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DEFINITION OF INNOVATIVE TECHNOLOGIES FOR MONITORING IMPACT AND BIOREMEDIATION INTERVENTIONS ON MARINE AQUACULTURE

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Sommario

SUMMARY

Aquaculture production has grown rapidly and significantly, overtaking fisheries production.

Organic loading from aquaculture activities, can impact on surrounding environments. The principal causes of this organic loading is due to uneaten food and excretory products from fish farm (Mavraganis *et al*., 2020).

As a result of the deposition of organic matter in the surface sediments located beneath fish cages, there is a reduction of oxygen penetration and an increase of mineralization rates (Tičina *et al*., 2020).

Nowadays there is a general agreement that production systems such as aquaculture should be sustainable (Valenti *et al*., 2018).

Because of increasing consumer demand for sustainably produced products, aquaculture has had the positive effect of significantly improving its environmental performance.

Large-scale retail production specifications have also introduced the evaluation of the effect of fish farming on the surrounding environment. Therefore, companies have started a series of paths that are leading them to a system of evaluation of the effects on the environment, allowing to monitor some environmental descriptors.

During the three years of my PhD thesis, I performed several experiments with the main goal of defining and validating techniques for monitoring the effects of aquaculture on the surrounding environment and identifying useful descriptors for aquaculture industries to allow them to independently conduct monitoring to ensure sustainable production.

For this purpose, I focused my attention on the Bluefin tuna facility located in the Gulf of Castellammare del Golfo (Sicily, Italy), operated from 2001 to 2009, investigating whether there were significant differences in the stations placed under or near the cages and control stations, for the considered indicators.

Organic matter (OM) over deposition can lead to hypoxic/anoxic conditions in the surface sediment and under these conditions sulfate-reducing bacteria decompose OM and produce free S_2^-. For this reason, in **Chapter 2** of this thesis, I validated the ion-selective electrode technique, which specifically detects S_2^-.

The results showed how the sulphides are sensitive and cost-effective chemical indicators of benthic organic enrichment. In fact, free sulphide is one of the most widely used geochemical variables for classifying the impact of organic matter deposition (Cranford *et al*., 2020).

Chapter 3 focused on the validation of a tool that would allow the simultaneous assessment of several descriptor parameters of environmental quality. Using near-infrared spectroscopy (NIRs) I was able to analyze parameters such as lipids, proteins, carbohydrates and the total organic matter (TOM) content of marine sediments, simply placing the dried sediment sample in a clear glass Petri dish placed in the NIRs measurement cell.

The results highlighted how this instrumental technique is reliable and transferable at aquaculture facilities, useful to quickly measure several parameters of marine sediments not using chemical laboratory analyses.

In **Chapter 4** of this thesis, I validated the gas chromatography technique, useful for determining the fatty acid profile.

Lipids is one of the main components of fish feed and Fatty acids are the common structural components of most lipids. For this reason in this part of the thesis I validated the gas chromatography technique.

The results showed that some fatty acids were present in the fatty acid profile of the station under the cages, whereas they were absent or less represented in the control station. This analysis is useful to trace aquaculture waste.

Chapter 5 is focused on validation of remote sensing technique, the methodology that allows to observe an object from space, to study its characteristics through the analysis and processing of electromagnetic radiation radiated by it and received by sensors on satellites.

This technique is useful for investigating chlorophyll-a (Chl-a) concentrations. In fact, Chl-a concentrations are used as early warning indicators to detect eutrophic trends in the marine environment, as phytoplankton growth is related to nutrient availability.

Remote sensing technique has proven useful for tracking and assessing the status and dynamics of aquaculture, and allows for low-cost, multi-temporal assessment compared to long-term field sampling.

CHAPTER 1

1. GENERAL INTRODUCTION

1.1. The State of World Fisheries and Aquaculture

Global demand for seafood is constantly growing, according to the most recent Food and Agriculture Organization (FAO) report, to meet the growth of the world population (FAO, 2020). It is forecasted that the world population will reach 8.5 billion in 2030. As fisheries cannot meet the ever-increasing demand for fish products, aquaculture will play an important role in compensating for this demand (FAO, 2020).

Global production of fish was about 179 million tons (mt) in 2018 (Figure 1.1), of which 82 mt were from aquaculture production (FAO, 2020). By 2029, annual aquaculture production is expected to exceed 100 mt, overcoming the capture sector (OECD & FAO, 2020).

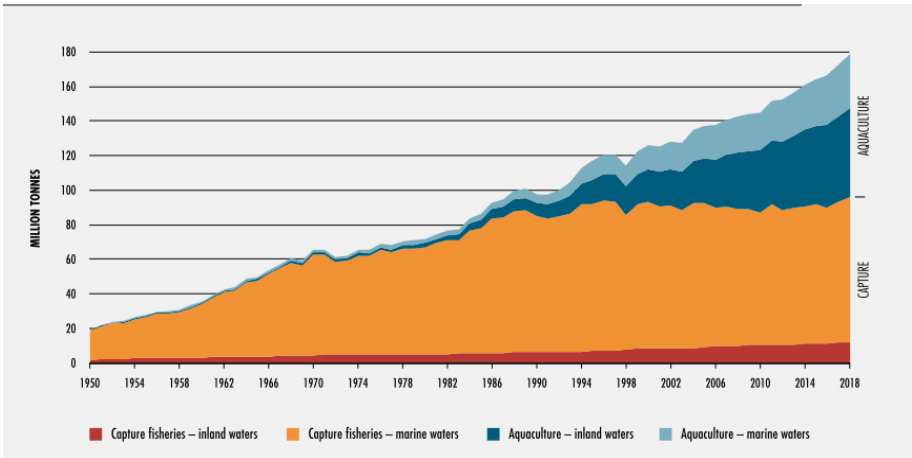

Figure 1.1 *World capture fisheries and aquaculture production. Source: (FAO, 2020)*

Global aquaculture production reached 29 mt in 1997, a threefold increase from 10 mt in 1987, and was based on about 300 animal and plant species (Naylor *et al.*, 2021) (Figure 1.2).

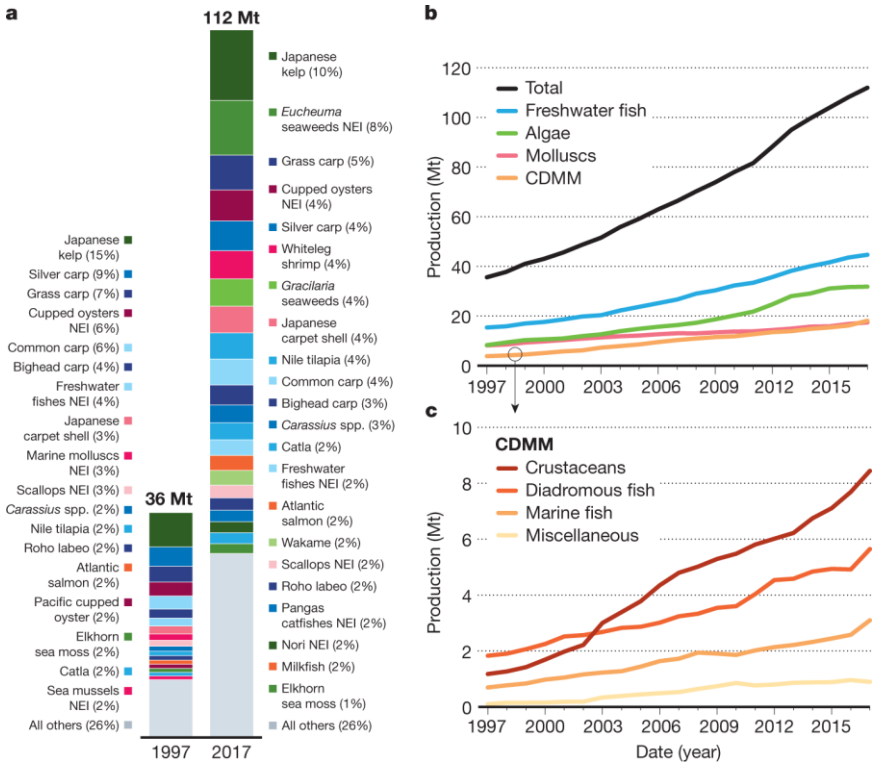

Figure 1.2. *Composition and growth of global live-weight aquaculture production. Source (Naylor et al., 2021)*

Over the next 20 years the growth trend was more rapid, reaching production values of 114.5 mt in 2018 (Figure 1.3), with a value of $263.6 billion. Total production consisted of 82.1 mt of aquatic animals ($250.1 billion), 32.4 mt of aquatic algae ($13.3 billion) and 26.000 t of ornamental shells and pearls ($179.000) (FAO, 2020).

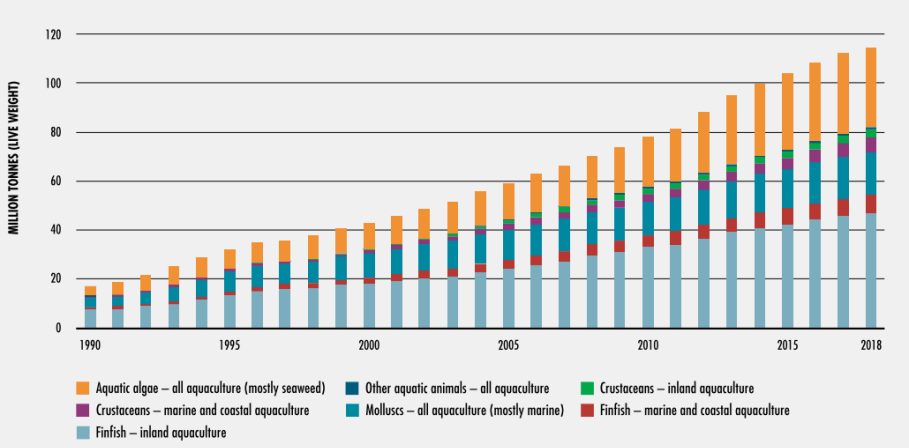

Figure 1.3. *World aquaculture production of aquatic animals and algae, 1990–2018. Source: (FAO, 2020)*

The contribution of aquaculture to the total aquatic products consumed by humans, from less than 5% in 1970 reached almost 50% in 2018, and in particular there has been an increase from 2006 to 2018 of 32% of the 622 farmed species involved in aquaculture production (FAO, 2020).

Aquaculture, at regional level, contributed for 16-18% of European Union (EU) total fish production (Figure 1.4) (FAO, 2020).

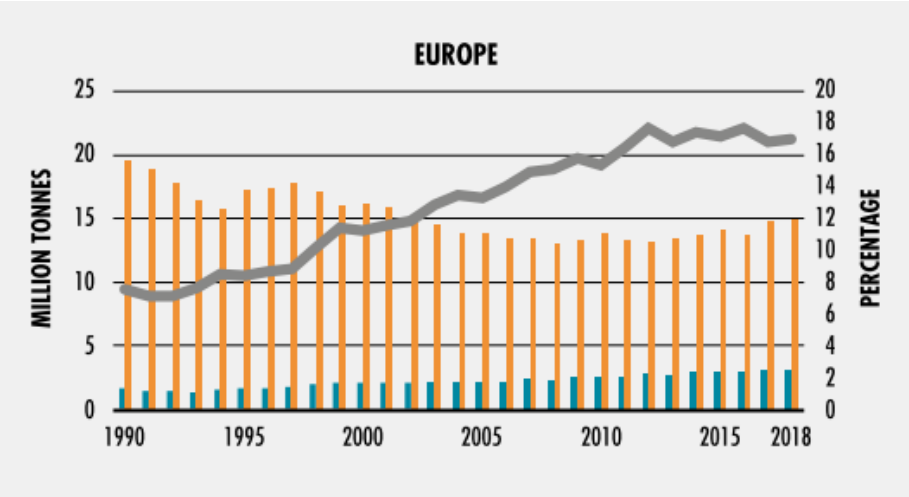

***Figure 1.4**. Contribution of Europe aquaculture in total production of aquatic animals. Source: (FAO, 2020)*

In Italy, in 2015, the total production from aquaculture was 2.347 t, representing almost 1.1% of total production (Sicuro, 2019).

Italian production is mainly focused on molluscs, it is the main producer of clams (of the species *Ruditapes philippinarum*), with 94,2% in volume and 91,6% in value and it covers two thirds of the EU production of mussels (species *Mytilus galloprovincialis*). Moreover it represents 45% of the production of sturgeons (family *Acipenseridae*) and about 20% of the production of rainbow trout (*Oncorhynchus mykiss*) (Gutiérrez *et al*., 2020; Passalacqua, 2017). Other cultivated species are the European sea bass (*Dicentrarchus labrax* Linnaeus, 1758), with a national production quote of 4.6% and gilthead sea bream (*Sparus aurata* Linnaeus, 1758), with a national production quote of 5.1% (Antonini *et al.*, 2019).

The regional Sicilian annual report on Fishing and Aquaculture (Regione Siciliana, 2017) details how, until 2010, aquaculture in Sicily provided more than 15% of National production. This production in 2013 rapidly declined, contributing to just under 10% of National production. This was as a result of

the closure of more than 50% of aquaculture facilities, going from 18 farms in 2008, to 12 in 2010, to 5 active farms in 2013. Of these, only two had hatcheries, the other three were fattening fish floating cages, producing a total of more than 2,000 tons of sea bass and sea bream (Figure 1.5) (Regione Siciliana, 2017).

