ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DOTTORATO DI RICERCA IN

TECNOLOGIE INNOVATIVE E USO SOSTENIBILE DELLE RISORSE DI PESCA E BIOLOGICHE DEL MEDITERRANEO (FISHMED-PHD)

Ciclo 37

Settore Concorsuale: 05/C1 - ECOLOGIA

Settore Scientifico Disciplinare: BIO/07 - ECOLOGIA

TOWARDS A SUSTAINABLE AND HEALTHY USE OF FISHERY RESOURCES: PROCESSES, TECHNOLOGIES, NUTRITIONAL QUALITY AND CONTAMINANTS

Presentata da: Polina Rusanova

Coordinatore Dottorato

Dr. Mauro Marini

Supervisore

Dr. Gioacchino Bono

Co-supervisore

Prof. Michele Casini

Prof. Stefano Goffredo

Esame finale anno 2025

Towards a sustainable and healthy use of fishery resources: processes, technologies, nutritional quality and contaminants

Abstract

With a direct impact on global food security and public health and providing essential nutrients to billions of people, seafood safety is a major concern in today's world. Contaminants such as microplastics, heavy metals, other chemicals and even preservation issues are increasingly jeopardizing the quality of seafood, posing a risk to both marine ecosystems and human consumers. Ensuring safe and sustainable seafood is the key to protecting both the environment and human well-being in the face of these growing challenges.

This dissertation explores sustainable approaches to the use of seafood, focusing on seafood safety, nutritional quality and environmental impact.

In the context of environmental challenges, the study highlights the increasing presence of microplastics and chemical contaminants in some seafood products, identifying their physiological effects on key species and the associated health risks to human consumers. Additionally, the study assesses the efficacy of technological advancements in seafood preservation, with particular emphasis on modified atmosphere packaging (MAP) and rapid freezing techniques as means for reducing the use of chemical additives while maintaining high product quality. These post-harvest technologies play a key role in preserving nutritional composition and ensuring safety along the supply chain.

This work addresses the sustainable use of fisheries and aquaculture resources from a holistic point of view, using seafood valorisation as a lever to compensate for the decline in fishery resources (and fishermen's profits). It provides ideas and explores innovative strategies for better preserving seafood products within the context of a growing global demand for safe and sustainable seafood.

Table of Contents

Introduction

1. Fishery and aquaculture

1.1 The state of the art

The fisheries and aquaculture sector is important for global food security and livelihoods, providing millions of people with essential sources of food, nutrition, income and employment. This sector not only supports economic growth through the harvesting, processing and marketing of aquatic products but also fosters community development and resilience. Many countries, particularly developing nations and small developing island states, rely heavily on fisheries and aquaculture, which are indispensable for the well-being of coastal, riverine, insular and inland populations. The development and progress of this sector is closely linked, for better or worse, to the issues of biodiversity conservation and sustainable management of aquatic resources. According to statistical data from FAO Yearbook 2021 ("Fish. Aquac. Stat. – Yearb. 2021," 2024), the combined production of fisheries and aquaculture reached a remarkable 218 million tons, marking a new record. This represents a notable increase of more than 2% compared to the 2020 results (FAO, 2022c).

As for fishery production by region, Asia emerged as the dominant force and was responsible for 70 percent of the global production of aquatic animals, with the Americas following at 13 percent, Europe at 9 percent, Africa at 7 percent, and Oceania at 1 percent. Capture fisheries accounted for 91.2 million tons, representing half of the total aquatic animal production, while aquaculture closely matched this figure with 90.9 million tons. The distribution of aquaculture varied across continents: Asia led with 63%, followed by Europe at 21%, the Americas at 19%, Africa at 18%, and Oceania at 14%. When factoring in algae production, aquaculture's share of total production rose to 58%. These statistics underscore the growing significance of aquaculture in meeting the global demand for aquatic products, while also highlighting regional differences in production methods and outputs ("Fish. Aquac. Stat. – Yearb. 2021," 2024).

Fishery and aquaculture products are now used in various sectors for direct human consumption or for processing into fishmeal and/or oil and, to a lesser extent, for various non-food uses. These non-food uses include ornamental fish, culturing fingerlings and fry, bait, pharmaceutical inputs, and feed for aquaculture, livestock and other animals. In 2021, nearly 89 percent of the total production of aquatic animals was used for direct human consumption, up from 72 percent in 1961. Additionally, 9 percent was processed into fishmeal and fish oil, while the remaining 2 percent was used for other non-food purposes. Of the aquatic products intended for human consumption, approximately 44 percent were sold as fresh products, 34 percent as frozen products, 12 percent as prepared and preserved items, and 10 percent as cured products (e.g. dried, salted or smoked) (FAO, 2022b).

