0.9	±	0.9
18:1*n*-9	10.1	±	0.7	5.9	±	5.4	3.8	±	2.0
18:1*n*-7	2.4	±	0.3	2.7	±	0.3	2.4	±	0.2
20:1*n*-9	0.1	±	0.1	0.2	±	0.0	0.3	±	0.2
22:1*n*-9	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0

Total monoenes	***12.9***	***±***	***0.9***	***9.2***	***±***	***5.8***	***7.5***	***±***	***2.9***
18:2*n*-6	42.7	±	6.3	69.4	±	17.2	54.6	±	16.3
20:2*n*-6	0.2	±	0.2	0.1	±	0.1	0.2	±	0.1
20:4*n*-6	0.3	±	0.2	0.5	±	0.7	0.5	±	0.2
Total n-6 PUFA	***43.2***	***±***	***6.3***	***70.0***	***±***	***17.8***	***55.3***	***±***	***16.4***
18:3*n*-3	4.3	±	0.7	12.6	±	7.1	12.5	±	7.8
20:5*n*-3 (EPA)	0.3	±	0.1	0.3	±	0.1	0.1	±	0.1
22:5*n*-3 (DPA)	0.2	±	0.3	1.0	±	0.3	0.4	±	0.5
22:6*n*-3 (DHA)	0.4	±	0.4	0.2	±	0.3	0.0	±	0.0
Total n-3 PUFA	***5.3***	***±***	***0.9***	***14.0***	***±***	***7.7***	***13.0***	***±***	***7.1***
Total PUFA	***48.4***	***±***	***7.2***	***84.0***	***±***	***10.0***	***68.3***	***±***	***23.5***

				***Posidonia oceanica* rhizomes**					
				May					
	IMTA			**R1**			**R2**		
n	3			3			3		
Lipid (mg $100g^{-1}$)	14.1	±	5.9	9.9	±	4.6	11.5	±	3.2
FA content (mg $100g^{-1}$)									
10:0	1.5	±	2.1	2.0	±	3.4	0.0	±	0.0
14:0	0.9	±	0.2	1.3	±	0.2	1.5	±	0.1
16:0	44.1	±	12.4	41.8	±	8.3	43.5	±	5.8
18:0	3.5	±	2.3	7.6	±	3.0	9.5	±	3.3
20:0	0.2	±	0.3	0.1	±	0.1	0.2	±	0.1
22:0	0.6	±	0.5	0.4	±	0.2	0.5	±	0.5

Total saturated[1]	***66.1***	±	***17.2***	***66.9***	±	***9.7***	***70.6***	±	***10.9***
16:1*n*-7	0.6	±	0.5	0.6	±	0.4	0.4	±	0.2
18:1*n*-9	12.0	±	2.5	5.7	±	2.3	7.6	±	2.9
18:1*n*-7	3.2	±	0.4	3.7	±	1.2	3.0	±	0.4
20:1*n*-9	0.2	±	0.1	0.3	±	0.2	0.1	±	0.1
22:1*n*-9	0.4	±	0.3	0.3	±	0.3	0.5	±	0.1
Total monoenes	***16.5***	±	***2.2***	***10.6***	±	***1.5***	***11.5***	±	***2.4***
18:2*n*-6	114.5	±	25.9	102.3	±	18.7	108.0	±	8.9
20:2*n*-6	0.2	±	0.2	0.2	±	0.2	0.3	±	0.1
20:4*n*-6	0.3	±	0.3	0.5	±	0.2	0.5	±	0.4
Total n-6 PUFA	***115.0***	±	***25.9***	***103.0***	±	***18.4***	***108.8***	±	***8.6***
18:3*n*-3	25.5	±	14.5	25.0	±	12.6	26.1	±	7.9
20:5*n*-3 (EPA)	0.2	±	0.2	0.1	±	0.1	0.2	±	0.3
22:5*n*-3 (DPA)	1.9	±	3.3	7.3	±	12.2	0.3	±	0.4
22:6*n*-3 (DHA)	0.0	±	0.0	0.5	±	0.1	0.0	±	0.0
Total n-3 PUFA	***27.6***	±	***12.3***	***32.9***	±	***24.5***	***26.6***	±	***8.2***
Total PUFA	***142.6***	±	***36.2***	***135.9***	±	***42.8***	***135.4***	±	***15.8***

	***Posidonia oceanica* rhizomes**								
	September								
	IMTA			**R1**			**R2**		
n	3			3			3		
Lipid (mg $100g^{-1}$)	18.7	±	1.2	17.5	±	2.2	4.9	±	2.3
FA content (mg $100g^{-1}$)									

10:0	0.1	±	0.1	0.1	±	0.0	0.2	± 0.1
14:0	2.0	±	0.3	2.0	±	0.0	1.3	± 0.3
16:0	49.3	±	16.9	61.2	±	5.8	37.6	± 5.8
18:0	15.9	±	5.3	12.3	±	2.2	4.1	± 0.9
20:0	1.1	±	0.3	1.0	±	0.2	2.2	± 0.1
22:0	0.4	±	0.4	0.7	±	0.2	1.1	± 0.1
Total saturated[1]	***80.1***	***±***	***22.8***	***86.5***	***±***	***7.6***	***68.4***	***± 8.2***
16:1*n*-7	0.5	±	0.3	0.3	±	0.1	0.0	± 0.0
18:1*n*-9	13.6	±	3.9	8.5	±	0.5	9.1	± 2.1
18:1*n*-7	3.0	±	0.2	2.7	±	0.1	2.1	± 0.2
20:1*n*-9	0.2	±	0.1	0.1	±	0.0	0.0	± 0.0
22:1*n*-9	0.3	±	0.3	0.4	±	0.1	0.0	± 0.0
Total monoenes	***17.7***	***±***	***4.3***	***12.0***	***±***	***0.4***	***11.2***	***± 2.0***
18:2*n*-6	119.3	±	35.3	151.2	±	11.1	97.1	± 13.0
20:2*n*-6	0.2	±	0.1	0.1	±	0.1	0.0	± 0.0
20:4*n*-6	0.3	±	0.3	0.0	±	0.0	0.1	± 0.1
Total n-6 PUFA	***119.8***	***±***	***35.0***	***151.3***	***±***	***11.2***	***97.2***	***± 13.0***
18:3*n*-3	21.1	±	11.2	39.2	±	5.9	19.8	± 5.8
20:5*n*-3 (EPA)	0.2	±	0.2	0.1	±	0.2	0.0	± 0.0
22:5*n*-3 (DPA)	0.2	±	0.4	0.0	±	0.0	0.0	± 0.0
22:6*n*-3 (DHA)	0.2	±	0.2	0.0	±	0.0	2.3	± 0.3
Total n-3 PUFA	***21.8***	***±***	***10.8***	***39.4***	***±***	***5.9***	***22.2***	***± 5.5***
Total PUFA	***141.6***	***±***	***45.8***	***190.6***	***±***	***17.0***	***119.4***	***± 18.2***

	Fish faeces					
	February			September		
n	2			2		
Lipid (mg 100g^{-1})	108.8	±	2.4	70.0	±	32.0
FA content (mg 100g^{-1})						
10:0	0.2	±	0.2	0.2	±	0.1
14:0	31.5	±	17.1	16.3	±	21.0
16:0	395.2	±	206.5	170.7	±	219.2
18:0	188.4	±	108.4	83.5	±	109.8
20:0	20.8	±	11.8	7.6	±	10.6
22:0	10.9	±	7.1	5.2	±	6.0
***Total saturated*[1]**	***682.7***	**±**	***370.5***	***303.3***	**±**	***393.5***
16:1*n*-7	25.7	±	19.5	17.2	±	22.7
18:1*n*-9	654.9	±	457.1	316.6	±	410.4
18:1*n*-7	61.5	±	43.8	31.0	±	37.7
20:1*n*-9	71.9	±	58.4	19.3	±	24.8
22:1*n*-9	13.9	±	11.9	4.5	±	5.8
Total monoenes	***828.0***	**±**	***590.8***	***388.6***	**±**	***501.4***
18:2*n*-6	341.9	±	278.4	189.6	±	241.6
20:2*n*-6	40.5	±	29.1	12.5	±	14.2
20:4*n*-6	32.1	±	28.7	10.7	±	14.2
Total n-6 PUFA	***414.5***	**±**	***336.2***	***212.8***	**±**	***270.0***
18:3*n*-3	56.9	±	42.1	37.0	±	48.2
20:5*n*-3 (EPA)	51.5	±	46.6	30.3	±	37.4
22:5*n*-3 (DPA)	95.1	±	74.8	36.3	±	44.6
22:6*n*-3 (DHA)	304.3	±	260.2	91.7	±	111.1
Total n-3 PUFA	***507.8***	**±**	***423.6***	***195.3***	**±**	***241.3***
Total PUFA	***922.3***	**±**	***759.8***	***408.0***	**±**	***511.3***