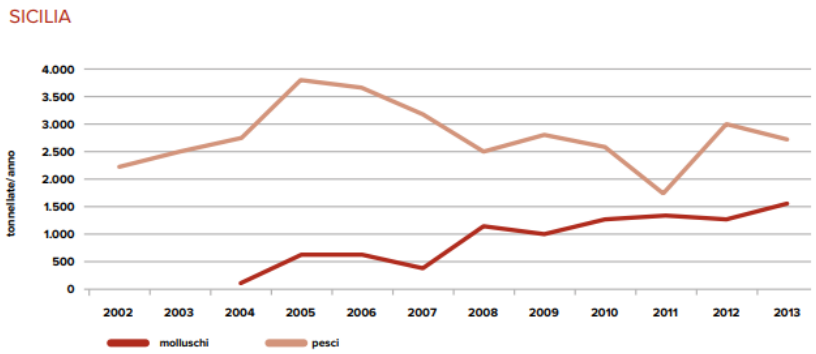

Figure 1.5. *Sicily aquacolture production trend 2002-2013. Source: MIPAAF (MIPAAF, 2014).*

Despite Sicilian aquaculture has suffered a decline in recent years, both nationally and globally, aquaculture will play a key role in ensuring food security and human nutrition in the future, especially as wild fisheries are insufficient to satisfy the demand for aquatic products (FAO, 2020).

However, aquaculture can lead to environmental risks, which can impact natural resources and ecosystems (Ahmed & Thompson, 2019).

Aquaculture, to be sustainable, must ensure the products of farming, useful for human sustenance, without causing impacts to existing ecosystems and without exceeding the global resources required for its production (Boyd *et al*., 2020).

To ensure the sustainability of our global food system and balance global resource demands with environmental limits, the environmental challenges that production will face as a result of intensification of aquaculture must be considered (FAO, 2020).

Constant monitoring is necessary both to safeguard the sustainability of the ecosystem and to protect the business (Mavraganis *et al*., 2020).

1.2. Aquaculture sustainability and eco-labels

Over the last 20 years, the increasing demand for increased sustainability to which aquaculture has been subjected has had the positive effect of significantly improving its environmental performance. The industry has generally embraced a commercial and social expectation of sustainable sound practices, in response to increasingly stringent regulations, but also independently for internal farm needs and, in particular, under market pressure, the industry has greatly improved the organoleptic and health characteristics of its products and its environmental performance (Chikudza *et al*., 2020). In fact, in order to meet the growing sensitivity of consumers to the issue of sustainability, aquaculture companies are implementing measures to reduce the environmental impacts associated with farming (Chikudza *et al*., 2020).

In a market aimed at product qualification, there is an increasing number of products qualified as "green", "sustainable" or "ecological", which refer to the reduction of environmental impacts associated with their manufacture (Chikudza *et al*., 2020; Jonell *et al*., 2016). This vision is in line with the many policies that impose standards to protect the environment (Chikudza *et al*., 2020).

A green label (Table 1.1) provides buyers with a guarantee that the products meet a predefined level of quality, safety and sustainability, certified by appropriate control bodies. These characteristics are controlled, monitored and guaranteed at farm level, with well-established systems, procedures and analyses enabling individual farmers to demonstrate compliance with the criteria standardized in the production specifications for large-scale distribution (Chikudza *et al*., 2020).

Table 1.1. *Sustainable aquaculture eco-label certifications*

FARMED RESPONSIBLY asc CERTIFIED ASC-AQUA.ORG TM	BEST AQUACULTURE PRACTICES CERTIFIED	FRIEND OF THE SEA ®
CERTIFIED SUSTAINABLE SEAFOOD MSC www.msc.org TM	GGN CERTIFIED AQUACULTURE	AquaGAP CERTIFIED QUALITY SUSTAINABLE AQUACULTURE

Recently, the production specifications for large-scale retail trade have also introduced, among the standardized parameters, the evaluation of the effect of the farm on the surrounding environment. Therefore, the companies have started a series of paths that, as for the other parameters considered,

evaluate the effects on the environment, using analytical techniques on environmental descriptors (Chikudza *et al*., 2020).

1.3. Blue Economy and Blue Growth

In view of the growing interest in the sustainability of the ocean economy, the terms “Blue Economy” and “Blue Growth” were introduced at Rio+20 United Nations Conference on Sustainable Development in 2012 (Mulazzani & Malorgio, 2017).

Aquaculture is one of the pillars of EU Blue Economy and its development is considered to play a key role in contributing to reaching the goals of Europe 2020 Strategy (European Commission, 2013).

EU, through the European Maritime and Fisheries Fund (EMFF), has invested €1.72 billion in the aquaculture sector for the period 2014-2020 (Guillen *et al.*, 2019).

The United Nations (UN) gave a general definition of "Blue Economy" as the economy of the oceans that is aiming to “the improvement of human well-being and social equity, while significantly reducing environmental risks and ecological scarcities” (UNCTAD, 2014).

The World Bank, more recently, defined the Blue Economy as “the sustainable use of ocean resources for economic growth, improved livelihoods and jobs while preserving the health of ocean ecosystem” (World Bank, 2017).

The World Bank’s definition is a concept that includes multiple aspects of ocean sustainability, from sustainable fisheries to ecosystem health and pollution prevention (Voyer *et al*., 2018).

Within the Blue Economy, aquaculture should include the value of natural capital for its sustainable development towards food security, sustainable livelihood and income (World Bank, 2017).

Further development of the Blue Economy of coastal and marine aquaculture could offer many social, economic and environmental benefits (Figure 1.6) (Ahmed & Thompson, 2019).

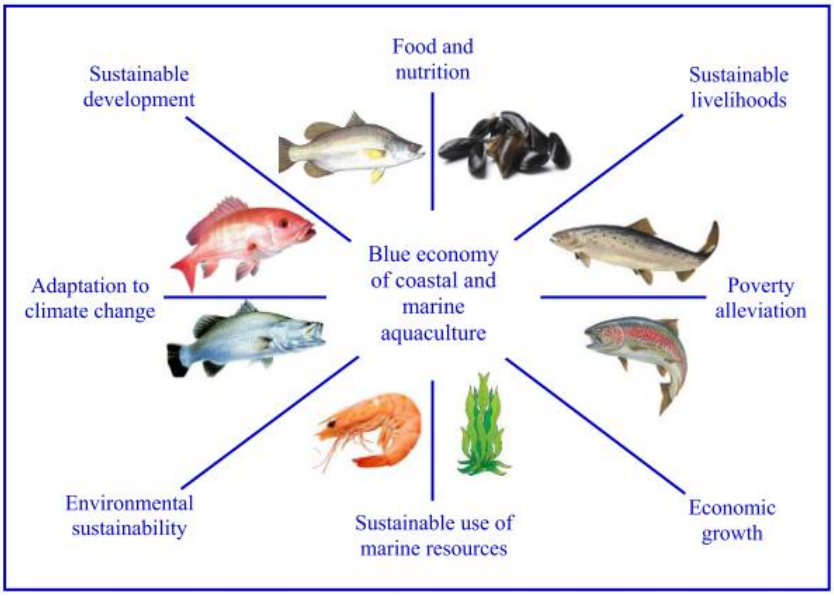

Figure 1.6. *Potential social, economic, and environmental benefits from finfish, shellfish, and seaweed production under the Blue Economy of coastal and marine aquaculture. Source: (Ahmed & Thompson, 2019)*

The Blue Economy concept has been endorsed by the World Bank, the EU, the African Union, the OECD and the UN, as coastal states assess the economic opportunities that exist both within their oceanic jurisdictions and beyond (Voyer *et al*., 2018).

In order to make the oceans places of development for coastal states (UNCSD *et al*., 2014), the UN leads negotiations for the management of deep sea resources located in areas beyond national jurisdiction (Warner, 2009),

To date, however, there is no commonly accepted definition of the Blue Economy, as the term has been used for a variety of purposes (Voyer & van Leeuwen, 2019).

According to Silver (Silver *et al*., 2015), Voyer (Voyer *et al*., 2018) and Voyer & van Leeuwen (Voyer & van Leeuwen, 2019) have been confirmed four Blue Economy "lens":

1. The 'oceans as natural capital' lens, concerning ecotourism, marine protected areas (MPAs) and payment for ecosystem services; the aim of this lens is to quantify the

conservation benefits and economic opportunities that arise from increased ocean protection;

2. The 'oceans as livelihoods' lens, whose main sectors are tourism and small-scale fisheries. This lens frames the Blue Economy as helping to address poverty and food security;
3. The 'oceans as good business' lens, which focuses on large multinational companies in the shipping and industrial fishing sectors, emphasises the scale of the economic contributions of ocean-based industries to global markets to put into play the importance of these sectors and their ability to provide increased growth;
4. The "oceans as a driver of innovation" lens, whose focus is on technical and technological innovations (ocean-based renewable energy, biotechnology and seabed mining), imagining the oceans as sources of new discoveries and wealth (Silver *et al*., 2015; Voyer *et al*., 2018; Voyer & van Leeuwen, 2019).

According to Blue Economy concepts, implementing sustainable practices enables benefits to be derived from the oceans and coastal regions, while respecting their carrying capacity to human activities.

For these reasons human activities must be handled to ensure the health of the oceans and the preservation of economic productivity, so that the potential they offer can be sustained over time (The EU Blue Economy Report, 2021).

“Blue Growth”, promoted by the 2014 FAO report, is defined as "a cohesive approach for environmentally compatible, integrated and socioeconomically sensitive management of aquatic resources including marine, freshwater, and brackish water environments". In particular, great emphasis is placed on the role of aquaculture in meeting growing nutritional needs through sustainable management (Moffitt & Cajas-Cano, 2014).

Similarly to the Blue Economy, Blue Growth does not have a single definition, but takes on very different meanings and approaches, depending on the social contexts in which it is used (Eikeset *et al*., 2018).

At the international level, the concepts of the Blue Economy and Blue Growth are gaining increasing visibility, given their prominent role in Rio+20. These concepts are part of Goal 2 (“End hunger,

achieve food security and improved nutrition and promote sustainable agriculture") and Goal 14 ("Conserve and sustainably use oceans, seas and marine resources for sustainable development") of the Sustainable Development Goals (SDGs) of the UN's 2030 Agenda (Ababouch & Carolu, 2015).

1.4. Ecosystem Approach to Aquaculture (EAA) and Precision Fish Farming (PFF)

1.4.1. Ecosystem Approach to Aquaculture (EAA)

Fish production in aquaculture has been growing rapidly, increasing the probability that this activity presents biological, economic and social challenges. These could affect the ability to maintain productive and environmentally friendly fish production, it is therefore important for the industry to monitor and control the effects of aquaculture (Brugère *et al*., 2019; Føre *et al*., 2018).

To bring the main principles of sustainable development to the attention of the aquaculture sector, FAO and UN have developed the Ecosystem Approach to Aquaculture (EAA) (Brugère *et al*., 2019).

EAA is defined as "a strategy to integrate the activity within the broader ecosystem so as to promote sustainable development, equity, and resilience of interconnected socio-ecological systems" (Brugère *et al*., 2019).

The three main goals of the EAA are: to ensure human well-being; to ensure ecological well-being; to facilitate human and ecological well-being in other sectors and policies (Custódio *et al*., 2020).

In the EAA aquaculture development and management should take into account all ecosystem functions and services without threatening the provision of these to society, to improve human welfare and to be developed in the context of other sectors, policies, and goals (Brugère *et al*., 2019). Indeed the EEA requires that aquaculture development not degrade ecosystem functions and services (Weitzman *et al*., 2019).

Aquaculture, in the Blue Growth Strategy (European Commission, 2017), is one of the five most important maritime economic activities, and to achieve sustainable Blue Growth, it is crucial to link both marine and coastal ecosystem services with the different sectors of the Blue Economy (Lillebø *et al.*, 2017).

Furthermore, according to the UN SDGs for 2030, sustainable aquaculture could contribute to the conservation of the seas and oceans and offer great opportunities to increase welfare and reduce hunger (Custódio *et al*., 2020).

1.4.2. Precision Fish Farming (PFF)

As the scale of production increases, a new framework to improve production in aquaculture needs to be developed. To meet the growing demand for fish products, it is important for the aquaculture industry to apply increasingly advanced and smarter methods to optimize production for higher volumes (Føre *et al*., 2018).

This implies the need to monitor and control the production process using technological tools for fish farming.

According to Føre *et al*. (Føre *et al*., 2018) the concept of Precision Fish Farming (**PFF**) has the purpose to apply control-engineering principles to fish production to improve the farmer's ability to monitor, control and document biological processes on fish farms.

PFF goals are to:

1) improve the accuracy, precision, and repeatability of farm operations;

2) facilitate more autonomous and continuous biomass/animal monitoring;

3) provide more reliable decision support;

4) improve the staff safety.

It is possible to imagine fish farming as 4 phases of different cyclical operational processes, where the responses are observed (Observe phase) and interpreted (Interpret phase) in the cage, obtaining a base for making decisions (Decide phase) on which actions to implement (Act phase) (Figure 1.7) (Føre *et al*., 2018).