Aquatic foods are among the most highly traded food commodities globally, with 225 states and territories reporting trading activities in fisheries and aquaculture products in 2020. In that year, world exports of aquatic products, excluding algae, amounted to approximately 60 million tons live weight, valued at 151 billion USD. This marked a significant decrease (8.4 percent in value and 10.5 percent in volume) from the record high of 67 million tons, worth USD 165 billion, achieved in 2018. From 1976 to 2020, the value of global exports of fisheries and aquaculture products (excluding algae) saw an average annual growth rate of 6.9 percent in nominal terms and 3.9 percent in real terms (adjusted for inflation), with an annual growth rate of 2.9 percent in terms of quantity over the same period. The global consumption of aquatic foods rose at an average annual rate of 3.0 percent from 1961 to 2019, nearly double the annual world population growth rate of 1.6 percent during the same period. Per capita consumption of aquatic animal foods increased by approximately 1.4 percent annually, climbing from 9.0 kg (live weight equivalent) in 1961 to 20.5 kg in 2019. Preliminary figures for 2020 indicate a slight decrease to 20.2 kg. In that year, aquaculture contributed 56 percent of the aquatic animal food production available for human consumption. Over recent decades, per capita consumption of aquatic foods has been significantly influenced by greater availability, shifting consumer preferences, technological advancements and income growth (FAO, 2022b, 2022a).

1.2 Challenges in the fishery sector

The fishery and aquaculture sector must address a variety of challenges. The specific issues faced by any given fishery or aquaculture production will depend on the category in question. These can include social, gender, economic, scientific, legal and environmental issues. ("Glob. Environ. Outlook – GEO-6 Heal. Planet, Heal. People," 2019).

Fisheries can be divided into two categories: small-scale fisheries (artisanal fisheries) and large-scale fisheries, each with distinct issues.

Small-scale fisheries are vital for employment, providing nearly 80% of the global fisheries workforce. They often use traditional fishing methods and gear and are typically managed at the local level. The role of women in this fishery activity is significant, particularly in developing countries and especially during post-harvest activities. However, their contributions are frequently underreported. Small-scale fisheries are also more vulnerable to external threats such as climate change and often cannot sustain households above the poverty line (Illuminating Hidden Harvests, 2022).

Large-scale fisheries, on the other hand, generate higher direct economic revenues but require greater capital investment. They tend to have a larger ecological footprint due to bycatch rates and habitat impacts from fishing gear (Illuminating Hidden Harvests, 2022).

Challenges in fisheries include difficulties in management, as systematic data collection for small-scale fisheries leads to inadequate monitoring of fishing effort and catches, with the consequent risk of unsustainable exploitation of marine resources. The lack of clear definitions, uneven adaptive capacity, gender dynamics, climate change impacts, health risks and seafood fraud contribute to economic, social and regulatory issues. Additionally, environmental concerns such as coral reef damage, heavy metal contamination and bycatch further threaten the sustainability and effective management of fisheries ("Glob. Environ. Outlook – GEO-6 Heal. Planet, Heal. People," 2019).

These challenges are interconnected and require a multifaceted approach to address the complexities of fisheries management and to secure the role of small-scale fisheries in sustainable development.

The specific focus of this PhD was on environmental issues, particularly the effects of contaminants on ecosystems and the sustainable use of seafood and fish. This includes investigating contaminant effects on marine ecosystems, assessing seafood nutritional quality and safety, and exploring innovative approaches to sustainable fisheries management.

1.3 Environmental Contaminants: Understanding the Impact of Pollutants on Seafood

Aquatic ecosystems are increasingly threatened by a wide variety of pollutants - especially those resulting from human activity - which can threaten the health of living organisms and degrade the environment by making water bodies unsuitable for use (Bashir et al., 2020; "Glob. Environ. Outlook – GEO-6 Heal. Planet, Heal. People," 2019).

The pollutants and their impact on aquatic ecosystems are presented below. Plastic-derived contaminants (i.e. nano- and microplastics) are described separately and in detail due to their urgent, real global threat to both the environment and food safety. In particular, as part of the PhD programme, a systematic review on this topic was published as a book chapter.