	Feed											
	February						May					
	Feed A			Feed C			Feed A			Feed B		
n	3			3			3			3		
Lipid (mg. 100g^{-1})	161.7	±	6.4	221.2	±	4.9	165.1	±	17.2	275.6	±	70.6
FA content (mg 100g^{-1})												
10:0	5.0	±	8.6	0.0	±	0.0	0.0	±	0.0	17.6	±	1.2
14:0	67.1	±	2.3	71.4	±	0.2	64.9	±	1.7	88.0	±	13.1
16:0	427.9	±	15.3	500.2	±	2.5	419.3	±	13.8	461.8	±	69.3
18:0	106.2	±	7.5	128.6	±	4.3	111.1	±	7.9	177.2	±	18.8
20:0	15.0	±	0.9	21.0	±	1.5	15.8	±	0.8	14.3	±	2.3
22:0	5.9	±	0.5	8.7	±	0.6	6.2	±	0.8	5.7	±	1.4
Total saturated[1]	***660.6***	***±***	***34.4***	***765.1***	***±***	***16.4***	***651.1***	***±***	***9.4***	***809.7***	***±***	***111.0***
16:1*n*-7	95.0	±	2.4	102.0	±	1.5	92.4	±	2.8	96.6	±	14.3
18:1*n*-9	1506.3	±	40.6	2179.6	±	12.0	1458.1	±	53.0	880.1	±	161.7
18:1*n*-7	105.0	±	2.3	141.1	±	2.4	102.2	±	3.6	84.1	±	12.3
20:1*n*-9	103.1	±	3.6	118.9	±	2.5	99.3	±	2.6	40.6	±	7.0
22:1*n*-9	14.8	±	0.9	20.3	±	1.0	14.0	±	0.4	5.6	±	0.9
Total monoenes	***1824.1***	***±***	***48.8***	***2562.0***	***±***	***18.8***	***1766.0***	***±***	***62.1***	***1107.0***	***±***	***196.3***
18:2*n*-6	838.7	±	25.7	1078.5	±	4.5	811.6	±	28.8	825.3	±	153.4
20:2*n*-6	26.1	±	1.0	26.9	±	0.2	25.6	±	0.4	5.6	±	1.0
20:4*n*-6	13.8	±	1.2	14.8	±	0.7	13.4	±	0.7	12.8	±	2.0
Total n-6 PUFA	***878.6***	***±***	***27.5***	***1120.3***	***±***	***5.4***	***850.6***	***±***	***29.7***	***843.7***	***±***	***156.4***
18:3*n*-3	249.1	±	7.0	313.2	±	0.9	239.6	±	8.3	153.5	±	28.9

20:5*n*-3 (EPA)	115.3	±	3.2	128.1	±	6.1	111.4	±	3.1	244.1	±	38.8
22:5*n*-3 (DPA)	64.0	±	10.3	73.2	±	3.7	56.0	±	2.4	52.9	±	8.8
22:6*n*-3 (DHA)	156.2	±	3.5	188.2	±	6.8	150.6	±	4.7	243.5	±	39.2
Total n-3 PUFA	***584.6***	***±***	***19.2***	***702.6***	***±***	***11.0***	***557.5***	***±***	***17.9***	***694.1***	***±***	***115.5***
Total PUFA	***1463.2***	***±***	***43.1***	***1822.9***	***±***	***16.3***	***1408.1***	***±***	***47.7***	***1537.8***	***±***	***271.9***

	September								
	Feed A			**Feed C**			**Feed A**		
n	3			3			3		
Lipid (mg. 100g^{-1})	165.0	±	4.4	178.3	±	10.9	241.8	±	21.5
FA content (mg. 100g^{-1})									
10:0	0.0	±	0.0	19.4	±	0.1	0.0	±	0.0
14:0	66.4	±	2.8	104.5	±	2.6	80.5	±	11.7
16:0	420.8	±	12.8	520.0	±	12.5	560.3	±	72.9
18:0	102.5	±	5.7	136.7	±	8.4	162.7	±	22.2
20:0	14.9	±	1.3	15.7	±	0.6	23.5	±	3.2
22:0	5.7	±	0.5	8.9	±	0.5	9.4	±	1.4
Total saturated[1]	***653.5***	***±***	***45.5***	***862.7***	***±***	***14.9***	***877.2***	***±***	***114.7***
16:1*n*-7	94.1	±	2.6	117.1	±	1.1	114.7	±	15.7
18:1*n*-9	1486.3	±	30.1	1063.8	±	14.1	437.4	±	65.5
18:1*n*-7	104.3	±	2.7	99.7	±	1.5	173.1	±	25.6
20:1*n*-9	102.2	±	4.6	48.8	±	2.6	133.6	±	19.5
22:1*n*-9	14.7	±	1.3	6.8	±	1.0	21.9	±	3.1
Total monoenes	***1801.5***	***±***	***41.2***	***1336.1***	***±***	***18.9***	***880.8***	***±***	***61.4***
18:2*n*-6	826.0	±	18.9	1013.0	±	15.9	1199.8	±	163.4
20:2*n*-6	26.0	±	1.4	7.1	±	0.5	30.1	±	4.9

20:4*n*-6	13.5	±	1.5	15.8	±	0.5	17.0	±	1.8
Total n-6 PUFA	***865.5***	**±**	***21.8***	***1035.9***	**±**	***15.0***	***1246.9***	**±**	***170.0***
18:3*n*-3	245.4	±	4.3	187.3	±	4.5	356.0	±	53.6
20:5*n*-3 (EPA)	115.2	±	4.5	293.8	±	3.6	149.5	±	22.9
22:5*n*-3 (DPA)	66.5	±	13.2	70.5	±	6.7	78.2	±	11.2
22:6*n*-3 (DHA)	155.6	±	4.7	295.1	±	3.5	217.9	±	31.4
Total n-3 PUFA	***582.7***	**±**	***26.7***	***846.7***	**±**	***10.5***	***801.6***	**±**	***111.9***
Total PUFA	***1448.2***	**±**	***48.6***	***1882.6***	**±**	***24.1***	***2048.5***	**±**	***275.1***

Means (± standard deviation)

[1] Includes Iso-15:0, Anteiso 15:0, 15:0, Anteiso 17:0, 17:0, 24:0, 26:0, 28:0, 30:0, 32:0.

Appendix 6.3. Results of similarity percentage analysis of fatty acid profiles of organic source groups for February, May, and September. Similarity within group (A) and dissimilarity between groups (B) are shown. Data was not transformed prior to analysis.

***Site* groups across all sampled organic *Source* groups in February**					
Group E0					
Average similarity: 95.46					
Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	29.5	29.5	3.2	30.9	30.9
18:2*n*-6	17.9	18.3	3.6	19.2	50.1
16:0	13.9	12.9	3.9	13.6	63.6
22:6*n*-3	5.7	5.2	2.4	5.5	69.1
18:3*n*-3	4.2	4.4	2.7	4.6	73.7
20:5*n*-3	4.2	4.1	2.0	4.3	78.1
18:0	3.9	3.1	2.3	3.3	81.3
Group E10					
Average similarity: 88.61					
Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	20.2	18.1	2.0	20.4	20.4
16:0	12.6	11.3	1.2	12.7	33.1
18:2*n*-6	9.1	7.9	11.0	8.9	42.0
20:4*n*-6	7.8	7.5	0.9	8.5	50.5
22:5*n*-3	6.4	6.5	1.1	7.4	57.8
20:5*n*-3	6.6	5.9	1.3	6.7	64.5
18:0	5.6	4.5	2.0	5.1	69.6
20:1*n*-9	3.8	3.8	1.6	4.3	73.8
18:1*n*-7	3.9	3.5	5.1	3.9	77.8
16:1*n*-7	3.2	2.4	1.8	2.8	80.5
Group E25					
Average similarity: 93.97					
Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	14.7	15.4	1.6	16.4	16.4

16:0	12.9	12.1	1.5	12.8	29.2
18:2*n*-6	13.8	11.3	1.2	12.0	41.2
20:4n6	5.8	7.0	7.0	7.5	48.7
18:3*n*-3	10.2	6.5	0.4	6.9	55.5
18:0	7.3	6.3	1.6	6.7	62.2
20:5*n*-3	5.3	6.0	1.0	6.4	68.6
22:5*n*-3	3.9	4.8	0.9	5.1	73.7
18:1*n*-7	3.1	3.1	2.2	3.3	77.0
20:1*n*-9	2.3	2.8	1.2	2.9	80.0
16:1*n*-7	2.2	2.3	1.6	2.4	82.4

Group R1

Average similarity: 86.71

Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
20:4*n*-6	8.9	10.6	0.7	12.2	12.2
16:0	13.0	10.3	1.6	11.9	24.1
20:5*n*-3	6.9	7.6	0.9	8.8	32.9
18:3*n*-3	11.2	6.8	0.4	7.8	40.7
18:2*n*-6	9.7	6.6	0.5	7.6	48.2
18:1*n*-7	5.1	4.8	1.3	5.6	53.8
18:0	6.5	4.8	1.3	5.6	59.3
16:1*n*-7	5.1	4.7	1.1	5.5	64.8
22:5*n*-3	4.2	4.6	0.8	5.3	70.1
18:1*n*-9	3.2	2.7	1.2	3.1	73.2
14:0	2.1	2.1	1.1	2.5	75.7
26:0	2.4	2.0	1.1	2.3	78.0
28:0	1.9	1.7	0.9	2.0	80.0
22:6*n*-3	1.5	1.6	1.1	1.8	81.8

Group R2

Average similarity: 84.93

Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
20:4*n*-6	9.9	12.1	0.8	14.3	14.3
16:0	10.6	9.2	1.1	10.9	25.1
20:5*n*-3	7.1	8.0	1.0	9.4	34.5

18:3*n*-3	12.1	7.5	0.4	8.8	43.3
18:2*n*-6	10.3	6.0	0.5	7.1	50.4
22:5*n*-3	4.1	4.9	0.8	5.8	56.2
18:1*n*-7	4.3	4.3	1.2	5.1	61.2
16:1*n*-7	4.5	4.3	1.0	5.0	66.2
18:0	6.2	3.1	1.4	3.7	69.9
18:1*n*-9	5.0	2.4	1.3	2.8	72.7
28:0	2.5	2.2	0.9	2.6	75.4
26:0	2.2	1.9	1.1	2.2	77.6
14:0	1.7	1.7	0.9	2.0	79.6
22:6*n*-3	1.7	1.6	0.9	1.9	81.5