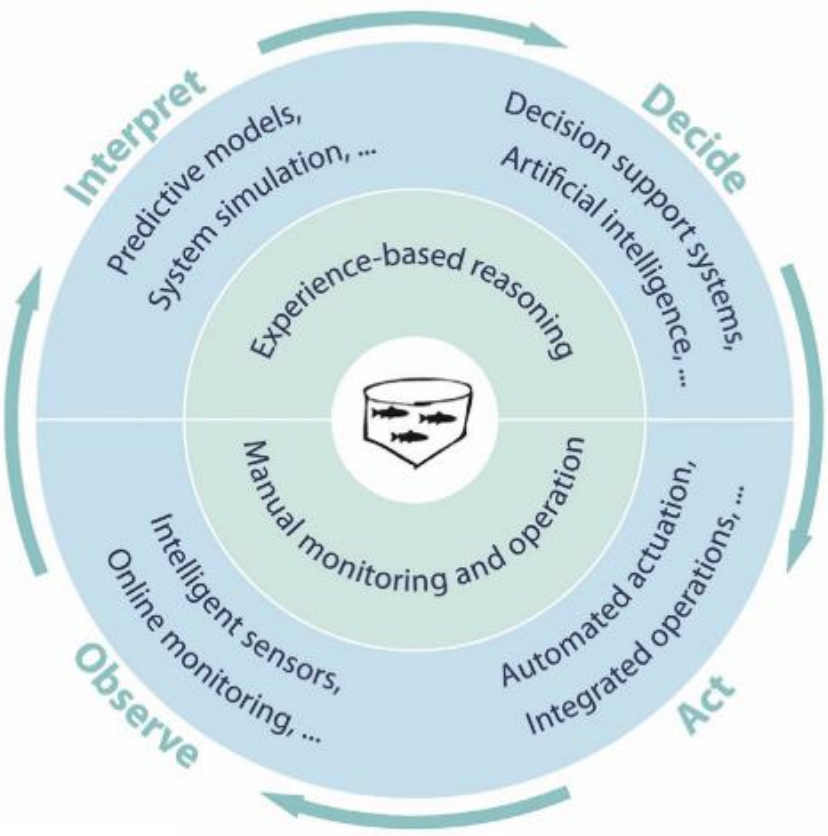

Figure 1.7. *A cyclical representation of PFF where operational processes are considered to consist of four phases: Observe, Interpret, Decide and Act. The inner cycle represents the present state-of-the-art in industry, with manual actions and monitoring, and experience-based interpretation and decision-making. The outer cycle illustrates how the introduction of PFF may influence the different phases of the cycle. Figure credits: Andreas Myskja (Føre et al., 2018)*

The best practice of PFF requires that methods are validated before they are released on the market. Through scientific studies, the PFF will allow greater confidence in the usefulness and efficiency of commercially available technologies (Føre *et al*., 2018).

1.5. Aquaculture effect

The release of nutrients, particulate matter, and chemicals, from marine aquaculture from caged fish farming, can affect the marine environment (Massa *et al*., 2017; Price *et al*., 2014).

Unconsumed feed and fish feces are deposited underneath the cages and spread by marine currents to the seafloor, where it becomes available to benthic communities in a biologically active form, constituting an organic load (Tomassetti *et al*., 2009).

The degree of organic enrichment depends both from chemical and physical factors, such as food type, currents and depth (Beveridge, 2008).

Low current speeds cause accumulation of organic matter and large organic enrichment, while high current speeds and deeper waters produce a larger spatial distribution that consequently reduces organic enrichment (Black & MacDougall, 2002; Lovatelli *et al*., 2013; Sarà *et al*., 2006).

The high velocity current also increases the input of oxygen, facilitating the aerobic decomposition of organic matter (Yokoyama, 2003).

Aquaculture facilities located in shallow, enclosed or semi-enclosed areas have less water exchange and more sedimentation of organic matter, which can lead to hypoxic or anoxic conditions (Tičina *et al*., 2020).

Reared species, stocking density and feeding strategy influence the magnitude of organic enrichment effects (Brooks & Mahnken, 2003).

The impact of uneaten food and nutrients on the environment is indeed a typical concern of aquaculture intensification (Massa *et al*., 2017).

Wastes from aquaculture are generally classified into solid wastes and dissolved wastes (Dauda *et al*., 2019).

Solid waste consists primarily of uneaten food and undigested substances, which, in fish, turns into fecal waste (Figure 1.8) (Turcios & Papenbrock, 2014).

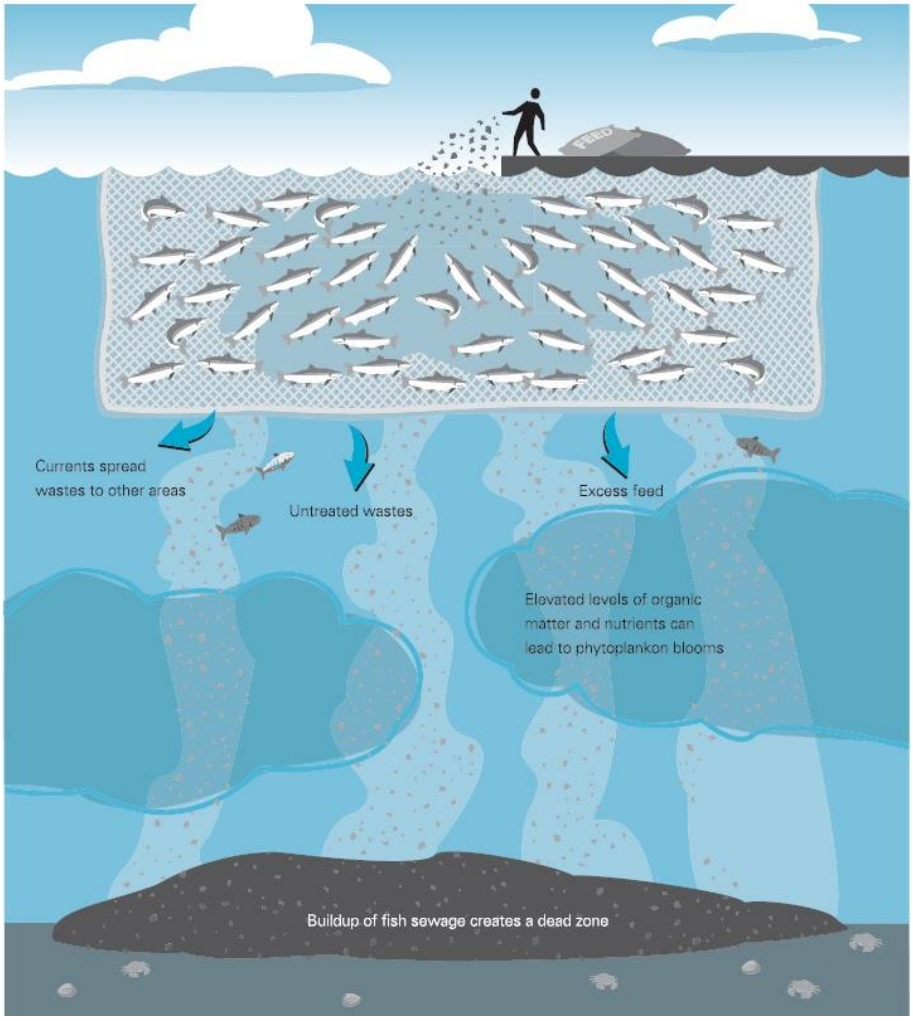

Figure 1.8. *Diagram illustrating the mechanisms by which aquaculture can contribute to eutrophication and hypoxia. Image Credit:* Sources of Eutrophication | World Resources Institute (wri.org)

The products of food metabolism in fish or decomposed and uneaten feed are considered as dissolved waste (Dauda *et al.*, 2019). The two main components of dissolved waste are:

- **phosphorus** (P), an important water nutrient, which is released into the water as particulate in feces as phosphate (Dauda *et al.*, 2019; Lazzari *et al.*, 2008).
- **nitrogen** (N), an important constituent of proteins, which is released into the water, mainly in dissolved form as ammonia (that can be further decomposed into nitrite and nitrate) (Chang & Zhang, 2017; Dauda *et al.*, 2019).

Proteins, lipids and carbohydrates are the main components of fish feed (Dauda *et al.*, 2019) and represent a high source of organic waste in fish farms (NOAA & USDA, 2011). Inclusion of **Carbohydrates** allows a reduction in fish feed costs (Moreira *et al.*, 2008).

Biochemical tracking of **Fatty Acids** (FA), common structural components of most lipids, can be used to detect aquaculture wastes in the environment (White *et al*., 2019).

FAs have the ability to provide a clear distinction between aquaculture output and other outputs, as the lipid profile of modern fish aquaculture feeds has changed greatly, especially increasing the use of terrestrially derived ingredients (White *et al*., 2019).

Depending on the type of farm, its fish production, feeding method, feed quality, as well as water depth, hydrodynamics and sediment geochemistry, there is variation in organic matter accumulation rates (Tomassetti *et al*., 2009; Mavraganis *et al*., 2020).

In coastal areas, excess nutrients and solid effluent loads from human activities (e.g. sewage and aquaculture) cause an enrichment of organic matter in surface sediments, oxygen depletion and an increase in sulphides, causing an effect on benthic habitat and microbial and faunal communities (Boyd *et al*., 2020; Tičina *et al*., 2020).

As oxygen consumption exceeds supply across the sediment-water interface, hypoxic or anoxic conditions are generated in surface sediments (Cranford *et al.*, 2017). **Sulphate**-reducing bacteria, under anoxic conditions, still decompose organic matter and produce hydrogen sulphide as a result of the end product of sulphate respiration (Cranford *et al.*, 2017).

Organic enrichment, which leads to changes in the structure of the macrofauna community, can be estimated in relation to the concentration of sulphides. Several categories of sediment (oxic, hypoxic and anoxic) can be defined depending on the sulphide concentration (Table 1.2) (Hargrave, 2010).

***Table 1.2**. Nomenclature for gradients in benthic organic enrichment based on previously published descriptions. Values for 'free' dissolved sulphides (ΣS_2^-, HS^-, H_2S) (S) and redox potentials (Eh_{NHE}) with thresholds for transitions between oxic sediment categories (bold) from Hargrave et al. (2008) (Hargrave, 2010).*

Benthic condition	Geochemical status	Oxygen stress	Sediment condition	Geochemical category	Macrofauna diversity	Oxic category	'Free' S (µM)	Eh_{NHE} (mV)
Normal	Oxic	Pre-hypoxic	Very good	Normal	High	Oxic A	100 150 250 400 625	225 200 175 150 125
						Oxic A/B threshold	**750**	**100**
Normal	Post-oxic	Aperiodic	Good	Oxic	Good	Oxic B	875 1250	75 25
						Oxic B/ hypoxic A threshold	**1500**	**0**
Transitory	Sulfidic	Moderate	Less good	Hypoxic	Moderate	Hypoxic A	1750 2500	-25 -75
						Hypoxic A/B threshold	**3000**	**-100**
Polluted	Sulfidic	Severe	Bad	Hypoxic	Poor	Hypoxic B	4000 5000	-150 -175
						Anoxic threshold	**6000**	**-185**
Grossly polluted	Methanic	Persistent anoxia	Very bad	Anoxic	Bad	Anoxic	7000 8500 10000	-195 -200 -210

Benthic eutrophication has effects and spatial extent that can be assessed through biochemical composition analysis (i.e., protein, lipids, and carbohydrates) and quantification of the organic matter in the sediment, as significant variables of benthic trophic status (Pusceddu *et al*., 2007).

To detect eutrophic trends in the marine environment, **chlorophyll-a** concentrations are used as early warning indicators (Rolland & Jacquet, 2010), as phytoplankton growth is linked to nutrient availability (Dalsgaard & Krause-Jensen, 2006).

Through interpretation of the radiance received at the sensor at different wavelengths, the concentrations of optically active water constituents, namely chlorophyll, colored dissolved organic matter, and total suspended solids, can be estimated from satellite imagery (Gordon & Morel, 2012). To obtain chlorophyll estimates, differential phytoplankton absorption in the visible region (0.4-0.7 mm) can be used (Rajitha *et al*., 2007).

Thus, studying physical and chemical aspects of the environment, can help to predict the impact of farming activity on the environment (Gentry *et al.*, 2017).

In order to ensure the sustainability of the industry, aquaculture stakeholders must protect the surrounding aquatic environment (Mavraganis *et al*., 2020).

To ensure proper management of aquaculture (Macuiane *et al*., 2016) and its sustainability (Lampadariou *et al*., 2005), it is important to monitor the impact that the activity could have on both water bodies and farmed fish through an effective monitoring system (Lampadariou *et al.*, 2005) that includes water quality analyses (Macuiane *et al*., 2016).

1.6. Aims and structure of the thesis

The aim of this thesis was to define techniques for monitoring production performances and marine aquaculture effects on the environment through effective, low-cost instrumentation and the subsequent transfer of the acquired techniques to the aquaculture industry.

The identification and use of appropriate descriptors allows the aquaculture industry to conduct its own monitoring to provide evidence of the sustainability of their production, thus responding to growing consumer demand for “Green products”.

To this purpose, I validated the NIRs and remote sensing technique.

The chapters of my thesis are briefly presented as follows:

In Chapter 1 I described the state of aquaculture and its possible effects on the environment.

In Chapter 2 I validated the ion-selective electrode technique, that allows the analysis of sulphides, sensitive and cost-effective chemical indicators of benthic organic enrichment.

In Chapter 3 I applied the NIR technique to predict the chemical components of the sediment, quickly and non-destructively.

In Chapter 4 I validated the gas chromatography technique, proved to be useful for distinguishing the origin of organic matter, whether from aquaculture, the marine environment, or other sources of anthropogenic impact.

In Chapter 5 I validated the remote sensing technique, a low-cost approach useful for tracking and assessing the status and dynamics of aquaculture through a multi-temporal analysis.

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CHAPTER 2

2. SULPHIDES

2.1. INTRODUCTION

Mariculture facilities offer benefits to national and local economies, but their implementation is often a source of concern over the effects they may have on the marine environment (Grigorakis & Rigos, 2011; Moraitis *et al*., 2013; Morata *et al*., 2015; Tsagaraki *et al*., 2011; Tsikopoulou *et al*., 2018).

The sustainability of these activities must be assessed taking into account the complex balance between the ecological, economic, social, political and cultural context in which the activity is conducted (Grigorakis & Rigos, 2011; Rabassó & Hernández, 2015; Tsikopoulou *et al*., 2018).