1.3.1 Plastic

The world generates approximately 350 million tons of plastic waste annually.

Nearly one quarter of the world's plastic waste, about 82 million tons, is mismanaged or littered. This means it is not stored in secure landfills, recycled or incinerated. Of this mismanaged waste, one quarter, or 19 million tons, leaks into the environment. This includes 13 million tons entering terrestrial environments and 6 million tons reaching rivers or coastlines. From here, 1.7 million tons eventually reach the ocean: 1.4 million tons are transported via rivers, and 0.3 million tons from coastlines. The remaining plastic waste that

leaks into aquatic environments accumulates in rivers and lakes. Approximately 0.5% of the world's plastic waste therefore ends up in the oceans (Hannah Ritchie, 2023).

Plastic pollution is largely influenced by fishing and aquaculture activities, primarily due to plastic nets abandoned at sea. In the short term, these discarded nets continue to trap marine fauna and cause physical damage to the environment, while in the long term they contribute to the increase in micro- and nanoplastics. The quantity of abandoned fishing gear and aquaculture waste in European waters is currently estimated at 11,000 tonnes per year (UNEP (United Nations Environment Programme), 2021).

Contamination of fish and shellfish by plastic and associated toxins poses risks to fundamental physiological processes of aquatic organisms such as cell cycle arrest, growth arrest and other oxidative stress (Anbumani et al., 2018). Furthermore, plastic contaminants affect the quality of seafood and fish products. The implications of plastic pollution on food security and community well-being are significant,and urgent action is required to mitigate these multifaceted challenges. The use of plastic materials has resulted in adverse consequences for the environment and food safety, which have collectively contributed to the deterioration of ecological systems. (Thomas et al., 2019).

1.3.2 Other emerging contaminants in seafood

Aquatic ecosystems are increasingly threatened by several other contaminants (e.g., heavy metals, organochlorine pesticides, polycyclic aromatic hydrocarbons [PAHs], pharmaceuticals and personal care products [PPCPs], phthalates and parabens), which pose significant risks to the physiological health of animals living in these environments, as well as to the people who rely on these resources for food ("Glob. Environ. Outlook – GEO-6 Heal. Planet, Heal. People," 2019).

Heavy metals are a serious environmental hazard due to their toxicity, persistence and bioaccumulation. These metals, including mercury, lead, cadmium, chromium and arsenic, enter water bodies through industrial discharges, urban runoff, atmospheric deposition and improper waste disposal. They pose significant risks to aquatic life, including immunotoxicity and neurotoxicity (United Nations Environment Programme [UNEP], 2023).

Organochlorine pesticides, classified as persistent organic pollutants (POPs), are likewise troubling. They persist in the environment, bioaccumulate in the food web and cause endocrine disruption, reproductive impairment, and immune system effects in marine life. These pesticides enter marine systems through historical use, agricultural runoff and atmospheric deposition, affecting top predators and marine mammals (Kaw & Kannan, 2017; Kurek et al., 2017).

Polycyclic Aromatic Hydrocarbons (PAHs), originating from incomplete combustion of fossil fuels and organic matter, enter marine environments through oil spills, atmospheric deposition and urban runoff. PAHs accumulate in sediments and biota, causing developmental abnormalities, liver damage and reduced growth in marine organisms (Arias et al., 2009).

Pharmaceuticals and personal care products (PPCPs) are emerging contaminants in aquatic ecosystems, entering water bodies through domestic wastewater, industrial discharges and agricultural runoff. PPCPs, including antibiotics and UV filters, disrupt physiological processes, induce antibiotic resistance and cause hormonal imbalances in aquatic organisms (Han et al., 2021; Pan et al., 2017).

Phthalates and parabens, used as plasticizers and preservatives, respectively, are also problematic. Found in various consumer products, they accumulate in aquatic systems, leading to hormonal disruptions and immune system effects in marine life (Zhang et al., 2021, 2022).