Groups E0 & E10

Average dissimilarity = 21.36

	Group E0	**Group E10**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:2*n*-6	17.9	9.1	3.3	1.8	15.3	15.3
18:1*n*-9	29.5	20.2	3.0	1.2	14.0	29.3
22:6*n*-3	5.7	2.6	1.8	2.3	8.6	37.9
18:0	3.9	5.6	1.7	2.4	7.9	45.7
20:5*n*-3	4.2	6.6	1.7	2.2	7.8	53.5
16:0	13.9	12.6	1.6	1.5	7.6	61.1
18:3*n*-3	4.2	1.5	0.9	1.8	4.2	65.3
18:1*n*-7	3.1	3.9	0.8	2.0	3.8	69.1
16:1*n*-7	3.0	3.2	0.7	0.9	3.2	72.3
22:5*n*-3	2.2	6.4	0.5	2.1	2.3	74.5
Iso-15:0	0.3	0.6	0.5	1.7	2.1	76.6
20:2*n*-6	0.7	2.0	0.4	0.8	2.0	78.6
14:0	2.3	2.2	0.4	1.4	1.7	80.2

Groups E0 & E25

Average dissimilarity = 21.55

	Group E0	**Group E25**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:2*n*-6	17.9	13.8	3.6	2.6	16.8	16.8

18:1*n*-9	29.5	14.7	2.3	1.1	10.6	27.4
18:0	3.9	7.3	1.8	1.9	8.3	35.7
22:6*n*-3	5.7	2.1	1.6	2.2	7.6	43.3
20:5*n*-3	4.2	5.3	1.4	2.6	6.6	49.9
16:0	13.9	12.9	1.4	1.3	6.3	56.1
18:1*n*-7	3.1	3.1	1.0	2.5	4.6	60.8
18:3*n*-3	4.2	10.2	0.7	1.7	3.4	64.2
20:2*n*-6	0.7	1.6	0.7	0.8	3.3	67.5
16:1*n*-7	3.0	2.2	0.7	0.7	3.0	70.6
Iso-15:0	0.3	0.6	0.6	2.7	2.7	73.3
14:0	2.3	1.2	0.5	2.6	2.5	75.8
22:5*n*-3	2.2	3.9	0.5	2.2	2.1	77.8
Anteiso15:0	0.3	0.4	0.5	1.9	2.1	79.9
20:4*n*-6	0.7	5.8	0.4	1.0	1.9	81.8

Groups E10 & E25

Average dissimilarity = 9.53

	Group E10	**Group E25**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	20.2	14.7	1.2	0.9	12.6	12.6
16:0	12.6	12.9	0.9	1.2	8.7	21.5
18:2*n*-6	9.1	13.6	0.8	1.0	6.2	29.7
20:5*n*-3	6.6	5.3	0.8	1.2	7.9	37.6
20:4*n*-6	7.8	5.8	0.7	0.8	6.9	44.5
18:0	5.6	7.3	0.6	1.1	6.6	51.0
16:1*n*-7	3.2	2.2	0.4	0.9	4.5	55.5
22:5*n*-3	6.4	3.9	0.4	0.9	3.6	59.2
14:0	2.2	1.2	0.3	1.0	3.5	62.6
22:6*n*-3	2.6	2.1	0.3	1.2	3.2	65.9
20:1*n*-9	3.8	2.3	0.3	0.9	3.1	68.9
18:1*n*-7	3.9	3.1	0.3	1.4	2.8	71.8
20:2*n*-6	2.0	1.6	0.2	0.5	2.4	74.1
21:0	0.7	0.4	0.2	1.0	2.2	76.4
18:3*n*-3	1.5	10.2	0.2	1.2	1.9	78.3
Iso-15:0	0.6	0.6	0.1	1.7	1.4	79.6
22:0	1.0	0.9	0.1	2.2	1.3	80.9

Groups E0 & R1						
Average dissimilarity = 44.60						
	Group E0	**Group R1**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	29.5	3.2	8.0	3.5	18.0	18.0
18:2*n*-6	17.9	9.7	5.6	2.4	12.5	30.5
18:1*n*-7	3.1	5.1	3.0	3.6	6.7	37.3
16:1*n*-7	3.0	5.1	2.9	2.5	6.5	43.7
26:0	0.2	2.4	2.0	5.6	4.4	48.2
16:0	13.9	13.0	1.9	1.2	4.3	52.5
28:0	0.1	1.9	1.7	1.7	3.8	56.3
18:0	3.9	6.5	1.5	1.0	3.4	59.7
18:3*n*-3	4.2	11.2	1.3	2.9	3.0	62.7
22:6*n*-3	5.7	1.5	1.3	2.2	2.8	65.5
30:0	0.1	1.2	1.2	2.1	2.7	68.1
Anteiso15:0	0.3	0.9	1.0	2.2	2.2	70.4
20:4*n*-3	0.5	1.2	1.0	0.7	2.2	72.6
32:0	0.1	0.9	1.0	2.0	2.1	74.7
20:5*n*-3	4.2	6.9	0.9	1.3	2.1	76.8
Iso-15:0	0.3	0.9	0.9	2.3	1.9	78.7
10:0	0.1	0.7	0.8	1.7	1.8	80.6

Groups E10 & R1						
Average dissimilarity = 39.80						
	Group E10	**Group R1**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	20.2	3.2	8.2	2.4	20.5	20.5
18:2*n*-6	9.1	9.7	3.5	3.0	8.8	29.3
20:4*n*-6	7.8	8.9	3.2	0.9	7.9	37.2
20:5*n*-3	6.6	6.9	2.6	1.7	6.4	43.6
16:0	12.6	13.0	2.0	1.0	4.9	48.5
16:1*n*-7	3.2	5.1	1.9	1.2	4.8	53.3
18:0	5.6	6.5	1.8	1.5	4.6	57.9

Groups E25 & R1						
Average dissimilarity = 24.01						
	Group E25	**Group R1**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	14.7	3.2	6.1	1.6	19.6	19.6
18:2*n*-6	13.8	9.7	2.9	1.9	9.4	28.9
20:4n6	5.8	8.9	2.1	0.7	6.8	35.7
18:0	7.3	6.5	2.0	1.5	6.3	42.0
16:1*n*-7	2.2	5.1	1.6	1.0	5.1	47.1
20:5*n*-3	5.3	6.9	1.5	1.1	4.8	51.8
16:0	12.9	13.0	1.3	0.7	4.3	56.1
18:1*n*-7	3.1	5.1	1.3	1.0	4.0	60.2
18:3*n*-3	10.2	11.2	1.3	0.7	4.0	64.2
20:1*n*-9	2.3	0.4	1.1	1.3	3.6	67.8
26:0	0.9	2.4	0.9	1.0	2.9	70.7
28:0	1.3	1.9	0.8	0.9	2.7	73.4
22:6*n*-3	2.1	1.5	0.7	1.2	2.1	75.5
20:2*n*-6	1.6	0.4	0.6	0.7	1.8	77.3
22:0	0.9	1.8	0.5	0.7	1.7	79.0
30:0	0.6	1.2	0.5	0.8	1.6	80.6

Groups E0 & R2						
Average dissimilarity = 45.05						
	Group E0	**Group R2**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	29.5	5.0	8.3	3.4	18.5	18.5
18:2*n*-6	17.9	10.3	6.2	3.0	13.7	32.1
16:1*n*-7	3.0	4.5	2.9	2.6	6.4	38.5
18:0	3.9	6.2	2.8	0.8	6.2	44.7
16:0	13.9	10.6	2.6	0.9	5.8	50.5
18:1*n*-7	3.1	4.3	2.5	2.1	5.6	56.1
28:0	0.1	2.5	2.0	1.9	4.5	60.5
26:0	0.2	2.2	1.6	2.2	3.6	64.1
30:0	0.1	1.5	1.3	2.5	2.8	67.0
22:6*n*-3	5.7	1.7	1.1	2.0	2.4	69.4

18:3*n*-3	4.2	12.1	1.0	2.0	2.3	71.6
Anteiso15:0	0.3	0.9	1.0	2.4	2.2	73.9
Iso-15:0	0.3	0.8	1.0	3.6	2.2	76.0
20:5*n*-3	4.2	7.1	0.9	1.7	2.1	78.2
32:0	0.1	0.9	0.9	1.9	2.0	80.2

Groups E10 & R2

Average dissimilarity = 38.58

	Group E10	**Group R2**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	20.2	5.0	7.9	2.0	20.5	20.5
20:4n6	7.8	9.9	3.8	1.0	9.7	30.2
18:2*n*-6	9.1	10.3	3.4	2.7	8.8	39.0
20:5*n*-3	6.6	7.1	2.4	1.9	6.2	45.2
16:0	12.6	10.6	2.2	1.2	5.6	50.8
18:0	5.6	6.2	2.0	0.7	5.3	56.1
16:1*n*-7	3.2	4.5	1.7	1.1	4.4	60.5
20:1*n*-9	3.8	0.7	1.7	1.6	4.3	64.8
18:1*n*-7	3.9	4.3	1.5	1.5	3.9	68.7
28:0	0.2	2.5	1.2	0.9	3.0	71.7
22:6*n*-3	2.6	1.7	0.9	2.6	2.4	74.2
26:0	0.3	2.2	0.9	1.0	2.4	76.6
30:0	0.2	1.5	0.7	1.0	1.9	78.5
Anteiso15:0	0.4	0.9	0.5	1.1	1.4	79.9
32:0	0.2	0.9	0.5	0.9	1.3	81.2