The effects of aquaculture on the environment can be analyzed on the water column, sediment geochemistry and benthic organisms (Aguado-Giménez *et al*., 2011; Forchino *et al*., 2011; Kalantzi & Karakassis, 2006; Mangion *et al*., 2018; Martinez-Garcia *et al*., 2013; Morata *et al*., 2015; Sarà *et al*., 2011; Vizzini & Mazzola, 2012).

Part of the organic matter that is deposited on the seabed, is consumed by heterotrophs aerobically and this causes a decrease in dissolved oxygen that is within the pore water of the sediment (Brown *et al*., 2011).

When there is no more oxygen, heterotrophic organisms continue to decompose excess organic matter through processes that use anaerobic respiration, these processes include sulphate reduction (Cranford *et al*., 2020).

Through sulphate reduction there is the production of sulphide ions, dissolved as H_2S, HS^- and S_2^- (Cranford *et al*., 2020). As H_2S concentrations in sediments increase, its toxic properties cause changes at the benthic level (Brown *et al.*, 2011), and these are used as indicators of physicochemical changes in sediments and added into the aquaculture monitoring programs of several organizations both private and government (Hargrave *et al*., 2008).

According to Brooks (2001) there is a correlation between free sulphides in sediments and changes in macrobenthic community structure (Brooks, 2001). Therefore, direct measurements of physical and chemical conditions in sediments can be used to monitor benthos effects (Brown *et al.*, 2011).

These determinations can be carried out by inexpensive and very rapid electrochemical methods with a favorable cost/benefit ratio (Cranford *et al*., 2017; B. T. Hargrave *et al*., 2008; Porrello *et al*., 2005; Sutherland *et al*., 2007).

Based on these considerations, the multi-annual (2001, 2007, 2011 and 2020) monitoring program of the effects on marine sediments of Bluefin tuna (*Thunnus thynnus*) farming located in the Gulf of Castellammare del Golfo (Sicily, Italy) included the assessment of the main chemical parameters describing the quality of sediments collected under cages and at control stations. Particular attention has been given to cost-effective descriptors, rapid and easy to use, such as sulphide content and redox potential.

This could contribute to the definition of guidelines for monitoring and evaluating the recovery of environmental conditions after the cessation of farming activity.

2.2. MATERIALS AND METHODS

2.2.1 Study area and sample collection

The surveyed farm was located in the Gulf of Castellammare del Golfo (Sicily, Italy) (Fig.2.1).

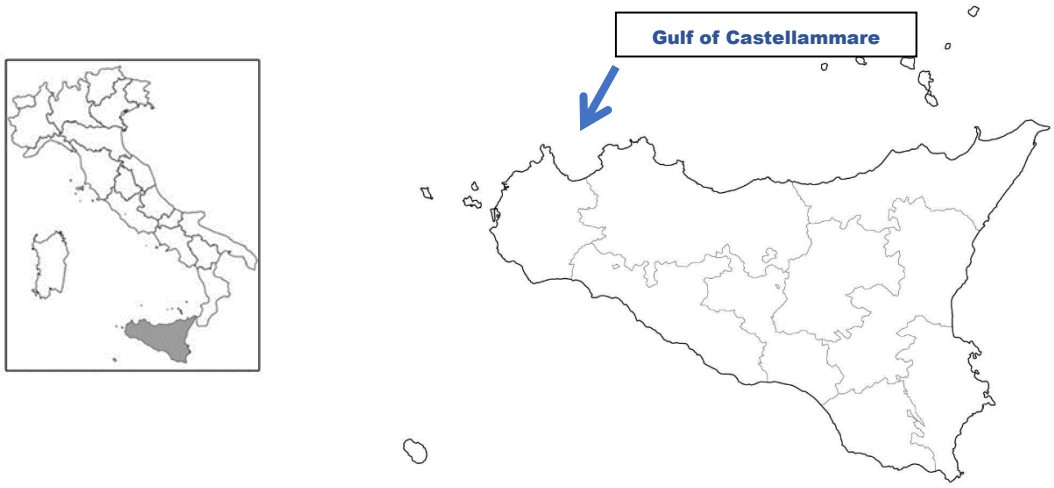

Fig.2.1. *Castellammare del Golfo Bluefin tuna location in the Gulf of Castellammare (Sicily, Italy)*

Capture based Bluefin tuna (*T. thynnus* Linnaeus, 1758) farming facility operated from 2001 to 2009. Farming area (18,000m^2) was divided into six round cages of 50m diameter, formed by a ring of high-density polyethylene from which a net was hung.

In July, tunas were put into the cages and daily fed with fresh or frozen fish, while in November the fattened tuna were caught and marketed. The farm produced about 700 metric tons of biomass of tunas per cycle. To validate the techniques, were used both frozen samples, collected before the beginning of my PhD (2001, 2007 and 2011) and stored at -80°C and fresh samples, collected during my PhD (2020), from different sampling stations located at different distances from the cages.

Six sampling stations (Fig.2.2), placed at a depth of 35-45m, where the bottom is composed of very fine sand to coarse silt, were identified around the fish farm, distributed along a transect, located along the direction of the dominant current (from North-West to South-East) to assess the spatial distribution of organic matter:

- CC, at the center of the cages;

A station along the upstream part of the transect:

- NW100, 100 meters from the cage line;

Two stations along the downstream part of the transect:

- SE50, 50 meters from the cage line;
- SE100, 100 meters from the cage line;

Two control stations:

- CT_1, 1000 meters from the cage line;
- CT_2, 500 meters from the cage line.

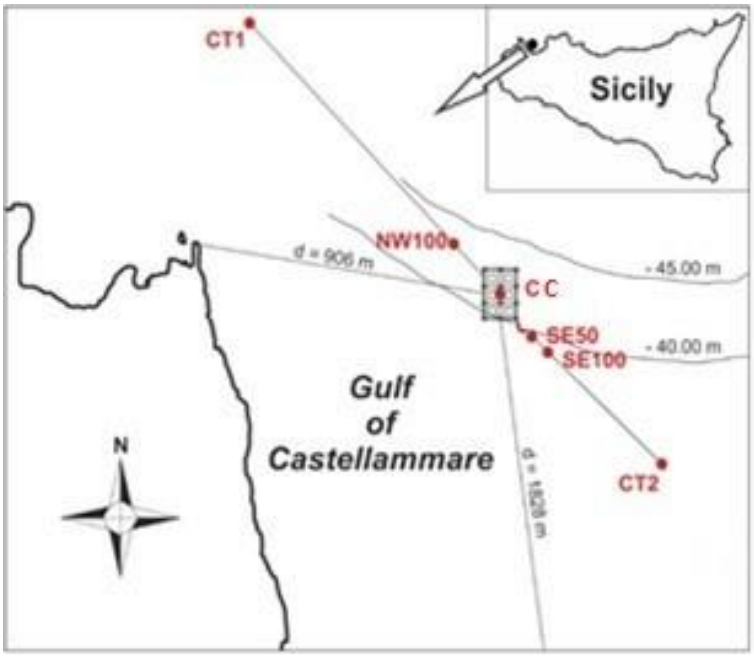

Fig.2.2. *Castellammare del Golfo Bluefin tuna sampling stations (in red) sited in the Gulf of Castellammare*

2.2.2. Determination of total free sulphides

The electrochemical determination of sulphides, as described by Wildish *et al*. (Wildish *et al*., 1999, 2001), was carried out on surface sediments (0-2 cm depth) with an ion-selective Ag^+/S_2^- 9616BN electrode (Orion), connected to a portable pH meter (Orion 290 A), a membrane electrode in which

the membrane potential is selective towards one or more ions and which measures the activity of the free ion, while the species to which the ion is bound, especially those that are not ionised, are not appreciated.

The concentration of sulphides was measured on samples defrosted at room temperature. The electrode was calibrated with three solutions of known title of Na_2S*9H_2O (Sigma-Aldrich) to which an equal volume of SAOB solution (antioxidant buffer solution, consisting of 20g NaOH and 17.9g EDTA + 8.75g L-ascorbic acid in a final volume of 250 ml) was added. To 10 ml of sediment sample were added 10 ml of SAOB. The solution consisting of SAOB + L-ascorbic acid remains stable for up to 3 hours, does not interfere with the reading and has the dual purpose of preventing oxidation of the sample and releasing the sulphide ions from bonding with the hydrogen ion so that the HS^- and H_2S forms are converted to S_2^-. As for the standards, the sediment samples were diluted 1:1 with the solution of SAOB + L-ascorbic acid. The resulting solution was stirred, and the electrode was inserted into the solution, allowing the concentration of the sulphides present to be read.

The measured potential, described by Nernst equation, refers to the amount of the measured ion in solution:

$E=E_0+S \log A$

Where

E = measured electrode potential

E_0= reference potential.

A = ion activity in solution.

S = electrode slope determined by standard calibration (Brown *et al*., 2011).

2.2.3. Statistical analysis

Data were analysed by a one-way variance analysis (ANOVA) using the STATISTICA version 6.0 statistical software.

Where the comparisons were significant, the differences between the averages were analysed using the Student-Newman-Keuls test (SNK test). Before proceeding with the analyses, the degree of heterogeneity of the variances was assessed using Cochran's test (Underwood *et al.*, 1997).

2.3. RESULTS AND DISCUSSION

Sulphides concentration has been proposed as a parameter to assess the quality of sediments and as a descriptor to predict sediments status, which can lead to effects on the benthic community (Cranford *et al.*, 2020; B. Hargrave *et al.*, 2008; Keeley *et al.*, 2012; Porrello *et al.*, 2005; Sutherland *et al.*, 2007; Wildish *et al.*, 2001).

Sediment's sulphides concentration (µM) comparison between the monitoring campaigns (2001-2007, 2011, and 2020) at the Bluefin tuna (*T. thynnus*) fish farm in Castellammare del Golfo is shown in Figure 2.3.

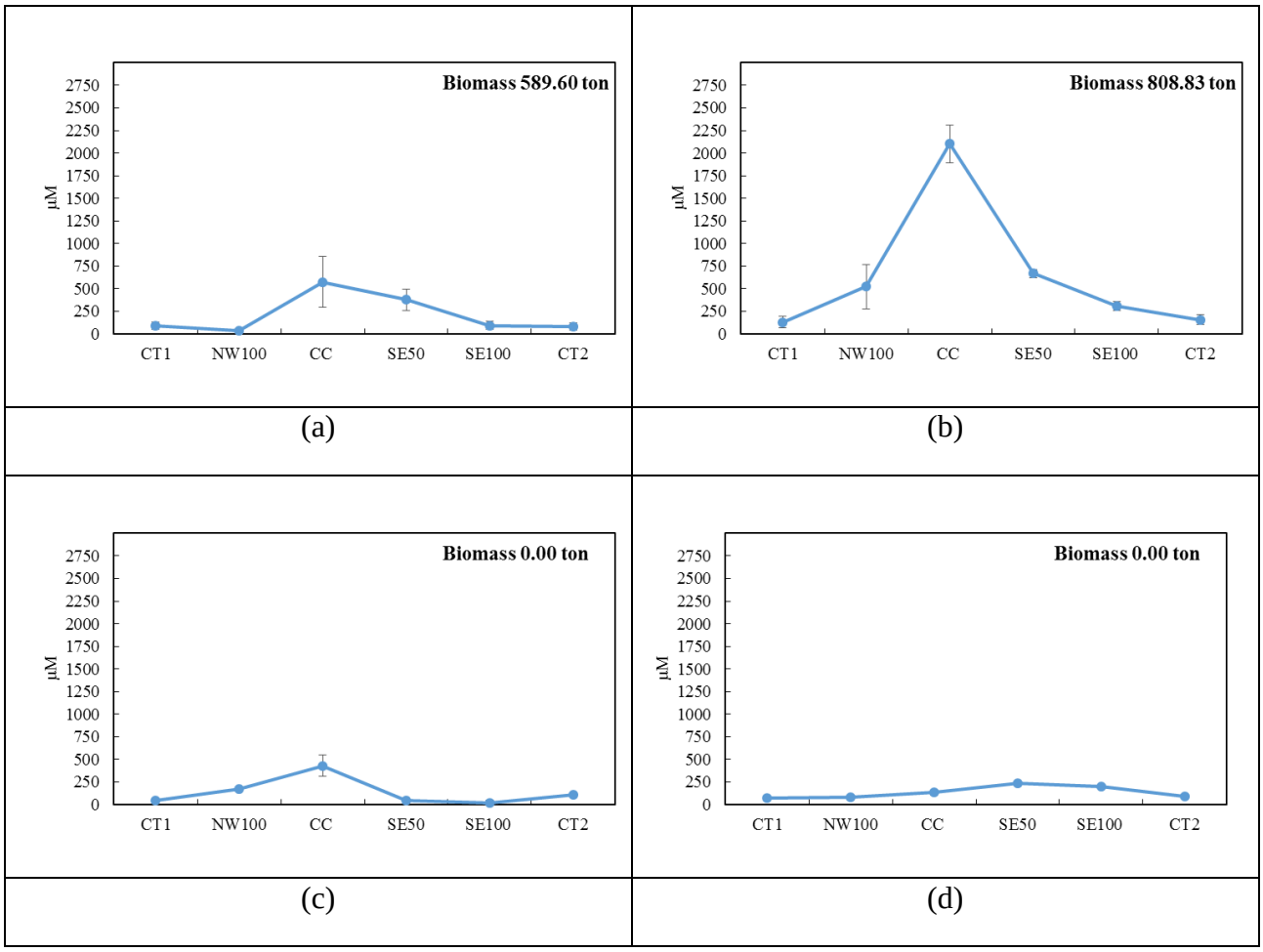

Fig.2.3. *Sediment Sulphide concentration and produced biomass in different years at Castellammare del Golfo Bluefin tuna sample stations: (a) 2001; (b)2007; (c)2011; (d)2020.*

Sulphide concentrations are related to the produced biomass.