2. Nutritional Quality and Safety of Fish and Seafood Products

The global seafood industry is a dynamic and multifaceted sector crucial to global food security and nutrition. As the demand for seafood rises, driven by its recognized health benefits and high nutritional value, it becomes very important to study and ensure the quality and safety of seafood products. The production and handling of fish and seafood require special attention due to their particular nature (Boziaris & Parlapani, 2017). Aquatic products are highly perishable because of their chemical composition, which includes a high content of non-protein nitrogen compounds (NPN), as well as high water activity and pH levels. These parameters create an ideal environment for microbial colonization and the activity of many spoilage microorganisms (Anagnostopoulos et al., 2022; Leroi & Joffraud, 2011). The sector's complexity is heightened by diverse supply chains, varying regulatory frameworks and the potential for contamination and fraud. Consequently, rigorous scientific research and advanced technological interventions are essential to maintain seafood quality and safety standards, safeguarding public health and sustaining the industry's growth (Bohnes et al., 2020; Cooney et al., 2023; Regueiro et al., 2022).

2.1 Nutritional Benefits of Seafood

The global demand for seafood has surged, driven by its recognized health benefits and nutritional value. According to the FAO (FAO, 2020b), aquaculture and fisheries together provided 17% of the total animal-source protein consumed by humans. Many countries rely on fish and aquatic products as their primary source of dietary protein. Fish plays a crucial nutritional role for many people: 3.1 billion individuals depend on fish for 20% of their daily protein intake, with some coastal communities relying on fish for over 70% of their protein needs (Sustainable Fisheries UW, 2023).

Seafood, including fish, marine mammals, shellfish and various marine flora, offers a rich source of high-quality protein and a variety of essential nutrients, notably long-chain omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). These nutrients are important in promoting health and play a significant role in the prevention of various cardiovascular diseases and in cognitive development and function. However, the perishable nature of seafood products calls for rigorous safety and quality measures from the point of capture to the consumer (Alasalvar et al., 2010; Jayasekara et al., 2020; Liu & Ralston, 2021; McManus & Newton, 2011; Nesheim & Yaktine, 2007; Reames, 2012).

During the PhD, I carried out research within the framework of the project CIRCLES (Controlling mIcRobiomes CircuLations for bEtter food Systems, - Improving food quality). The main task was to estimate the impact of different diets on the nutritional parameters of gilthead sea bream (*Sparus aurata*) in aquaculture production. This topic is expanded upon in the publication 'Nutritional Quality and Safety of Fish and Seafood Products'.

2.2 Challenges in Seafood Safety

Seafood presents a unique set of safety challenges that must be addressed due to the highly perishable nature of these products and their susceptibility to various contaminants. These challenges are further complicated by the global scale of the seafood industry, with complex supply chains spanning multiple countries and regulatory environments. Addressing these safety concerns is essential to ensure that seafood remains safe for consumption while retaining its nutritional benefits.

2.2.1 Chemical and microbiological spoilage

Seafood products are subject to rapid chemical and microbiological degradation/spoilage. One of the main challenges in managing such products is to maintain their freshness (improving shelf-life), nutritional value and, above all, safety. There are three different types of fish spoilage (Microbe Notes, 2023):

- Oxidative spoilage: the most common kind of spoilage for fish with high oil/fat contents. Lipid oxidation leads to protein denaturation, alterations in protein structure and changes in electrophoretic profiles, nutritional degradation and depletion of endogenous antioxidant systems (Geng et al., 2023).

- Enzymatic spoilage: after capture, dead fish undergo biological and chemical changes catalyzed by various enzymes naturally present in their tissues. Proteolysis causes protein degradation, leading to fish spoilage through microbial growth (Microbe Notes, 2023).

- Microbial growth: the internal tissue of fish is considered sterile, while bacteria are commonly found on the slime layer of the skin, gill surfaces and in the intestine. Microbial growth in fish is the primary cause of spoilage, resulting in the production of amines, biogenic amines, organic acids, alcohols, aldehydes and ketones that impart unpleasant and off flavors. The development of microbiological communities depends on the temperature of the products and the method of preparation (Microbe Notes, 2023).

An important step for minimalizing spoilage degradation of seafood and fish products is to use correct methods of preservation for maximizing shelf life and minimizing risks (Microbe Notes, 2023).

I studied this topic in depth during my doctoral studies and published research papers on the optimal methods of preservation and packaging of shrimp products.

2.3 Supply Chain Complexity

The seafood industry is characterized by a highly complex supply chain that spans from the capture or cultivation of seafood to its processing, distribution and sale to the end consumer. This complexity is driven by several factors, including the global nature of the industry, the perishability of seafood products and the stringent regulatory requirements for safety and quality (Denham et al., 2015).