Groups E25 & R2

Average dissimilarity = 29.98

	Group E25	**R2**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	14.7	5.0	5.8	1.4	19.3	19.3
18:2*n*-6	13.8	10.3	2.7	1.8	9.2	28.4
20:4*n*-6	5.8	9.9	2.5	0.7	8.3	36.7
18:0	7.3	6.2	2.2	0.8	7.2	43.9
16:1*n*-7	2.2	4.5	1.4	0.9	4.7	48.6

20:5*n*-3	5.3	7.1	1.4	1.2	4.5	53.1
16:0	12.9	10.6	1.3	1.1	4.4	57.6
18:3*n*-3	10.2	12.1	1.2	0.6	4.1	61.7
20:1*n*-9	2.3	0.7	1.1	1.2	3.6	65.3
18:1*n*-7	3.1	4.3	1.0	1.1	3.5	66.7
28:0	1.3	2.5	1.0	0.9	3.3	72.0
26:0	0.9	2.2	0.8	1.0	2.5	74.5
22:6*n*-3	2.1	1.7	0.7	1.3	2.2	76.7
30:0	0.6	1.5	0.6	0.9	1.9	78.6
22:5*n*-3	3.9	4.1	0.4	1.5	1.5	80.1

Groups R1 & R2

Average dissimilarity = 15.33

	Group R1	**Group R2**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:0	6.5	6.2	1.7	0.8	11.3	11.3
18:1*n*-9	3.2	5.0	1.5	0.5	9.9	21.3
16:0	13.0	10.6	1.5	0.5	9.4	30.7
18:2*n*-6	9.7	10.3	1.0	0.6	6.5	37.2
20:5*n*-3	6.9	7.1	0.7	0.9	4.9	42.0
20:4*n*-6	8.9	9.9	0.7	0.8	4.6	46.7
18:3*n*-3	11.2	12.1	0.7	0.7	4.6	51.3
16:1*n*-7	5.1	4.5	0.6	0.8	4.2	55.4
18:1*n*-7	5.1	4.3	0.5	0.7	3.5	59.0
22:5*n*-3	4.2	4.1	0.5	1.1	3.1	62.0
20:4*n*-3	1.2	0.3	0.4	0.3	2.6	64.6
26:0	2.4	2.2	0.4	0.7	2.5	67.1
22:0	1.8	1.2	0.4	0.6	2.3	69.3
28:0	1.9	2.5	0.3	0.8	2.1	71.5
Anteiso17:0	0.9	0.5	0.3	0.6	2.1	73.6
22:6*n*-3	1.5	1.7	0.3	0.7	2.0	75.6
30:0	1.2	1.5	0.3	0.6	1.6	77.2
20:2*n*-6	0.4	1.0	0.2	0.4	1.6	78.8
14:0	2.1	1.7	0.2	0.8	1.5	80.3

***Site* groups across all sampled organic *Source* groups in May**

Group E0					
Average similarity: 96.67					
Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	21.1	20.8	1.9	21.5	21.5
16:0	19.4	18.6	1.2	19.3	40.8
18:2*n*-6	13.6	13.3	1.5	13.8	54.6
18:0	7.2	4.9	1.8	7.2	61.8
22:6*n*-3	5.2	5.0	3.7	5.2	66.9
20:5*n*-3	4.4	4.3	2.4	4.4	71.1
14:0	4.2	4.0	1.6	4.2	75.5
18:1*n*-7	3.5	3.5	2.2	3.6	79.1
16:1*n*-7	3.5	3.4	3.1	3.5	82.6

Group E10					
Average similarity: 88.86					
Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
16:0	15.4	14.6	1.3	16.4	16.4
18:1*n*-9	13.4	12.7	2.7	14.3	30.7
20:5*n*-3	8.0	7.8	1.7	8.2	38.9
18:2*n*-6	7.0	6.4	4.3	7.2	46.1
20:4*n*-6	7.6	6.0	1.0	6.8	52.9
22:5*n*-3	6.3	5.8	1.3	6.6	59.5
18:0	6.3	5.3	1.7	5.9	65.3
18:1*n*-7	4.1	3.8	3.4	4.3	69.7
22:6*n*-3	3.8	3.3	2.8	3.7	73.4
16:1*n*-7	2.4	2.7	1.9	3.1	76.5
20:1*n*-9	3.3	2.5	1.6	3.0	79.5
14:0	2.8	2.7	1.3	2.9	82.5

Group E25					
Average similarity: 88.59					
Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
18:2*n*-6	16.4	15.4	0.9	17.3	17.3
18:3*n*-3	14.4	13.4	0.6	15.1	32.4
16:0	13.0	12.1	1.9	13.6	46.0
18:1*n*-9	9.1	8.2	1.3	9.3	55.3
20:4*n*-6	6.1	5.5	0.6	6.2	61.4
20:5*n*-3	5.0	4.5	0.9	5.1	66.5
22:5*n*-3	4.3	3.8	0.8	4.3	70.8
18:0	4.4	3.8	1.3	4.3	75.1
18:1*n*-7	3.4	3.0	1.4	3.3	78.4
16:1*n*-7	2.7	2.3	1.1	2.6	81.0

Group R1					
Average similarity: 85.61					
Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
18:3*n*-3	15.6	15.0	0.6	17.5	17.5
16:0	13.3	12.7	1.9	14.9	32.3
18:2*n*-6	12.3	11.6	0.6	13.6	45.9
20:4*n*-6	7.4	6.5	0.5	7.6	53.5
18:1*n*-7	5.6	4.9	1.0	5.7	59.1
20:5*n*-3	5.6	4.7	0.7	5.4	64.6
16:1*n*-7	4.3	3.8	0.8	4.4	68.9
18:0	5.5	3.0	0.6	3.5	72.5
26:0	2.9	2.5	1.1	2.9	75.4
22:5*n*-3	3.2	2.2	0.5	2.6	78.0
28:0	2.4	1.9	1.3	2.3	80.2

Group R2					
Average similarity: 91.13					
Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
18:3*n*-3	15.3	14.6	0.6	16.1	16.1
16:0	13.7	13.1	1.9	14.4	30.5

18:2*n*-6	12.6	12.1	0.6	13.2	43.7
20:4*n*-6	8.5	7.4	0.6	8.1	51.8
16:1*n*-7	6.0	5.7	0.9	6.3	58.0
20:5*n*-3	5.6	5.0	1.0	5.5	63.5
18:1*n*-7	4.2	4.0	1.1	4.4	67.9
18:0	3.5	2.7	1.2	2.9	70.8
22:5*n*-3	2.8	2.5	0.5	2.7	73.6
28:0	2.4	2.2	1.5	2.4	76.0
18:1*n*-9	2.5	2.1	1.6	2.3	78.3
26:0	2.2	2.0	1.7	2.2	80.5

Groups E0 & E10

Average dissimilarity = 17.79

	Group E0	**Group E10**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
16:0	19.4	15.4	2.7	1.8	15.4	15.4
18:1*n*-9	21.1	13.7	2.5	5.8	1.4	29.2
18:0	7.2	6.3	2.1	1.2	11.8	41.0
14:0	4.2	2.8	1.2	3.6	6.5	47.5
18:2*n*-6	13.6	7.0	1.1	1.5	5.9	53.4
22:6*n*-3	5.2	3.8	0.9	1.4	4.8	58.2
20:2*n*-6	0.5	2.3	0.8	1.0	4.6	62.8
20:4*n*-6	0.8	7.6	0.7	1.1	3.8	66.6
20:5*n*-3	4.4	8.0	0.7	1.7	3.6	70.3
22:5*n*-3	2.7	6.3	0.5	1.6	2.9	73.1
17:1*n*-7	0.3	1.0	0.5	1.1	2.5	75.7
18:1*n*-7	3.5	4.2	0.4	2.0	2.3	78.0
16:1*n*-7	3.5	3.0	0.4	1.1	2.1	80.1

Groups E0 & E25

Average dissimilarity = 24.88

	Group E0	**Group E25**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
16:0	19.4	13.0	5.1	3.3	20.5	20.5
18:1*n*-9	21.1	9.1	2.7	2.1	11.0	31.5

14:0	4.2	1.6	1.7	2.6	6.9	38.3
18:0	7.2	4.4	1.3	1.5	5.2	43.6
18:2*n*-6	13.6	16.4	1.2	1.3	4.9	48.5
22:5*n*-3	2.7	4.3	1.1	2.3	4.2	52.7
18:1*n*-7	3.5	3.4	1.0	2.1	4.1	56.8
16:1*n*-7	3.5	2.7	1.0	3.5	4.1	60.9
20:5*n*-3	4.4	5.0	0.8	2.2	3.2	64.0
20:4*n*-6	0.8	6.1	0.8	1.4	3.1	67.1
22:6*n*-3	5.2	2.1	0.7	1.2	3.0	70.1
20:2*n*-6	0.5	1.0	0.5	0.7	2.0	72.1
26:0	0.3	1.3	0.5	1.2	1.9	74.0
20:1*n*-9	1.8	1.5	0.5	2.0	1.9	75.9
Iso-15:0	0.4	0.7	0.4	1.7	1.6	77.5
28:0	0.2	1.3	0.4	1.2	1.6	79.1
Anteiso15:0	0.4	0.6	0.4	2.2	1.6	80.7