At CC and SE50 stations there was an increase in the concentration of sulphides at the end of the first breeding cycle (Fig.2.3 a).

In 2007 which, among the analysed years was the one of maximum productivity, the highest sulphide concentration values, above 2000 μM, were detected at the CC station (Fig.2.3 b).

These values returned below 1000 μM 2 years (Fig. 2.3 c) and 11 years (Fig.2.3 d) (respectively 2011 and 2020) after the suspension of the aquaculture activity, when tunas were no longer present in the farming cages and then biomass was zero.

Sulphide concentrations were higher in sediments sampled underneath the cages (CC) and decreased with increasing distance from the cages. These values were comparable to those reported by Brown *et al*., (2011).

These results are in agreement with He *et al*. (2019), whose study involved collecting sediment from 6 different fish farms in Canada at different distances from the cages (0 m, 15 m, 30 m, 60 m, 90 m, 125 m and 500 m). In 4 of the 6 fish farms, sulphides concentration were higher at the station at 0 m distance from the cages than other stations (He *et al*., 2019).

As observed in our study, the increase in sulphides concentration is related to the presence of the aquaculture facility, as demonstrated by Jiang *et al.* (2021). These authors observed that sulphides were significantly higher in an aquaculture area characterised by reduced conditions, than in non-aquaculture areas (Jiang *et al*., 2021).

These results show that after an increase of sulphides concentration in 2007, in 2011 the recovery process was evident: sulphides values decreased rapidly compared to those found in previous years and, at CC, did not differ significantly from those found at the same station before the rearing start in 2001. The results obtained in 2020 further confirmed the recovery of the environmental conditions.

Suphides concentration values confirmed that a restoration of initial conditions, with a partial recovery of sediment quality, is expected after a temporary suspension of farming activities (Piedecausa *et al*., 2010).

2.4. CONCLUSION

Among the analytical methods aimed at detecting sulphides content (colorimetric, spectroscopic, chromatographic and electrochemical), the electrochemical technique that I used has the advantage of easy application, low cost and direct, sensitive and fast detection even in the presence of low sulphides concentration (Gomez *et al*., 2018).

As expected, the permanent cessation of farming activities led to a full recovery of the qualitative, chemical and biological characteristics of the sediment (Aguado-Giménez *et al*., 2012; Brooks & Mahnken, 2003).

This shows that the results obtained from data collection on a very long-term scale (2001-2020) over a specific area, for the assessment of the effects of fish farming, are able to provide an useful information on the relationships between local cumulative effects and significant changes in environmental conditions (Tsikopoulou *et al*., 2018).

2.5. REFERENCES

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CHAPTER 3

3. NEAR INFRARED SPECTROSCOPY

3.1. INTRODUCTION

Marine sediments can be considered recorders of processes affecting the overlying water column, since they integrate, spatially and temporally, a good part of the ecological processes involving the surrounding systems and represent a useful descriptor for the study of the effect of floating cage systems on the marine environment (Hargrave, 2010; 2008; Kalantzi & Karakassis, 2006).

Analysis of sediment composition is very important for assessing potential impacts (Galasso *et al*., 2017). The usual chemical laboratory analyses, to assess the quality of marine sediments, apply standard methods that can be very expensive and time-consuming. An alternative to standard chemical analytical techniques is near-infrared spectroscopy (NIRs), a rapid and non-invasive method useful for the assessment of physical and chemical composition, which also reduces the cost of routine analysis (Galasso *et al.*, 2017).

NIRs, a fast and precise technique (Downey, 1996), through the use of near-infrared waves (700nm-2500nm), exploits the different absorbance of the main organic compounds (e.g. lipids and proteins) and, in particular, the vibrations of molecular bonds (C-H, O-H, N-H, S-H) stimulated by near-infrared electromagnetic radiation (Fernández Pierna *et al*., 2018).

NIRs is a type of vibrational spectroscopy that uses the energy of photons in the energy range of $2.65x10^{-19}$ to $7.96x10^{-20}$ J (corresponding to the wavelength range of 750 to 2,500 nm) (Figure 3.1) (Pasquini, 2003, 2018).

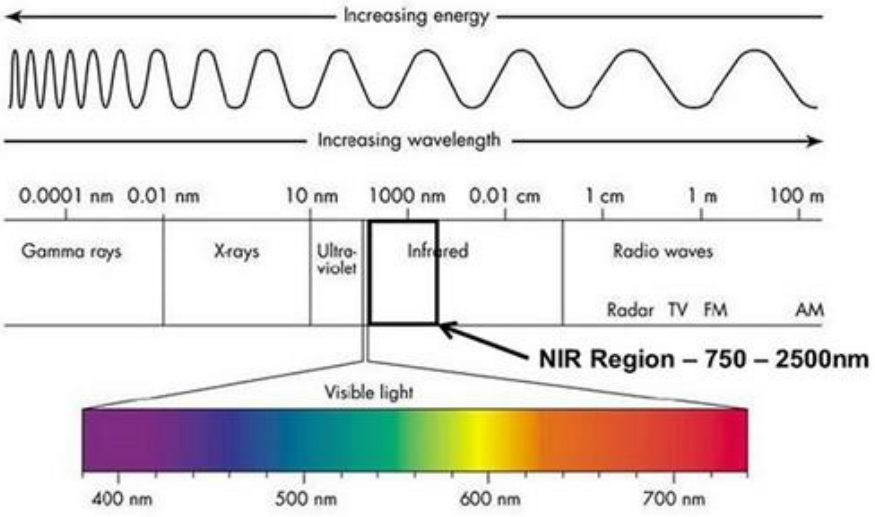

Fig.3.1. *Electromagnetic spectrum*

The possible applications for the NIRs are quantitative (to determine the concentration of an analyte in a matrix) and qualitative (aiming at sample classification) (Stark *et al*., 1986). Quantitative techniques represented the first applications of NIRs, qualitative techniques were subsequently developed with the objective of discerning between samples with different characteristics through the application of multivariate statistics algorithms to spectral data (Cen & He, 2007).

Studies have shown that this technique has promising potential for the rapid and accurate determination of TOC in a wide variety of sediment types (Chang *et al*., 2005; Leach *et al*., 2008; Yu *et al.*, 2017).

The aim of this study was to evaluate the potential of NIRs in assessing the composition of marine sediments from floating cage farms.

3.2. MATERIALS AND METHOD

240 samples of marine sediment, collected at the Bluefin tuna fish farm in Castellammare del Golfo, were analyzed by chemical methods and by NIR spectrometer technique. Samples were divided into two groups: 180 were used for the calibration of the instrument, 60 were used for external validation to test the predictive ability of NIRs.

NIRs technique have been used to assess lipids, proteins, carbohydrates and the total organic matter (TOM) content of marine sediments.

3.2.1. Chemical Analysis

3.2.1.1. Lipids Analysis

I have defined three techniques for extracting lipids from sediments:

1) Chemical extraction
2) Supercritical fluid extraction (SFE)
3) High pressure liquid extraction (PLE)

Chemical extraction

Total lipids were determined according to Folch *et al.*(Folch *et al.*, 1957). 240 ml of methylene chloride: methanol (2:1) with 0.01% BHT were added to 5 g of sample.

Samples were put in the refrigerator overnight in shaking to 130 rpm to allow the lipids extraction. Subsequently samples were filtered with filter paper in a separatory funnel and 60 ml of 0.73% NaCl solution were added to facilitate phases separation.

Samples were left in the refrigerator overnight to allow better phase separation.

Lipids were dried by rotavapor, determined gravimetrically, then resuspended in 5 ml N-hexane and stored under nitrogen atmosphere at –20◦C until further analysis.

Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE), an alternative method to conventional lipids extraction, is a separation technology that uses a supercritical fluid as a solvent (each fluid is characterized by a critical point, defined in terms of critical temperature and pressure) (Sahena *et al.*, 2009).

Carbon dioxide is the main supercritical solvent used (critical conditions = 30.9 ◦C and 73.8 bar). It is cheap, environmentally friendly and easily settable. In addition, CO_2 is gaseous at room temperature and pressure, making analyte recovery very easy and providing analytes solvent-free (Herrero *et al*., 2010).

Supercritical technology was applied to the dry sediment matrix using supercritical (SC)-CO_2 with a standardized protocol (Messina *et al*., 2015; Messina *et al*., 2019; Messina *et al*., 2019b). A supercritical extraction unit (SFE System model HELIX, Applied Separations Allentown, PA, USA) equipped with a CO_2 pump unit and a steel vessel with a volume of 50 mL was used.

The extraction was done as follows: 10 grams of the dried sediment with a hydroscopic dispersant reagent (Applied Separations, Allentown, PA, USA) (1:2 (w/w)) were in the extraction vessel sandwiched with defatted glass wool forming a fixed bed in the vessel. The unit was pressurized and the sample was kept in contact with SC–CO_2 (50°C) and pressure (270 bar) for 20 min in static mode. Dynamic extraction was carried out with a CO_2 flow of 4 lpm (liter per minute) for 40 min. The extract was obtained in the sample vial collector and stored at −20°C

High pressure liquid extraction (PLE)

Pressurized liquid extraction (PLE) uses solvents at high temperatures, above its boiling point and below its critical point, under enough pressure to keep them in a liquid state (Alvarez-Rivera *et al*., 2020).

In PLE, the sample is placed in a closed container, and is added sand, sodium sulphate, or Hydromatrix as a dispersant in the cell and the solvent is then added to the cell at the beginning of the heating cycle and pumped in and out of the cell to maintain pressure and perform the number of static cycles indicated by the user (Schantz, 2006).

PLE has established itself as a high-throughput, from natural sources, extraction technique for sustainably extracting bioactive compounds and determining a wide variety of analytes of interest in environmental samples (Alvarez-Rivera *et al*., 2020).

Sediment extraction was performed using the Pressurized Liquid Extraction System (FMS, USA). Ten grams of dry sediment were mixed with 10 g of sand (hydroscopic dispersing agent, Applied Separation, USA) and loaded in stainless steel tubes between two 30 ml layers of sand. Sediment samples were extracted with n-hexane applying the following extraction program: tubes were filled, pre-heated, pressurized to 2000 ± 100 psi and heated to 100 °C for 15 min static extraction, cooled

(10 min), depressurized and washed with solvent. This cycle was repeated three times and finally the two lines used for extraction were drying using pressurized N_2 gas (5 min) (White *et al*., 2009 with modifications). The extracts were evaporated to dryness and the residues weighed. Lipids were dried by rotavapor and determined gravimetrically.

3.2.1.2. Protein Analysis

The crude protein content was determined using the Kjeldahl method.

0.5 g of marine sediment were subjected to digestion with 96% sulfuric acid at 400°C, for a mineralization step. Then, a direct distillation with 2% boric acid and a titration with 0.1N hydrochloric acid (HCl) was performed, using the Kjeltec Distillation Unit B-324 (BUCHI Labortechnik AG).

Percent crude protein was measured by multiplying the percent nitrogen by a factor of 6.25, considering the normality of the HCl used for titration (AOAC, 1992).

3.2.1.3. Carbohydrates Analysis

The concentration of total carbohydrates was estimated using the phenol-sulfuric acid method (Dubois *et al*., 1956).

To 10 mg of sediment sample, 1 ml of distilled water was added and the sample sonicated for 30 seconds. 1 ml of phenol (5% w/v in distilled water) and 5 ml of concentrated H_2SO_4 were added. The samples were cooled for 60 min.

Absorption was measured at 485 nm with a spectrophotometer (Varian spectrophotometer, model Cary 50 scan, Netherlands). Glucose was quantified using a standard curve of glucose.

3.2.1.4. TOM Analysis

TOM was determined by ashing. 0.5 g of sediment was ashed for 5 hours at 500°C. The difference between the initial and final weights were used to calculate the TOM.

3.2.2. Sample Treatments for NIR Spectral Acquisition

The sediment samples were dried at 60°C overnight to minimize the influence of water content on their reflectance spectra (Chang *et al.*, 2005). The spectra were recorded the following day.

Sediment samples were placed in a Petri dish, made of transparent glass. The dish was placed in a measurement cell that included a rotating sample holder that allows three readings at three different locations on the plate containing the sample. The measurement was performed in reflectance mode.

3.2.3. Near-infrared-spectroscopy

All chemometric analyses, including calibration and validation, were performed using NIRCal 5.5 (Buchi, Flawil, Switzerland). The raw optical data were pretreated. For each property, on which a calibration curve was made, different pre-treatments were performed using NIRCal 5.5 (Buchi, Flawil, Switzerland).

In the calibration phase, the spectral data acquired with the NIRs were related to the chemical analyses (Lipids, Proteins, Carbohydrates and TOM) that you want to estimate using appropriate regression algorithms. The properties have been preliminarily determined by official standard or reference methods.

Cross-validation, or internal validation, was carried out as default software output, using the blockwise procedure, sharing out the calibration set into 3-fold blocks, and testing in turn one block as the validation set and the others as calibration sets. The software computed a series of calibration models and automatically selected the best (Tamburini *et al.*, 2017).

The Büchi NIR spectrometer system, in order to gain maximum benefit from the measured region, applies principal component analysis (PCA). For quantitative analyses partial least squares regression (PLS) was used as a regression model to compare reference data and expected NIRs results (Tamburini *et al*., 2017).

The accuracy of the calibration equations is evaluated by the instrument software NIRCal 5.5 (Buchi, Flawil, Switzerland) by several statistical parameters.