Groups E10 & E25

Average dissimilarity = 17.08

	Group E10	**Group E25**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
16:0	15.4	13.0	1.9	1.0	11.2	11.2
20:4*n*-6	7.6	6.1	1.8	0.8	10.5	21.7
18:1*n*-9	13.7	9.1	1.4	1.5	8.2	29.9
18:0	6.3	4.4	1.3	1.3	7.8	37.6
18:2*n*-6	7.0	16.4	1.0	1.2	6.1	43.7
20:5*n*-3	8.0	5.0	1.0	1.6	5.5	49.2
18:1*n*-7	4.2	3.4	0.7	1.3	4.0	53.2
20:1*n*-9	3.3	1.5	0.7	0.9	3.9	57.1
16:1*n*-7	3.0	2.7	0.7	1.7	3.9	61.0
22:5*n*-3	6.3	4.3	0.6	1.8	3.7	64.7
20:2*n*-6	2.3	1.0	0.4	0.7	2.4	67.1
14:0	2.8	1.6	0.4	1.0	2.3	69.4
22:6*n*-3	3.8	2.1	0.4	1.3	2.1	71.5
26:0	0.4	1.3	0.3	0.8	1.9	73.4
22:4*n*-6	0.8	0.2	0.3	0.7	1.8	75.2
Iso-15:0	0.8	0.7	0.3	1.6	1.6	76.8

Anteiso15:0	0.7	0.6	0.3	1.6	1.6	78.3
28:0	0.2	1.3	0.3	0.7	1.4	79.8
17:1*n*-7	1.0	0.5	0.3	0.6	1.4	81.2

Groups E0 & R1

Average dissimilarity = 46.10

	Group E0	**Group R1**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
16:0	19.4	13.3	4.5	1.9	9.7	9.7
18:0	7.2	5.5	3.9	3.0	8.4	18.2
18:1*n*-9	21.1	2.5	3.8	4.0	8.2	26.3
18:1*n*-7	3.5	5.6	3.6	2.6	7.8	34.2
26:0	0.3	2.9	2.5	1.8	5.5	39.7
16:1*n*-7	3.5	4.3	2.5	1.3	5.3	45.0
20:5*n*-3	4.4	5.6	2.3	3.2	4.9	49.9
18:2*n*-6	13.6	12.3	2.2	6.0	4.7	54.6
28:0	0.2	2.4	1.9	2.4	4.2	58.8
22:5*n*-3	2.7	3.2	1.8	2.7	3.8	62.6
22:0	0.3	1.7	1.6	1.2	3.4	66.0
22:6*n*-3	5.2	1.4	1.3	1.7	2.9	68.9
14:0	4.2	1.9	1.2	1.7	2.6	71.5
20:0	0.8	1.4	1.0	1.5	2.3	73.8
20:4*n*-6	0.8	7.4	1.0	2.5	2.2	75.9
20:1*n*-9	1.8	0.3	0.9	4.8	1.9	77.8
30:0	0.2	0.9	0.8	2.0	1.8	79.6
15:0	0.5	1.1	0.8	1.3	1.6	81.3

Groups E10 & R1

Average dissimilarity = 43.87

	Group E10	**Group R1**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	13.7	2.5	5.4	3.3	12.3	12.3
18:0	6.3	5.5	3.8	1.4	8.6	20.9
20:4*n*-6	7.6	7.4	3.4	1.0	7.8	28.7
18:2*n*-6	7.0	12.3	3.0	3.7	6.9	35.7

20:5*n*-3	8.0	5.6	2.9	2.8	6.5	42.2
18:1*n*-7	4.2	5.6	2.5	1.3	5.7	47.8
16:0	15.4	13.3	2.3	1.1	5.3	53.2
16:1*n*-7	3.0	4.3	1.9	1.2	4.4	57.6
26:0	0.4	2.9	1.7	1.0	3.9	61.5
20:1*n*-9	3.3	0.3	1.5	1.6	3.3	64.8
22:6*n*-3	3.8	1.4	1.3	1.4	3.0	67.9
28:0	0.2	2.4	1.3	1.1	2.9	70.8
22:5*n*-3	6.3	3.2	1.2	2.2	2.7	73.5
22:0	1.0	1.7	1.2	0.9	2.7	76.2
20:2*n*-6	2.3	0.2	1.0	1.1	2.2	78.4
20:0	1.3	1.4	0.9	1.3	2.1	80.5

Groups E25 & R1

Average dissimilarity = 25.91

	Group E25	**Group R1**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	9.1	2.5	3.4	1.2	12.9	12.9
18:2*n*-6	16.4	12.3	2.2	1.6	8.5	21.4
18:0	4.4	5.5	2.1	1.0	8.3	29.6
18:3*n*-3	14.4	15.6	1.5	0.9	5.8	35.5
20:5*n*-3	5.0	5.6	1.4	1.0	5.3	40.8
18:1*n*-7	3.4	5.6	1.3	0.7	4.9	45.7
20:4*n*-6	6.1	7.4	1.2	0.7	4.6	50.3
16:1*n*-7	2.7	4.3	1.1	0.9	4.2	54.5
22:5*n*-3	4.3	3.2	1.1	1.1	4.1	58.6
16:0	13.0	13.3	1.0	1.0	3.7	62.2
26:0	1.3	2.9	0.9	0.7	3.5	65.8
28:0	1.3	2.4	0.8	0.8	2.9	68.7
22:6*n*-3	2.1	1.4	0.7	0.8	2.8	71.4
22:0	0.8	1.7	0.6	0.6	2.4	73.8
20:1*n*-9	1.5	0.3	0.6	1.1	2.4	76.2
20:0	0.7	1.4	0.5	0.7	1.9	78.1
20:2*n*-6	1.0	0.2	0.4	0.7	1.6	79.7
14:0	1.6	1.9	0.4	1.3	1.6	81.3

Groups E0 & R2						
Average dissimilarity = 42.45						
	Group E0	**Group R2**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
16:1*n*-7	3.5	6.0	4.7	7.3	11.0	11.0
16:0	19.4	13.7	4.3	1.9	10.1	21.0
18:1*n*-9	21.1	2.5	3.9	7.3	9.2	30.2
18:0	7.2	3.5	3.8	2.8	9.0	39.2
18:2*n*-6	13.6	12.6	2.0	6.9	4.8	44.0
18:1*n*-7	3.5	4.2	2.0	1.7	4.7	48.7
22:5*n*-3	2.7	2.8	1.7	2.5	4.0	52.7
28:0	0.2	2.4	1.7	9.8	4.0	56.7
26:0	0.3	2.2	1.4	7.5	3.3	60.0
20:5*n*-3	4.4	5.6	1.3	1.9	3.1	63.2
30:0	0.2	1.6	1.3	7.3	3.1	66.2
20:4*n*-6	0.8	8.5	1.2	2.2	2.9	69.1
32:0	0.2	1.2	1.1	7.5	2.6	71.7
22:6*n*-3	5.2	1.5	1.0	2.2	2.2	73.9
20:4*n*-3	0.5	1.0	1.0	1.0	2.2	76.2
20:1*n*-9	1.8	0.2	0.9	4.3	2.1	78.3
14:0	4.2	2.1	0.9	1.3	2.1	80.4

Groups E10 & R2						
Average dissimilarity = 38.98						
	Group E10	**Group R2**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	13.7	2.5	5.5	3.6	14.1	14.1
20:4*n*-6	7.6	8.5	3.7	0.9	9.4	23.4
16:1*n*-7	3.0	6.0	3.4	1.5	8.6	32.0
18:2*n*-6	7.0	12.6	3.0	4.6	7.6	39.6
16:0	15.4	13.7	2.3	1.3	5.9	45.5
18:0	6.3	3.5	2.2	1.7	5.6	51.1
20:1*n*-9	3.3	0.2	1.5	1.6	3.8	54.9
18:1*n*-7	4.2	4.2	1.4	1.4	3.6	58.5
28:0	0.2	2.4	1.1	1.4	2.9	61.4

22:5*n*-3	6.3	2.8	1.1	2.0	2.8	64.2
20:5*n*-3	8.0	5.6	1.0	1.6	2.7	66.9
22:6*n*-3	3.8	1.5	1.0	1.7	2.5	69.4
20:2*n*-6	2.3	0.2	1.0	1.1	2.5	71.9
26:0	0.4	2.2	1.0	1.5	2.5	74.4
30:0	0.3	1.6	0.9	1.3	2.2	76.6
20:4*n*-3	0.5	1.0	0.8	0.9	2.0	78.6
32:0	0.2	1.2	0.7	1.3	1.8	80.4

Groups E25 & R2

Average dissimilarity = 23.03

	Group E25	**Group R2**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	9.1	2.5	3.3	1.2	14.5	14.5
18:2*n*-6	16.4	12.6	2.1	1.7	9.2	23.7
16:1*n*-7	2.7	6.0	1.9	0.9	8.2	31.9
18:0	4.4	3.5	1.6	1.3	7.1	39.0
20:4*n*-6	6.1	8.5	1.4	0.6	6.0	45.0
18:3*n*-3	14.4	15.3	1.2	0.9	5.2	50.2
16:0	13.0	13.7	0.9	0.8	3.8	53.9
22:5*n*-3	4.3	2.8	0.8	1.2	3.6	57.5
18:1*n*-7	3.4	4.2	0.7	1.0	3.1	60.6
28:0	1.3	2.4	0.6	0.9	2.7	63.3
20:1*n*-9	1.5	0.2	0.6	1.1	2.7	66.0
20:4*n*-3	0.3	1.0	0.5	0.6	2.3	68.3
20:5*n*-3	5.0	5.6	0.5	0.8	2.3	70.6
14:0	1.6	2.1	0.5	1.4	2.2	72.8
22:6*n*-3	2.1	1.5	0.5	0.9	2.1	74.9
26:0	1.3	2.2	0.5	0.9	2.1	77.0
30:0	0.8	1.6	0.5	0.9	2.0	78.9
20:2*n*-6	1.0	0.2	0.4	0.7	1.8	80.7