Q-Value, is an estimate of the quality of the calibration, its range varies from 0 to 1. Usually the value 1 is the maximum value but only theoretically achievable; values such as 0.75 are considered acceptable, those between 0.5 and 0.75 are not very accurate, those below 0.5 are considered useless (Tamburini *et al*., 2017) (Durbin & Watson, 1950).

Q-Value is evaluated automatically by the software during the calibration phase as:

$$Qvalue = \frac{1}{1 + \sum_{i=1}^{n} wivi}$$

where "w" is the weight assigned for each statistical measure, "v" is the corresponding value of the statistical measures and "i" is the number of measures (Tamburini *et al*., 2017).

BIAS is the mean of the differences between predicted values (yi) and reference values (xi) (Bittner *et al*., 2011).

$$BIAS = \frac{\sum_{i=1}^{n}(x_i - y_i)}{n}$$

If the BIAS value is high, it means that there are some systematic errors between the calibration and prediction datasets, a perfect correlation will produce a bias of 0 (Cantor *et al*., 2011).

The accuracy of NIRs-predicted data sets was measured as Squared correlation coefficient $\mathbf{r^2}$: for evaluating the difference between reference and measures values (Bittner *et al.*, 2011; Tamburini *et al.*, 2017).

SEP (Standard error of prediction) and **SEC** (Standard error of calibration) denote the standard deviation of the differences between the reference values and the NIRs results in the validation set and the calibration set, respectively (Bittner *et al*., 2011).

$$SEP = \sqrt{\frac{\sum_{i=1}^{n}(x_i - y_i - BIAS)}{n - 1}}$$

$$SEC = \sqrt{\frac{\sum_{i=1}^{n}(x_i - y_i - BIAS)}{n - 1}}$$

In addition, for the determined model to be acceptable, the SEC and SEP values must be of the same order of magnitude. In fact, their ratio should be approximately close to the value 1 (Bittner *et al*., 2011; Tamburini *et al*., 2017; Williams & Norris, 1987).

The software calculates a number of calibrations models and automatically selects the best one by comparing different statistical parameters (Tamburini *et al*., 2017).

3.3. RESULTS AND DISCUSSIONS

3.3.1. Calibration Phase

Using the reference values of TOM, Carbohydrates, Lipid and Protein obtained by chemical analysis on 180 sediment samples and the original and pre-treated spectral data (Næs *et al*., 2002), calibration models were generated with the PLS regression method by the NIRcal 5 software. Table 1 shows the statistical parameters obtained from the individual calibration curves.

Table 3.1. *The optimal forecast results of the PLS model for the properties (TOM, Carbohydrates. Lipid, Protein) analyzed on the sediments and the corresponding statistical parameters of the various single and combined pre-treatments.*

Property	Pre-Treatment Applied	N° Factors	N° Outlier	Q-value	r^2 (C-Set)	r^2 (V-Set)	SEC	SEP	BIAS (V-Set)
TOM	sg9[1]	16	0	0.78	0.952	0.947	0.45	0.45	0.0198
Carbohydrates	db1[2], ncl[3]	8	0	0.74	0.906	0.901	0.22	0.22	-0.0009
Lipid	dg2[4],SNV[5]	8	0	0.76	0.818	0.812	0.10	0.08	-0.0009
Protein	ilg[6]	10	0	0.804	0.925	0.922	0.06	0.06	-0.0056

[1]Smooth Savtzky-Golay 9 Points
[2]First Derivate BCAP
[3]Normalization by Closure
[4]Second Derivate Savtzky-Golay 9 Points
[5]Strandard Normal Variate
[6]Absorbance inverse 1/(10^x)

The PLS analysis provided good regression models between measured and predicted values for TOM, Carbohydrates, Lipid and Protein, r^2 values were obtained for cross-validation of 0.952, 0.906, 0.818 and 0.925 respectively (Table 3.1), confirming PLS to be the best regression model for NIRs (Pasquini, 2018).

These results suggest that the calibration curves obtained can be considered acceptable and could be used for routine analysis (Inagaki *et al*., 2012). These results are in agreement with the ones obtained by Galasso *et al.* (2017) in which there was a strong linear relationship between the values predicted by the NIR in the calibration set and the values obtained with the laboratory analysis (Galasso *et al*., 2017).

3.3.2. Validation Phase

After developing a calibration model, the performance of the model was validated using an external validation on 60 different sediment samples before applying the calibrations in routine analyses (Tamburini *et al.*, 2017). This procedure is mainly used for complex and heterogeneous samples, strongly influenced by composition and structure, for which cross-validation only is not sufficient to consider the performance of the model reliable (Tamburini *et al.*, 2017).

The analysis showed a good validation model for all parameters examined with R^2 values between 0.802 and 0.839 (Figure 3.2).

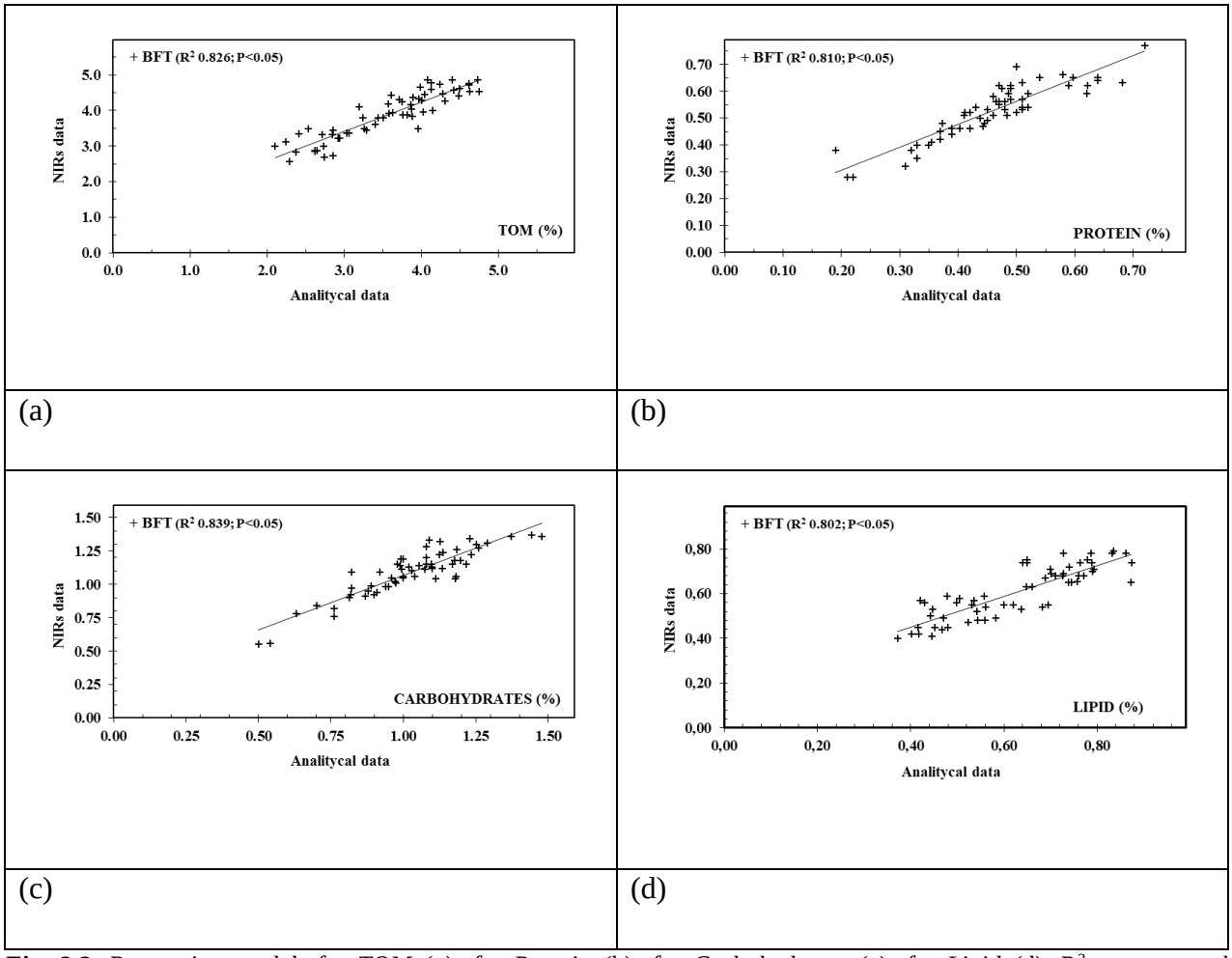

Fig. 3.2. *Regression model: for TOM (a); for Protein (b); for Carbohydrates (c); for Lipid (d). R^2 represents the regression coefficient*

The results indicate that external validation is efficient to define the quality of a regression model (Pasquini, 2018).

Table 3.2 shows for all examined properties a Pearson's correlation coefficient "r" significantly higher than 0.9 ($p<0.05$), indicating a high positive correlation between NIRs data and analytical data.

Table 3.2. *Correlation between NIRs and analytical data*

	NIRs Data	Analitycal Data	r (Pearson)	p
Property	mean ±sd	mean ±sd		
TOM	3.91±0.65	3.61±0.72	0.909	**
Protein	0.54±0.12	0.47±0.12	0.927	**
Carbohydrates	1.11±0.23	1.05±0.23	0.932	**
Lipids	0.56±0.15	0.59±0.16	0.906	**

**p<0,01

3.3.3. Field Application

After validating the calibration curves, further sediment samples from different sampling stations were analysed using NIRs.

The results obtained were compared with chemical analysis.

Figure 3.3 shows that the results obtained by NIRs were the same results obtained by laboratory analysis.

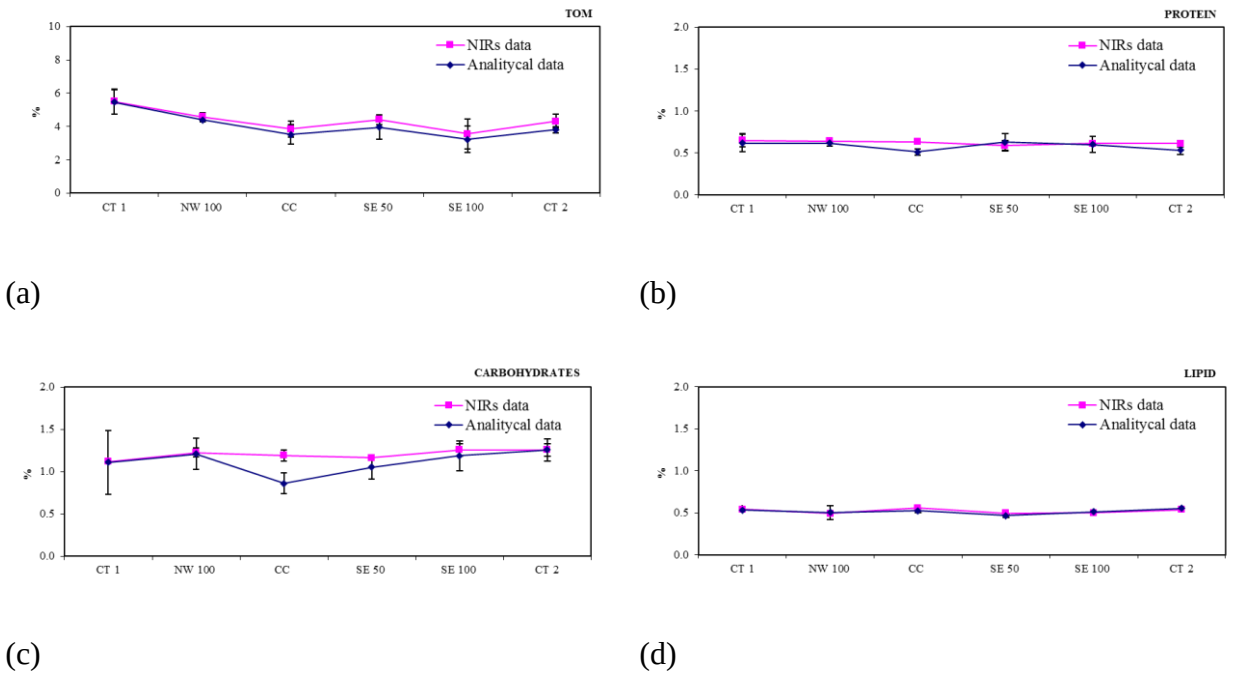

Fig.3.3. *Correlation between NIRs and analytical data: TOM (a); Protein (b); Carbohydrates (c); Lipid (d).*

The results obtained showed that NIRs is a valid technique for the investigation on these matrices, in agreement with Galasso *et al*., (2017) and Malley & Williams (2014). These authors developed calibration models for the assessment of organic matter content, total organic carbon, total organic nitrogen, carbon-nitrogen ratio, total phosphorus and lipid content from farmed sea bass faeces and sediments. Thus demonstrating a strong relationship between NIRS predictions and values measured by laboratory analyses (Galasso *et al*., 2017; Malley & Williams, 2014)

3.4. CONCLUSION

NIRs provides a "fingerprint" of the sample (Cozzolino, 2016) and allows to analysed samples in real time, making it a valuable tool (Malley & Williams, 2014). Indeed, NIR spectroscopy is increasingly being used to replace traditional analytical methods (Fernández Pierna *et al*., 2018), as it allows accurate results to be obtained easily, cheaply and without damaging the sample, preserving it for further analysis. Nevertheless, this technique implies the need to acquire hundreds of spectra to obtain a valid calibration curve (Fernández Pierna *et al*., 2018).

The results obtained indicate that NIRs technology contribute greatly to the monitoring of aquatic ecosystems due to savings on analysis, speed of analysis, and the ability to analyze multiple properties simultaneously (Malley & Williams, 2014), proving to be a reliable and transferable technique.

The use of NIRs in ecological research and aquaculture depends on the development of robust calibration models that allow to assess, with good accuracy, the composition of a wide range of wastes (Galasso *et al.*, 2017).

Marine fish farms, due to uneaten food and fish feces, could cause environmental disturbance on the sediment (Martinez-Garcia *et al.*, 2013); quantification of this organic matter is important to assess the effects of aquaculture facilities on the surrounding environment.

This study confirms the potential of NIRs methodology to predict the organic composition of aquaculture facility wastes in terms of lipid, protein, carbohydrate, and TOM contents.

3.5. REFERENCES

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CHAPTER 4

4. FATTY ACIDS

4.1. INTRODUCTION

Aquaculture, among primary industries, is one of the fastest growing food sectors globally (FAO, 2020; Perdikaris *et al*., 2016). This leads to increased feed intake for farmed species. Although efforts are being made to minimise aquaculture waste through investment in improved feed composition and maximised digestion, cage farming results in unconsumed feed, fish digestion and excretion products being released directly into the environment (Mavraganis *et al*., 2017; Tacon & Forster, 2003; White *et al*., 2019). The release of these substances results in an organic enrichment of the sediment beneath the cages and can lead to anoxic and reducing conditions (Fernandez-Jover *et al*., 2011; George & Parrish, 2015; Tičina *et al*., 2020; White *et al*., 2019).

Monitoring programmers in aquaculture usually involve the detection of parameters such as pH, redox potential, grain size and benthic community studies. It is also important to know the chemical composition of the sediment, such as lipids, which can give information on the transformation of organic matter (Van Biesen & Parrish, 2005).

In marine ecosystems, lipids provide the densest form of energy, which is transferred through the food web (Parrish *et al*., 2015). In particular, among the various fatty acids, polyunsaturated essential fatty acids of the long-chain omega-3 series (PUFA omega-3), are important for ecosystem health and stability (Parrish *et al*., 2015).

Lipids can be used as biomarkers to assess the dispersion of organic waste from a sea cage facility. In particular, fatty acids can be used to assess the dispersion of these wastes. These markers can be used to track the sources of both natural production (Budge & Parrish, 1998) and aquaculture organic waste in the marine environment (White *et al*., 2017; Woodcock *et al*., 2017).

Fatty acids are used as dietary tracers (Dalsgaard *et al*., 2003; Kelly & Scheibling, 2012). It has been shown that both individual molecules and fatty acid groups bio-accumulate through food webs and can be traced back to specific origins. In fact, particular long-chain (≥C20) PUFA omega-3 such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are produced by marine phytoplankton (Dalsgaard *et al*., 2003). Terrestrial plants, on the other hand, are the predominant producers of PUFA (≤C18) (Turchini *et al*., 2009), in particular oleic acid (OA, 18:1n-

9), linoleic acid (LA, 18:2n-6), and α-linolenic acid (ALA, 18:3n3) (Woodcock *et al*., 2019) and do not produce any long-chain (≥C20) PUFA omega-3. For this reason, marine and terrestrial ecosystems have markedly different fatty acid profiles (White *et al*., 2017).

In this context, fatty acid analysis could be used as a tool for existing monitoring programs (White *et al*., 2017) and provide significant results on the origin of impacts in aquaculture sediments (White *et al*., 2019).

4.2 MATERIALS AND METHODS

4.2.1 Lipids Analysis

I have defined three techniques for extracting lipids from sediments (Chapter 3):

1) Chemical extraction
2) Supercritical fluid extraction (SFE)
3) High pressure liquid extraction (PLE)

4.2.2. Fatty acids analyses

Fatty acids methyl esters (FAMEs) were obtained from the total lipids extracted by PLE (Chapter 3) according to Lepage & Roy (Lepage & Roy, 1984). The methyl esters were determined by gravimetric method, dissolved at 1% in cycloexane and analyzed by gas chromatography. The instrument was a gas chromatographer (Clarus 580 GC, PerkinElmer,USA) equipped with a silica capillary column (30 m × 0.32 mm × 0.25 μm Omegawax 320, Supelco, Bellefonte, PA, USA) and a flame ionization detector. Helium was used as the carrier gas (25 cm s^{-1}). The column temperature was programmed at 200◦C, injector and detector were maintained at 250◦C and 300◦C, respectively. Individual FAMEs were identified by comparison of known standard (mix PUFA of fish oil,Supelco, Bellefonte, PA, USA).

4.2.3. Statistical analysis

One-way analysis of variance (ANOVA) was performed Significance was accepted at probabilities $P < 0.05$. Analyses were performed by STATISTICA version 6.0.

4.3. RESULTS AND DISCUSSION

4.3.1 Lipid analysis

Among the used techniques for lipid extraction, PLE proved to be the most efficient in terms of cost and benefit. In fact, this technique gave a better yield than SFE extraction and overcame the extraction problems encountered with the Folch method (data not shown). The Folch method, in fact, led to in an overestimation of the lipid content, due to the presence of residual particulate matter that was not removed during filtration.

After verifying its effectiveness, all samples were extracted using PLE technique. From the total lipids, fatty acids were obtained.

4.3.2. Fatty acids

Fatty acids (FAs), used as biomarkers, play an important role in tracking the dispersal of aquaculture wastes (uneaten food, faeces etc.) (White *et al*., 2019).

Although there are few studies regarding the use of FAs as sediment biomarkers to track waste from aquaculture (White *et al*., 2019).

In my study I evaluated the FAs profile of sediments collected from two different stations: Central Cage (CC) and Control 2 (CT_2).

The obtained results are shown in Figure 4.1.

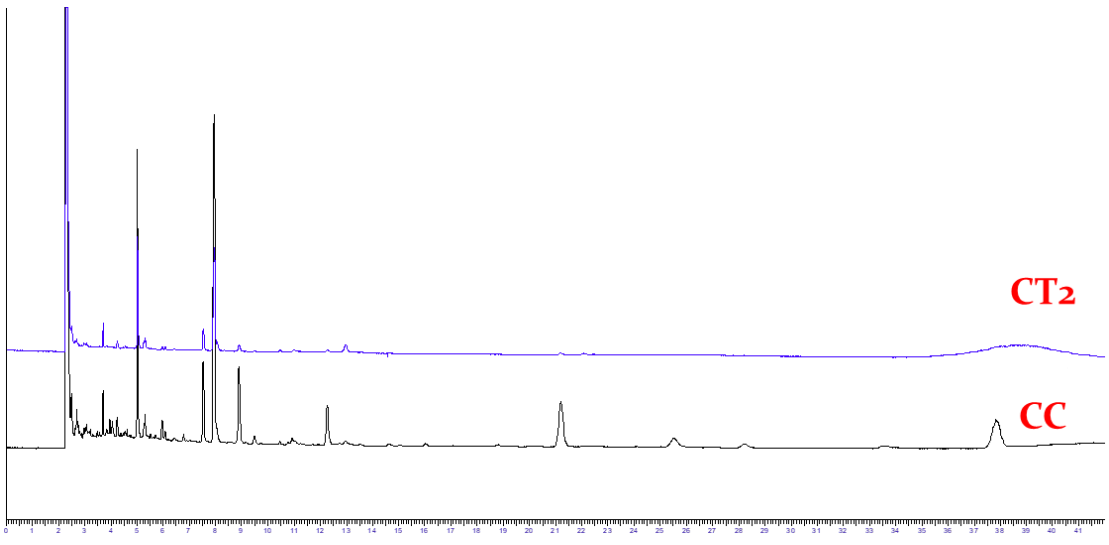

Fig.4.1. *Sediment profile of fatty acids collected from the central cages station (CC) and from a control station (CT_2)*

The fatty acid profiles of CC and CT_2 were statistically different ($P<0.05$). CC shows the presence of fatty acids, not detected in CT_2.

The main difference between the two stations is in the n-3 PUFAs; the CC sediment showed a significantly ($P<0.05$) higher content (28.48 ± 0.90 data not shown) than the CT_2 sediment (0.90± 0.23 data not shown). The fatty acids most representative of this class were EPA and DHA, in fact in CC these values were 11.35±0.27 and 12.96 ± 0.39 respectively, while these fatty acids were not found in CT_2.

Results are in agreement with Woodcock *et al.* (2019) who observed, in sediments collected underneath farmed salmons cages, that EPA and DHA fatty acid concentrations were higher at the station at 0 m distance from the cages than other stations (Woodcock *et al*., 2019).

The present study showed that the composition of the sediments near the tuna cages is influenced by the intake of waste from the cages and in particular by the not ingested food (Black *et al*., 2012; Henderson *et al*., 1997; Johnsen *et al*., 1993; White *et al*., 2017; Woodcock *et al*., 2017).

4.4. CONCLUSION

The study conducted, allows to highlight that FA analysis can be used to monitor the accumulation of aquaculture wastes in the marine environment.

FA has an increasingly recognized wide applicability in tracking aquaculture wastes in marine ecosystems, allowing the distinction between terrestrial FA and FA of marine origin. The increase in some fatty acids such as 18:1n9 and 18:2n6 indicate an increase in plant components of terrestrial origin in the diet. While a decrease in EPA and DHA is a primary response due to a decrease in n-3 PUFA in the diet (White *et al*., 2019).

The application of FA biomarkers to trace aquaculture wastes is still a relatively new method. Further studies are needed to confirm the efficacy of this biomarker.

These biomarkers are very useful, as they allow the origin of organic enrichment and its potential impact to be defined, providing researchers with a useful tool for examining the overall influence of aquaculture on marine ecosystems and for sustainable management of this activity.

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CHAPTER 5

5. REMOTE SENSING

5.1. INTRODUCTION

The term ‘remote sensing’ refers to the technique (or techniques) for the acquisition of physical data of an object without touch or contact (Lintz & Simonett, 1976; Rajitha *et al*., 2007).

The introduction of this technique has changed the approach to monitoring marine ecosystems, because it allows the quantification of phytoplankton biomass through a synoptic view of the environmental variables influencing it (Volpe *et al*., 2012).

Coastal monitoring programs and oceanographic campaigns involve the collection of samples *in situ* and for limited periods, ensuring the acquisition of accurate but punctiform results. This limitation can be overcome by remote sensing, which allows data to be acquired over large temporal and spatial scales, significantly changing the way marine ecosystem dynamics are investigated (Devi *et al*., 2015; Robinson, 2004; 2010).

However, a limitation of remote sensing data acquisition is related to the difficulty of observing properties at depth in water column. Indeed, the detection capability of satellite observations depends on the band and the measurement principle considered (Sammartino *et al*., 2020; Volpe *et al*., 2012).

At first, remote sensing applications were linked to the assessment of global changes in land cover (Ramankutty & Foley, 1999; Skole & Tucker, 1993). Then, this technique allowed to map carbon stocks (Asner *et al*., 2010) and bird habitats (Goetz *et al*., 2010; Rodrigues *et al*., 2012).

In recent years, remote sensing research has focused on the study of ecological response variables ranging from extinction probability (Di Marco *et al*., 2014) to genotype (Madritch *et al*., 2014).

Regarding the ecological response variables, one of the fields of application of the remote sensing technique is related to the study of marine ecosystems and their primary productivity (Rose *et al*., 2015).

Marine Primary productivity is linked to phytoplankton growth in relation to nutrient availability (Dalsgaard & Krause-Jensen, 2006).

Phytoplankton is at the basis of the marine food web (Rinaldi *et al*., 2014). If it grows excessively, it causes a lack of oxygen during the respiration process, becoming dangerous for aquatic life. Monitoring the dynamics of phytoplankton is crucial for the functioning of the marine ecosystem (Mavraganis *et al*., 2020; Righetti *et al*., 2019; Rinaldi *et al.*, 2014).

In remote sensing, phytoplankton biomass can be detected through ocean color measurements. Consequently, these measurements allow for a quantitative assessment of chlorophyll-a concentrations (Rose *et al*., 2015; Volpe *et al*., 2012).

Satellite systems require a combination of temporal frequency (once a day to once a year), spatial resolution (size of measured area; from 2 meters to 1.2 km pixels), spectral resolution (number, size and location of spectral bands) and radiometric resolution. The latter is able to determine the lowest radiance or reflectance range that the sensor can detect and reliably discriminate by spectral band (Dekker *et al*., 2019).

Using the global coverage and spatial and temporal resolution of satellite observations, it is possible to map changes from small to large scales and in particular, high temporal resolution imagery is used to capture changes in chlorophyll levels associated with algal blooms (Rose *et al*., 2015).

Satellite imagery, through interpretation of the received at-sensor radiance at different wavelengths, can estimate chlorophyll concentrations (Gordon & Morel, 2012), using phytoplankton absorption in the visible region (0.4-0.7 mm) (Rajitha *et al*., 2007).

Through optical means in the visible/near infrared (VIS/NIR; ~400-900 nm) spectral region, it is possible to define water quality (Dekker *et al.*, 2019).

Light reaches the water surface and is reflected or refracted; dissolved and particulate water constituents scatter the light and some of it is reflected up through the atmosphere and observed by satellite sensors (Figure 5.1) (Dekker *et al.*, 2019).