Groups R1 & R2						
Average dissimilarity = 16.22						
	Group R1	**Group R2**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:0	5.5	3.5	2.1	0.7	13.0	13.0
20:5*n*-3	5.6	5.6	1.6	1.0	9.6	22.7
16:1*n*-7	4.3	6.0	1.1	0.8	6.9	29.5
20:4*n*-6	7.4	8.5	0.9	0.7	5.6	35.2
18:1*n*-7	5.6	4.2	0.9	0.6	5.6	40.8
18:3*n*-3	15.6	15.3	0.6	0.7	3.9	44.7
22:5*n*-3	3.2	2.8	0.6	0.6	3.7	48.4
26:0	2.9	2.2	0.6	0.6	3.6	52.0
18:1*n*-9	12.3	12.6	0.5	0.8	3.3	55.4
16:0	13.3	13.7	0.5	1.3	3.2	58.5
20:4*n*-3	0.0	1.0	0.5	0.5	3.1	61.7
18:1*n*-9	2.5	2.5	0.5	0.9	2.9	64.5
22:0	1.7	1.1	0.5	0.6	2.8	67.3
20:0	1.4	0.8	0.4	0.6	2.6	69.9
30:0	0.9	1.6	0.4	0.6	2.6	72.5
22:6*n*-3	1.4	1.5	0.4	1.0	2.3	74.8
28:0	2.4	2.4	0.3	0.7	2.1	76.9
32:0	0.6	1.2	0.3	0.6	2.0	78.9
18:4*n*-3	0.5	0.8	0.3	0.7	1.8	80.7

***Site* groups across all sampled organic *Source* groups in September**

Group E0					
Average similarity: 96.68					
Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	25.1	24.2	2.5	25.0	25.0
18:2*n*-6	21.0	21.2	3.2	21.9	46.9
16:0	15.4	15.2	2.8	15.8	62.7
18:3*n*-3	5.0	5.0	1.9	5.2	67.9

22:6*n*-3	5.3	4.7	2.2	4.9	72.7
18:0	4.8	4.4	2.3	4.6	77.3
20:5*n*-3	3.6	3.5	1.6	3.6	80.9

Group E10

Average similarity: 92.62

Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	14.7	13.8	1.4	14.9	14.9
16:0	12.3	11.9	1.5	12.8	27.7
18:2*n*-6	9.9	9.3	2.1	10.1	37.8
20:4*n*-6	9.6	8.2	1.0	8.9	46.7
22:5*n*-3	7.6	7.4	1.2	8.0	54.7
18:0	7.5	6.8	2.4	7.4	62.0
20:5*n*-3	5.9	5.3	1.3	5.7	67.8
18:1*n*-7	3.9	3.5	4.4	3.8	71.6
20:1*n*-9	3.1	2.8	3.4	3.1	74.6
22:0	2.6	2.6	2.4	2.8	77.4
22:6*n*-3	2.4	2.1	3.4	2.3	79.7
16:1*n*-7	2.3	2.1	3.0	2.3	82.0

Group E25

Average similarity: 90.83

Species	**Average Abundance**	**Average Similarity**	**Sim/SD**	**Contribution %**	**Cumulative %**
18:2*n*-6	19.9	21.6	1.3	22:6*n*-3	23.8
16:0	18.3	18.4	3.3	20.3	44.0
18:3*n*-3	9.5	10.2	0.8	11.2	55.2
18:0	9.0	7.2	1.6	7.9	63.2
18:1*n*-9	7.5	7.1	1.2	7.8	70.9
18:1*n*-7	3.3	2.6	1.1	2.8	73.8
16:1*n*-7	2.5	2.1	1.0	2.3	76.1
20:4*n*-6	4.1	2.1	0.4	2.3	78.3
14:0	1.9	1.7	1.2	1.4	80.2

Group R1

Average similarity: 86.01

Species	Average Abundance	Average Similarity	Sim/SD	Contribution %	Cumulative %
18:2*n*-6	19.6	19.2	0.9	22.3	22.3
16:0	19.9	18.6	4.4	21.6	43.9
18:3*n*-3	14.0	13.1	0.8	15.2	59.2
18:0	11.6	7.6	1.4	8.8	68.0
16:1*n*-7	4.8	3.9	1.0	4.6	72.5
18:1*n*-7	4.0	3.3	1.1	3.8	76.3
18:1*n*-9	2.8	2.6	4.0	3.0	79.3
20:5*n*-3	3.1	2.4	0.9	2.8	82.1

Group R2

Average similarity: 81.12

Species	Average Abundance	Average Similarity	Sim/SD	Contribution %	Cumulative %
18:2*n*-6	13.3	11.5	0.7	14.2	14.2
20:4*n*-6	9.5	10.0	0.8	12.4	26.6
16:0	13.6	9.6	1.3	11.9	38.5
18:3*n*-3	8.5	7.0	0.6	8.6	47.1
20:5*n*-3	5.5	5.7	0.9	7.0	54.0
18:1*n*-7	5.5	4.7	1.0	5.8	59.8
18:0	7.7	4.0	0.9	4.9	64.7
22:5*n*-3	3.8	3.8	0.8	4.7	69.4
16:1*n*-7	4.2	3.4	0.9	4.2	73.6
18:1*n*-9	2.7	2.0	1.5	2.5	76.1
14:0	2.4	1.8	0.9	2.2	78.3
17:0	1.8	1.5	1.9	1.8	80.1

Group E0 & E10

Average similarity: 11.77

	Group E0	Group E10				
Species	Average Abundance	Average Abundance	Average Dissimilarity	Diss/SD	Contribution %	Cumulative %
16:0	15.4	12.3	2.7	9.8	22.6	22.6
18:1*n*-9	25.1	14.7	1.5	1.6	12.8	35.3

22:0	0.6	2.6	1.0	15.0	8.3	43.7
18:2*n*-6	21.0	9.9	0.9	3.3	7.9	51.6
18:0	4.8	7.5	0.7	2.3	6.0	57.6
14:0	2.3	1.8	0.6	10.0	5.3	62.9
16:1*n*-7	2.3	2.3	0.5	8.6	4.2	67.1
22:6*n*-3	5.3	2.4	0.5	7.4	3.9	71.1
Iso-15:0	0.3	1.8	0.5	11.3	3.8	74.9
20:5*n*-3	3.6	5.9	0.4	6.8	3.0	77.9
20:1*n*-9	2.3	3.1	0.3	6.6	2.6	80.4

Group E0 & E25

Average similarity: 17.35

	Group E0	**Group E25**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	25.1	7.5	5.1	6.0	29.6	29.6
22:0	0.6	1.6	1.3	11.4	7.6	37.2
18:0	4.8	9.0	1.3	4.0	7.4	44.6
16:1*n*-7	2.3	2.5	1.1	23.8	6.3	50.9
16:0	15.4	18.3	1.1	4.3	6.2	57.1
18:2*n*-6	21.0	19.9	1.0	11.2	5.9	63.0
20:1*n*-9	2.3	0.9	0.7	15.7	3.9	66.9
Iso-15:0	0.3	1.1	0.6	9.8	3.6	70.5
22:6*n*-3	5.3	1.3	0.5	6.5	3.1	73.6
20:5*n*-3	3.6	2.1	0.5	4.7	3.0	76.6
20:0	0.6	0.9	0.4	5.0	2.1	78.7
17:0	0.7	1.4	0.3	9.6	1.7	80.3

Group E10 & E25

Average similarity: 15.54

	Group E10	**Group E25**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	14.7	7.5	2.9	2.6	18.9	18.9
18:2*n*-6	9.9	19.9	1.6	2.7	10.5	29.4
16:0	12.3	18.3	1.4	3.6	9.2	38.5
20:4*n*-6	9.6	4.1	1.2	0.7	7.8	46.4

18:0	7.5	9.0	1.1	1.3	7.1	53.5
20:5*n*-3	5.9	2.1	0.6	0.8	3.8	57.3
16:1*n*-7	2.3	2.5	0.5	2.3	3.1	60.4
18:1*n*-7	3.9	3.3	0.5	2.2	3.0	63.4
17:0	1.1	1.4	0.4	1.8	2.6	66.0
20:1*n*-9	3.1	0.9	0.4	2.5	2.4	68.4
14:0	1.8	1.9	0.4	1.5	2.3	70.7
22:0	2.6	1.6	0.3	2.3	2.1	72.9
22:4*n*-6	0.5	0.5	0.3	0.8	2.0	74.9
24:0	0.6	1.1	0.3	1.3	1.7	76.5
22:5*n*-3	7.6	2.7	0.2	0.9	1.5	78.1
18:4*n*-3	0.1	0.2	0.2	2.3	1.5	79.6
20:2*n*-6	1.8	0.4	0.2	0.8	1.5	81.1

Groups E0 & R1

Average dissimilarity = 46.87

	Group E0	**Group R1**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	25.1	2.8	11.3	13.1	24.2	24.2
18:2*n*-6	21.0	19.6	5.1	14.9	10.8	35.0
18:0	4.8	11.6	3.8	1.1	8.1	43.1
16:1*n*-7	2.3	4.8	3.0	3.0	6.3	49.4
16:0	15.4	19.9	2.8	1.3	5.9	55.3
10:0	0.1	1.2	2.4	1.7	5.0	60.3
18:1*n*-7	3.2	4.0	2.2	3.0	4.7	65.0
20:5*n*-3	3.6	3.1	2.2	2.7	4.7	69.7
22:6*n*-3	5.3	1.5	1.8	1.9	3.8	73.5
20:1*n*-9	2.3	0.1	1.3	28.1	2.7	76.2
8:0	0.1	0.6	0.9	1.2	2.0	78.2
18:4*n*-3	0.7	0.5	0.7	8.4	1.4	79.6
30:0	0.0	0.8	0.7	5.6	1.4	81.0

Groups E10 & R1

Average dissimilarity = 44.75

	Group E10	Group R1				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	14.7	2.8	9.9	15.2	22.1	22.1
18:2*n*-6	9.9	19.6	6.0	14.2	13.4	35.5
18:0	7.5	11.6	3.4	1.1	7.7	43.2
16:*1n*-7	2.3	4.8	2.5	2.5	5.5	48.7
18:1*n*-7	3.9	4.0	2.4	3.3	5.4	54.1
10:0	0.1	1.2	2.3	1.7	5.2	59.2
20:5*n*-3	5.9	3.1	1.8	2.2	4.1	63.3
16:0	12.3	19.9	1.8	1.6	4.1	67.4
22:0	2.6	0.2	1.6	10.6	3.5	70.8
22:6*n*-3	2.4	1.5	1.3	1.4	2.9	73.8
14:0	1.8	2.4	1.2	5.6	2.7	76.4
20:1*n*-9	3.1	0.1	1.0	42.7	2.1	78.6
8:0	0.0	0.6	0.9	1.2	2.1	80.6

Groups E25 & R1

Average dissimilarity = 21.77

	Group E25	Group R1				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	7.5	2.8	2.8	1.2	12.9	12.9
18:3*n*-3	9.5	14.0	2.6	1.1	11.7	24.7
18:0	9.0	11.6	2.5	0.9	11.5	36.1
18:2*n*-6	19.9	19.6	2.1	1.3	9.6	45.7
16:0	18.3	19.9	1.6	1.3	7.3	53.0
16:1*n*-7	2.5	4.8	1.0	0.8	4.6	57.6
20:5*n*-3	2.1	3.1	0.9	0.7	3.9	61.5
18:1*n*-7	3.3	4.0	0.8	0.9	3.8	65.2
22:0	1.6	0.2	0.6	0.8	2.9	68.1
10:0	0.2	1.2	0.6	0.5	2.8	70.9
22:6*n*-3	1.3	1.5	0.5	0.7	2.4	73.3
Iso-16:0	0.8	0.3	0.4	1.2	1.9	75.2
28:0	1.2	1.2	0.4	1.4	1.7	76.9

26:0	0.8	1.3	0.3	1.6	1.5	78.4
32:0	0.8	0.8	0.3	1.0	1.3	79.7
14:0	1.9	2.4	0.3	1.0	1.3	81.0

Groups E0 & R2

Average dissimilarity = 55.16

	Group E0	**Group R2**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	25.1	2.7	12.0	12.4	21.8	21.8
18:0	4.8	7.7	8.7	1.1	15.8	37.6
16:0	15.4	13.6	5.2	1.1	9.4	47.0
18:2*n*-6	21.0	13.3	5.2	21.0	9.3	56.3
18:1*n*-7	3.2	5.5	5.0	6.6	9.1	65.4
16:1*n*-7	2.3	4.2	2.9	8.8	5.2	70.6
22:5*n*-3	2.0	3.8	1.4	1.5	2.5	73.1
20:1*n*-9	2.3	0.6	1.2	5.9	2.3	75.4
14:0	2.3	2.4	1.2	1.7	2.1	77.5
22:6*n*-3	5.3	1.4	1.1	1.4	1.9	79.4
17:0	0.7	1.8	0.9	2.1	1.7	81.1

Groups E10 & R2

Average dissimilarity = 39.47

	Group E10	**Group R2**				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:1*n*-9	14.7	2.7	5.9	1.4	15.0	15.0
20:4*n*-6	9.6	9.5	4.4	0.9	11.2	26.2
18:0	7.5	7.7	4.3	0.7	10.9	37.2
18:2*n*-6	9.9	13.3	4.1	2.2	10.3	47.5
16:0	12.3	13.6	3.0	1.0	7.5	55.0
18:1*n*-7	3.9	5.5	2.7	1.1	6.8	61.7
20:5*n*-3	5.9	5.5	1.8	1.2	4.6	66.4
16:1*n*-7	2.3	4.2	1.4	1.5	3.6	69.9
22:5*n*-3	7.6	3.8	1.2	1.7	2.9	72.8
22:0	2.6	1.1	1.0	1.5	2.6	75.4
20:1*n*-9	3.1	0.6	1.0	3.7	2.5	77.7

14:0	1.8	2.4	1.0	1.1	2.4	80.3

Groups E25 & R2

Average dissimilarity = 27.55

	Group E25	Group R2				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:0	9.0	7.7	3.9	0.9	14.1	14.1
16:0	18.3	13.6	2.5	0.9	9.0	23.1
18:2*n*-6	19.9	13.3	2.4	1.6	8.8	31.9
18:1*n*-9	7.5	2.7	2.4	0.9	8.6	40.5
20:4*n*-6	4.1	9.5	1.3	0.5	4.9	45.3
18:1n7	3.3	5.5	1.3	0.7	4.8	50.2
20:5*n*-3	2.1	5.5	1.2	0.7	4.4	54.6
18:3*n*-3	9.5	8.5	1.1	1.0	3.9	58.4
16:1*n*-7	2.5	4.2	0.9	1.1	3.4	61.8
22:0	1.6	1.1	0.7	0.8	2.4	64.1
22:5*n*-3	2.7	3.8	0.6	0.8	2.1	66.2
32:0	0.8	1.4	0.6	1.1	2.0	68.2
17:1*n*-7	0.4	0.9	0.5	0.7	1.8	70.0
17:0	1.4	1.8	0.5	1.3	1.7	71.7
14:0	1.9	2.4	0.5	0.8	1.7	73.3
Iso-15:0	1.1	0.8	0.4	1.1	1.6	74.9
22:6*n*-3	1.3	1.4	0.4	1.0	1.5	76.5
24:0	1.1	1.4	0.4	1.6	1.5	78.0
22:4*n*-6	0.5	0.7	0.4	1.2	1.4	79.4
2-OH14:0	0.3	0.9	0.4	0.4	1.4	80.8

Groups R1 & R2

Average dissimilarity = 24.15

	Group R1	Group R2				
Species	**Average Abundance**	**Average Abundance**	**Average Dissimilarity**	**Diss/SD**	**Contribution %**	**Cumulative %**
18:0	11.6	7.7	4.6	0.8	19.1	19.1
16:0	19.9	13.6	3.1	1.1	12.8	31.9
18:3*n*-3	14.0	8.5	1.7	1.1	7.0	38.9
18:2*n*-6	19.6	13.3	1.4	0.7	5.9	44.8

18:1*n*-7	4.0	5.5	1.1	0.8	4.7	49.5
32:0	0.8	1.4	0.7	1.5	3.1	52.6
20:5*n*-3	3.1	5.5	0.7	0.8	2.8	55.3
28:0	1.2	1.5	0.7	1.9	2.8	58.1
16:1*n*-7	4.8	4.2	0.6	0.9	2.5	60.6
18:1*n*-9	2.8	2.7	0.6	1.4	2.5	63.1
30:0	0.8	0.8	0.5	0.9	2.3	65.3
22:5*n*-3	0.3	3.8	0.5	0.7	2.2	67.5
17:1*n*-7	0.2	0.9	0.5	0.7	2.2	69.7
22:6*n*-3	1.5	1.4	0.5	0.8	1.9	71.6
2-OH14:0	0.4	0.9	0.5	0.4	1.9	73.5
10:0	1.2	0.4	0.4	0.5	1.8	75.3
24:0	0.9	1.4	0.4	1.2	1.8	77.0
Iso-15:0	0.6	0.8	0.4	1.2	1.7	78.7
26:0	1.3	1.0	0.4	1.1	1.6	80.3

Appendix 6.4. Lipid content and fatty acid composition of *Holothuria poli* in IMTA (E10 and E25) and at reference sites (R1 and R2), in February, May and September.

	February			
	E10	**E25**	**R1**	**R2**
n	3	3	3	3
Lipid (mg 100g^{-1})	29.2 ± 21.8	40.4 ± 5.7	27.5 ± 11.1	28.9 ± 4.4
FA content (mg 100g $^{-1}$)				
14:0	4.0 ± 1.9	7.7 ± 1.0	5.4 ± 1.3	4.5 ± 2.8
16:0	23.3 ± 11.4^{ab}	29.6 ± 4.6^{a}	14.0 ± 1.6^{ab}	7.2 ± 0.7^{b}
18:0	29.3 ± 15.1	27.5 ± 2.9	25.1 ± 3.5	15.6 ± 1.0
20:0	11.1 ± 4.5	13.5 ± 1.2	12.3 ± 3.3	12.5 ± 2.9
22:0	15.8 ± 8.4	18.3 ± 1.2	17.4 ± 4.9	17.7 ± 1.2
Total saturated [1]	***123.6 ± 49.0***	***152.4 ± 21.1***	***110.6 ± 18.2***	***87.3 ± 13.0***
16:1*n*-7	9.6 ± 2.8	18.0 ± 4.5	10.8 ± 2.4	6.8 ± 3.2
18:1*n*-9	87.9 ± 32.7^{a}	98.8 ± 8.7^{a}	5.8 ± 0.7^{b}	8.5 ± 1.0^{b}
18:1*n*-7	25.9 ± 6.7^{ab}	31.7 ± 7.7^{a}	14.9 ± 3.0^{bc}	11.1 ± 2.9^{c}
20:1*n*-9	58.8 ± 21.7^{a}	58.4 ± 7.9^{a}	6.9 ± 0.5^{b}	6.9 ± 0.9^{b}
22:1*n*-9	18.4 ± 7.2^{a}	18.7 ± 1.4^{a}	2.4 ± 0.3^{b}	3.1 ± 0.7^{b}
Total monenes	***200.6 ± 3.5 ^{a}***	***225.6 ± 28.0 ^{a}***	***40.8 ± 1.7 ^{b}***	***36.4 ± 6.8 ^{b}***
18:2*n*-6	65.3 ± 23.9	59.1 ± 7.3	6.5 ± 0.5^{a}	7.5 ± 0.2^{a}
20:2*n*-6	21.4 ± 7.7^{ab}	23.0 ± 1.8^{a}	10.0 ± 1.0	10.6 ± 2.6^{b}
20:3*n*-6	15.2 ± 7.2	18.7 ± 3.3	16.5 ± 4.5	16.3 ± 3.4
20:4*n*-6	160.2 ± 81.6	197.9 ± 10.5	201.2 ± 52.2	199.1 ± 17.7
Total n-6 PUFA [2]	***267.3 ± 119.6***	***304.4 ± 22.6***	***238.2 ± 58.1***	***237.8 ± 24.0***
18:3*n*-3	12.0 ± 5.0^{a}	11.8 ± 1.2^{a}	1.3 ± 0.4^{b}	1.2 ± 0.1^{b}
20:5*n*-3 (EPA)	101 ± 30.6	141.6 ± 13	119.9 ± 11.7	109.2 ± 19.8
22:5*n*-3 (DPA)	113.2 ± 44.2^{ab}	123.2 ± 11.6^{a}	79.1 ± 14.1^{ab}	78.8 ± 13.0^{b}
22:6*n*-3 (DHA)	20.7 ± 6.4^{a}	27.8 ± 5.2^{a}	7.3 ± 0.7^{b}	4.6 ± 1.1^{b}
Total n-3 PUFA [3]	***256.5 ± 87.3***	***316 ± 30.1***	***218.1 ± 25.3***	***204.2 ± 34.0***
Total PUFA	***523.8 ± 206.9 ^{a}***	***620.4 ± 50.4 ^{a}***	***456.2 ± 82.5 ^{b}***	***442.1 ± 57.9 ^{b}***