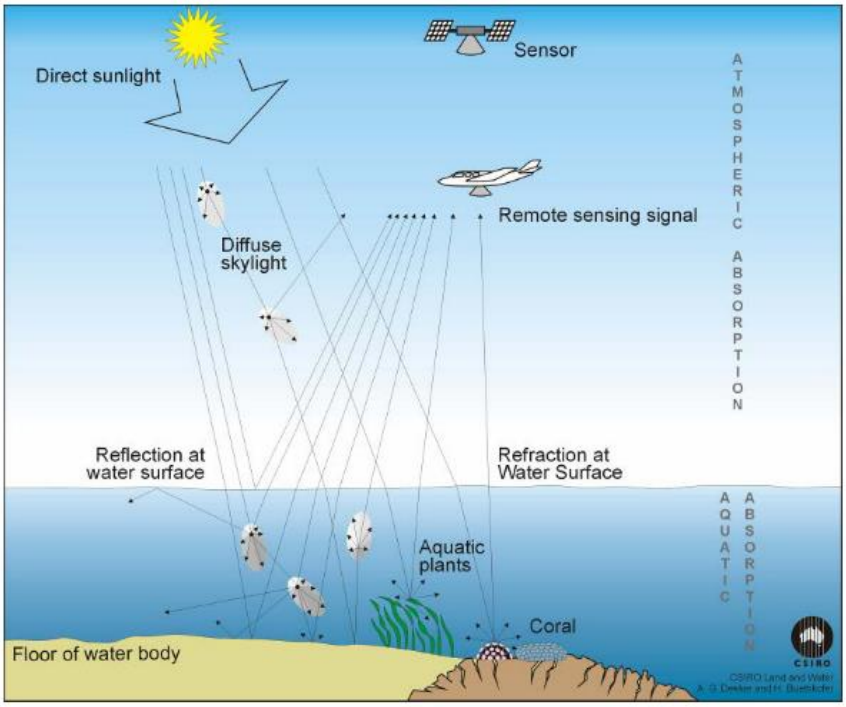

Fig.5.1. *Schematic of the light interactions that drive optical Earth Orbit involving the air, water and substrate (Dekker et al., 2019).*

Remote sensing techniques could be included in environmental monitoring programs, as they allow the detection of an imbalance in marine phytoplankton through quantitative estimates of chlorophyll in water bodies. Thus contributing to improving and preventing these imbalances in the marine environment (Adhikary *et al*., 2021; Rose *et al*., 2015).

5.2. MATERIALS AND METHODS

5.2.1 Study area

The study area of this research was in the coordinate location 38°03'36"-38°02'45" N and 12°52'03"-12°53'38" E.

I have used 2 differences satellite remote sensing to study the distribution of chlorophyll-a (chl-a) variations among 1997-2009.

The distribution of chlorophyll-a variations was obtained from several data on Landsat 5 and Landsat 7 images.

The characteristics of the two satellites sensors used are:

Landsat 5 Spatial Resolution (m) = 30 multispectral,120 thermal.

Spectral Range (nm) = 450e12500

Number of Bands = 7

Landsat 7 Spatial Resolution (m) = 15 panchromatic,30 multispectral,60 thermal.

Spectral Range (nm) = 450e12500

Number of Bands = 8

(Schroeder *et al*., 2019).

Landsat data used in this study downloaded from http://glovis.usgs.gov/app and ENVI image analysis software was used to process (atmospheric corrections) the Landsat data.

After downloading the images and processing them, the few field data available were used to choose the algorithm for calculating chl-a concentrations.

In this study, we have selected the Oceanic Chlorophyll 2 algorithm (OC2), that is a modified cubic polynomial (MCP) function that uses Rrs490/Rrs555, where Rrs is remote sensing reflectance and 490 and 555 are wavelengths and the formula is

$$\mathrm{C} = [10\text{^}(a_0 + a_1\mathrm{R} + a_2R^2 + a_3R^3)] + a_4$$

where:

$R = \log\left(\frac{Rrs490}{Rrs555}\right)$

$\boldsymbol{a_0} = 0.3410 \quad \boldsymbol{a_1} = -3.0010 \quad \boldsymbol{a_2} = 2.8110 \quad \boldsymbol{a_3} = -2.0410 \quad \boldsymbol{a_4} = -0.0400$ (O'Reilly *et al*., 1998).

5.2.2. Statistical analysis

To investigate whether there were differences in chlorophyll-a concentration between years ("Years"), before (1997 to 2000) and after (2001 to 2009) the start of aquaculture activities ("Before VS After"), a Permutational Analysis of Variance, PERMANOVA (M. Anderson & Braak, 2003) was performed using the "PRIMER 7" software.

PERMANOVA was based on Euclidean distance matrix with 9999 permutations and was chosen because this method does not assume a normal distribution of errors, allows for factorial designs and accounts for interaction effects (Anderson *et al*., 2008).

5.3. RESULTS AND DISCUSSION

This study investigated if there were differences in chlorophyll-a concentrations in the Bluefin tuna farm at the Castellammare del Golfo facility, during the period 1997-2009.

This work presented some limitations due to:

1. the presence of clouds that did not allow the acquisition of Chl-a concentrations at certain times of the year;
2. the failure of the Landsat 7 Scan Line Corrector, this failure occurred in 2003 and resulted in a 22% loss of pixels in imagery (Chen *et al*., 2011).

These limitations did not allow an analysis to be performed for each sampling station as had been planned.

An evaluation of a large number of semi-analytical and empirical algorithms, for data collected from a variety of sources and locations, was conducted by O'Reilly and colleagues (O'Reilly *et al*., 1998). Based on their analysis, it was found that most of the empirical algorithms performed better than the semi-analytical algorithms and among the empirical algorithms the best of all were the cubic polynomial formulations such as the Ocean Chlorophyll 2 (OC2) and Ocean Chlorophyll 4 (OC4) models (Chauhan *et al*., 2002).

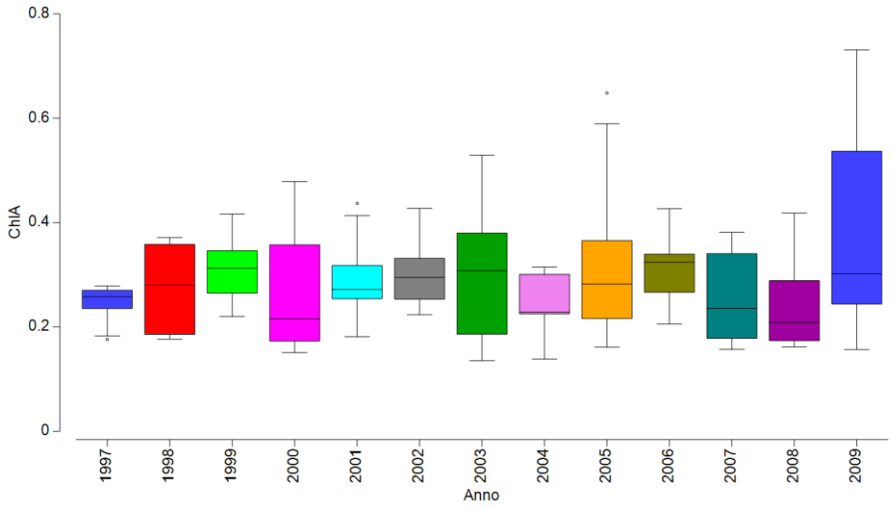

Fig. 5.2. *Distribution of Chl-a concentration at the tuna farm sited in the Gulf of Castellammare from 1997 to 2009*

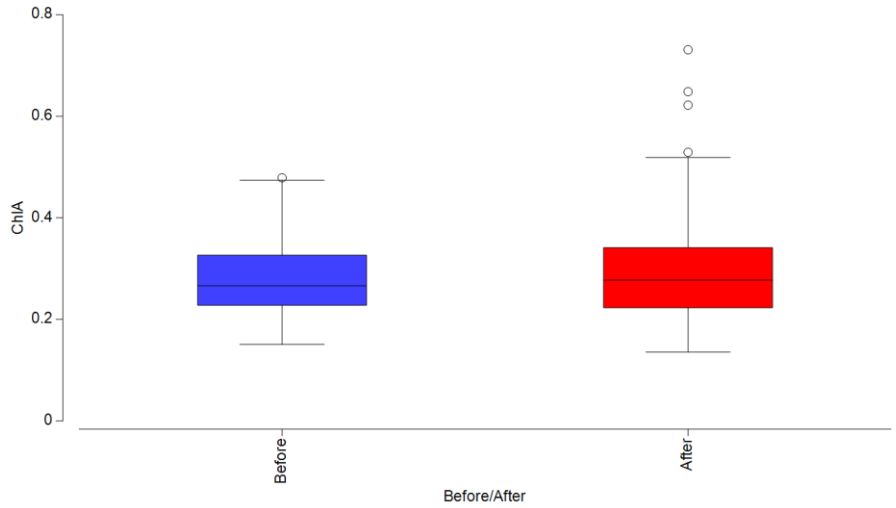

Fig.5.3. *Distribution of Chl-a concentration at the tuna farm sited in the Gulf of Castellammare before (1997 to 2000) and after (2001 to 2009) the start of the aquaculture farm activities.*

Table 5.1. *Results of PERMANOVA analysis for Chl-a concentration in different Years*

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Years	12	0.16781	0.013984	1.1841	**0.3026**	9930
p > 0.05						

Table 5.2. *PERMANOVA analysis on the Chl-a concentration, associated with Before VS After*

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Before VS After	1	0.0066737	0.0066737	0.46737	**0.5388**	9826
p > 0.05						

As “p” is > of 0.05 it means that there are no significant differences in Chlorophyll-a values either between “Years” or “Before VS After” (PERMANOVA, p > 0.05) (Table 5.1; Table 5.2; Fig. 5.2; Fig. 5.3).

The results are in agreement with Fanelli (2007) in which chl-a concentrations were measured monthly by satellites in the Gulf of Castellammare, from December 2003 to June 2005. Values ranged from about 0.1 to about 0.7 mg/m^3 were observed (Fanelli, 2007).

According to these results, in the Tičina *et al*. (2020), regarding the impacts of marine aquaculture on marine biota in oligotrophic environments of the Mediterranean Sea, several authors reported to have found no significant differences, between aquaculture and control sites, on phytoplankton

assemblages in the Mediterranean Sea based on in situ analyses (Tičina *et al*., 2020),particularly in La Rosa *et al*. (La Rosa *et al.*, 2002) did not observe significant differences between the cage and control sites (located at about 1 km far from the cage) in chlorophyll-a concentration in sea water samples at a fish farm located in the Gaeta Gulf in the Tyrrhenian Sea (Western Mediterranean) (La Rosa *et al*., 2002).

Gohin *et al*., (2020) results confirm good overall agreement between satellite-derived Chl-a observations and in situ data.

The use of satellite imagery has the advantage of multi-temporal assessment and low cost compared to prolonged field sampling, so it is considered an excellent instrument for water monitoring (Rajitha *et al.*, 2007).

The interaction of light with chlorophyll, in the visible spectrum, changes the shape and amount of the reflected signal (Kirk, 2011) and these changes are used by water quality algorithms (Dekker *et al*., 2019).

5.4. CONCLUSION

Chlorophyll, in the pelagic environment, has been considered the most significant biological variable to detect eutrophic conditions (Tičina *et al*., 2020).

Satellites are recognized all over the world as important instruments to estimate the trophic state of oceans and seas (Cherif *et al*., 2021).

Data on the extent of aquaculture areas, obtained by remote sensing, are very useful, both for scientists and for public authorities, in order to monitor and evaluate the status and dynamics of aquaculture, so as to provide useful data to develop appropriate measures for environmental conservation and natural resources management (Ottinger *et al*., 2016).

Also the Marine Strategy Framework Directive includes the use of remote sensing to assess the state of eutrophication and biodiversity of European seas (Cherif *et al*., 2021).

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6. CONCLUDING REMARKS

During the three years of my research activity, I validated a series of descriptors and indicators that can be used by the aquaculture industries to independently conduct monitoring to ensure sustainable production and in the laboratory to identify the origin of sediments.

The choice of an indicator is important, as it can allow to synthesize information from complex phenomena. For this reason, it must to be accessible and measurable (Fezzardi *et al*., 2013). An indicator has three main functions: simplification, quantification and communication (Fezzardi *et al.*, 2013).

The indicators studied have been sulphides and fatty acids.

Regarding the use of sulphide content determination, obtained results made it possible to carry out a space-time assessment of the effect on the environment of a Bluefin tuna sea cage farm.

The electrochemical technique I used to detect the sulphide content has the advantage of easy application, low cost and direct, sensitive and fast detection even in the presence of low sulphide concentration (Gomez *et al*., 2018). These characteristics make it an easily transferable technique to industry.

The use of fatty acids as an indicator of the environmental status of the sediments made it possible to identify spatial differences over the same sampling years. Significant differences were observed between the fatty acid profiles of the sediments under the cages compared to the control stations.

The fatty acid profile was determined by means of gas chromatographic analysis (section 4.2.2). The Gas Chromatograph allows rapid and specific analysis of fatty acids being a precise, sensitive and reproducible tool (Tang & Row, 2013). This technique is not transferable to the farm because it is expensive, requires laboratory equipment and qualified personnel. However, it has been validated because fatty acid analysis can distinguish whether the organic matter is from aquaculture, the marine environment, or other sources of anthropogenic impact.

The assessment of the composition of marine sediments from floating cage farms was successfully investigated using the above mentioned indicators (sulphides and fatty acids) and NIRs technology. NIRs technology provides fast and accurate information on sediment composition in terms of several parameters, proving to be a reliable and transferable technique.

Another useful technology for tracking and assessing the status and dynamics of aquaculture is Remote Sensing, allowing multi-temporal and large-scale monitoring at low cost compared to long-

term field sampling. This technology, already included in the Marine Strategy Framework Directive to assess the state of eutrophication and biodiversity of European seas, was used as early warning indicator to detect eutrophic trends in the marine environment, as it allows the detection of an imbalance in marine phytoplankton through quantitative estimates of chlorophyll in water bodies.

The use of these indicators and technologies will allow the aquaculture industry to conduct easily and at low cost its own monitoring to provide evidence of the sustainability of their production.

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