Means (± standard deviation) assigned different superscript letters in the same row are significantly different (ANOVA, $p < 0.05$).

[1]Includes Iso-15:0, Anteiso 15:0, 15:0, Iso-16:0, Iso-17:0, Anteiso 17:0, 17:0, 19:0, 21:0, 24:0; [2] Includes 22:4*n*-6; [3] Includes 18:4*n*-3, 20:3*n*-3, 20:4*n*-3.

	May			
	E10	**E25**	**R1**	**R2**
n	3	3	3	3
Lipid (mg 100g^{-1})	20.4 ± 5.3	25.8 ± 10.1	12.7 ± 2.9	19.3 ± 5.2
FA content (mg 100g $^{-1}$)				
14:0	5.8 ± 5.4	4.7 ± 2.8	3.0 ± 3.5	2.8 ± 2.1
16:0	29.4 ± 27.9	22 ± 11.7	8.4 ± 6.0	13.8 ± 11.6
18:0	32.5 ± 32.2	27.1 ± 12.1	13.8 ± 9.2	31.5 ± 24.3
20:0	11.4 ± 5.5	12.9 ± 6.8	6.4 ± 4.1	10.3 ± 7.1
22:0	13.3 ± 6.5	15.0 ± 6.7	7.1 ± 5.6	11.3 ± 7.1
Total saturated [1]	***140.8 ± 113.8***	***132.2 ± 75.7***	***56.5 ± 39.7***	***93.7 ± 67.8***
16:1*n*-7	10.8 ± 10.4	11.2 ± 9.1	8.2 ± 10.3	4.0 ± 3.0
18:1*n*-9	74.6 ± 60.9^{a}	33.8 ± 13.0^{ab}	3.7 ± 3.0^{b}	4.2 ± 2.5^{b}
18:1*n*-7	26.6 ± 21.0	22.6 ± 16.0	8.5 ± 7.9	8.7 ± 5.6
20:1*n*-9	52.4 ± 46.3^{a}	24.0 ± 12.9^{ab}	3.4 ± 3.5^{b}	4.5 ± 3.4^{b}
22:1*n*-9	14.1 ± 12.0^{a}	8.9 ± 4.4^{ab}	1.1 ± 1.4^{b}	2.4 ± 0.7^{ab}
Total monenes	***178.5 ± 150.4 [a]***	***100.5 ± 55.4 [ab]***	***24.9 ± 26.1 [b]***	***23.8 ± 14.7 [b]***
18:2*n*-6	53.9 ± 41.9	24.6 ± 6.4	2.9 ± 2.5^{a}	3.6 ± 2.7^{a}
20:2*n*-6	19.5 ± 15.9^{a}	13.1 ± 5.9^{ab}	4.5 ± 5.7^{ab}	4.8 ± 3.5^{b}
20:3*n*-6	12.8 ± 6.3	14.3 ± 4.2	7.0 ± 5.3	11.5 ± 8.3
20:4*n*-6	117.5 ± 48.8	166 ± 53.9	93.0 ± 62.1	132.8 ± 84.0
Total n-6 PUFA [2]	***208.7 ± 115.3***	***222.1 ± 71.6***	***109.7 ± 76.9***	***155.8 ± 99.9***
18:3*n*-3	9.7 ± 9.7	4.3 ± 1.6	0.7 ± 0.7	1.3 ± 1.3
20:5*n*-3 (EPA)	101.5 ± 53	94.4 ± 34.7	63.2 ± 62.2	58.5 ± 38.4
22:5*n*-3 (DPA)	92.3 ± 52.4	86.5 ± 31.9	38.9 ± 37.0	50.3 ± 31.7
22:6*n*-3 (DHA)	20.7 ± 18.2	13.1 ± 5.3	4.7 ± 4.8	3.9 ± 3.3
Total n-3 PUFA [3]	***231.2 ± 134.5***	***205.4 ± 75.5***	***111.8 ± 109.2***	***120.2 ± 79.9***
Total PUFA	***439.9 ± 249.7***	***427.4 ± 147.1***	***221.5 ± 186***	***276.1 ± 179.1***

Means (± standard deviation) assigned different superscript letters in the same row are significantly different (ANOVA, $p < 0.05$).

[1]Includes Iso-15:0, Anteiso 15:0, 15:0, Iso-16:0, Iso-17:0, Anteiso 17:0, 17:0, 19:0, 21:0, 24:0; [2] Includes 22:4*n*-6; [3] Includes 18:4*n*-3, 20:3*n*-3, 20:4*n*-3.

	September								
	E10			**E25**			**R2**		
n	3			2			3		
Lipid (mg 100g^{-1})	41.3	±	16.6	20.2	±	6.0	23.3	±	2.0
FA content (mg 100g $^{-1}$)									
14:0	6.7	±	2.5	3.5	±	1.4	3.3	±	2.6
16:0	35.2	±	9.0	17.3	±	13.6	10.0	±	3.6
18:0	35.7	±	0.6	19.1	±	20.5	15.0	±	7.6
20:0	10.1	±	2.7	5.8	±	0.7	6.7	±	2.8
22:0	11.5	±	4.7	7.4	±	0.3	8.2	±	3.2
Total saturated [1]	***161.9***	***±***	***50.1***	***96.0***	***±***	***35.7***	***67.8***	***±***	***19.6***
16:1*n*-7	12.8	±	8.3	4.0	±	1.4	6.8	±	3.2
18:1*n*-9	42.1	±	21.4	7.8	±	2.9^{a}	2.4	±	0.4^{a}
18:1*n*-7	35.3	±	21.4	14.8	±	5.1	9.7	±	5.6
20:1*n*-9	27.9	±	13.1^{a}	10.7	±	2.7^{ab}	6.1	±	3.1^{b}
22:1*n*-9	9.2	±	3.5^{a}	2.5	±	2.0^{ab}	1.0	±	0.8^{b}
Total monenes	***127.3***	***±***	***67.3 ^{a}***	***39.8***	***±***	***14.1 ^{ab}***	***26.0***	***±***	***7.6 ^{b}***
18:2*n*-6	42.7	±	20.1^{a}	12.5	±	5.7^{a}	2.3	±	2.0
20:2*n*-6	22.8	±	11.9^{a}	7.0	±	2.5^{ab}	4.4	±	1.6^{b}
20:3*n*-6	17.2	±	5.6	9.7	±	2.3	9.7	±	3.6
20:4*n*-6	122.9	±	25.2	72.2	±	9.2	89.4	±	32.2
Total n-6 PUFA [2]	***211.4***	***±***	***63.9***	***109.1***	***±***	***2.1***	***109.6***	***±***	***22.9***
18:3*n*-3	6.2	±	3.1^{a}	2.3	±	1.7^{ab}	0.7	±	0.6^{b}
20:5*n*-3 (EPA)	70.8	±	38.9	23.9	±	0.1	41.4	±	23.3
22:5*n*-3 (DPA)	92.1	±	40.6	42.1	±	2.6	34.3	±	19.1
22:6*n*-3 (DHA)	15.5	±	12.4	4.6	±	0.8	5.0	±	4.5
Total n-3 PUFA [3]	***187.1***	***±***	***96.8***	***75.5***	***±***	***7.1***	***83.3***	***±***	***27.4***
Total PUFA	***398.4***	***±***	***160.7***	***184.5***	***±***	***5.0***	***192.9***	***±***	***58.2***

Means (± standard deviation) assigned different superscript letters in the same row are significantly different (ANOVA, $p < 0.05$).

[1]Includes Iso-15:0, Anteiso 15:0, 15:0, Iso-16:0, Iso-17:0, Anteiso 17:0, 17:0, 19:0, 21:0, 24:0; [2] Includes 22:4*n*-6; [3] Includes 18:4*n*-3, 20:3*n*-3, 20:4*n*-3.