Global Distribution and Trade: Seafood is one of the most internationally traded food commodities. According to the FAO report, around 38% of global seafood production is traded internationally (FAO, 2020a). The global distribution network for seafood involves numerous stakeholders, including fishermen, farmers, processors, wholesalers, retailers and exporters. Each link in this chain must coordinate effectively to ensure that seafood products are transported and handled in a manner that maintains their quality and safety (Anderson et al., 2018; Straume et al., 2024).

Perishability and Logistical Challenges: Seafood is highly perishable, requiring specific temperature and handling conditions to maintain freshness and prevent spoilage (Panebianco et al., 2024). Transport logistics from the point of capture or harvest to the consumer involves rapid transportation, cold storage and efficient customs procedures. Any delays or failures in these logistics can lead to significant losses in quality and increases in waste, such that effective supply chain management is crucial (Bita & Sharifian, 2024).

Regulatory Compliance: Navigating the complex regulatory landscape is a significant challenge within the seafood supply chain. Different countries have varying regulations concerning food safety, quality, labeling and sustainability. For instance, the EU has stringent regulations that govern every aspect of seafood handling and processing (Gordon & Williams, 2020), as outlined in the document.

Traceability and Consumer Demand: Modern consumers demand transparency and traceability in their seafood products. Customers want to know where and how their food was caught or farmed, and whether it is sustainable (Ibáñez, 2015).

During my doctoral studies, I completed an internship at the United Nations Industrial Development Organization (UNIDO), contributing to the Post-harvest Fisheries Development Project (CapFish) by undertaking specific tasks pertaining to the complex supply chains of fish and fish products. One such task is Official Food Safety Control, which involves establishing an official control framework with the necessary tools and procedures and the institutional capacity to train qualified fishery officers and inspectors. The Cambodia Quality Seal should be implemented with official control at private sector operations to ensure

compliance with international food safety standards and should be supported by increasing national laboratory resources and testing capacity (Capfish Postharvest Newsletter, 2023).

2.3.1 Seafood fraud

Deceptive practices intended to mislead consumers regarding the origin, freshness, safety, species, or quality of the seafood products they buy constitutes seafood fraud. This is an important issue due to its implications for consumer safety, economic trust and environmental sustainability (Chai et al., 2021; Donlan & Luque, 2019; Horreo et al., 2017).

The seafood supply chain is vulnerable to various types of fraud at multiple stages, from source to consumption (Fox et al., 2018).

Species Substitution: This is widespread and involves replacing one type of seafood with another, often a less expensive or inferior species. Studies have shown that mislabeling rates can range from 25-60% for commonly substituted species like red snapper, wild salmon and Atlantic cod. This not only deceives consumers but can also pose serious health risks, especially when allergenic or toxic species are involved (Miller et al., 2012; Ryburn et al., 2022).

IUU Fishing: Illegal, unreported and unregulated fishing practices contribute significantly to seafood fraud. They make it difficult to trace and verify the origin and the sustainability of seafood, leading to overfishing and ecological imbalance. Overall, the global economic cost of this phenomenon is estimated at between $36 billion and $50 billion per year. This includes losses from legitimate catch and revenue, as well as income and country tax losses (Sumaila et al., 2020).

Animal Welfare and Ethical Concerns: The conditions under which seafood is harvested can also be a part of fraudulent practices, where claims about animal-friendly methods are falsely made to fetch premium prices (Ali et al., 2015).

Regulatory and Monitoring Challenges: The global nature of the seafood trade, combined with visually similar species and processing methods that obscure species identity, makes regulation and monitoring challenging. This is exacerbated by inadequate systems for documenting catch landings and non-transparent supply chains, particularly in major seafood exporting and importing countries (Fox et al., 2018; Lawrence et al., 2022).

During my PhD, I conducted research at the University of Thessaly in relation to the seafood fraud project: microbiome analyses were used to distinguish the highly valued Norway lobster (*Nephrops norvegicus*) caught in the Mediterranean Sea from, for example, N. lobsters caught in the seas of northern Europe. The main objective of the research was to provide comprehensive information on the authenticity and traceability of Mediterranean Norway lobster along the supply chain, as well as to detect possible differences between the trophic webs of the three populations, which could be used for ecological studies.

3. Summary of the sections that form the backbone of my thesis

Considering all of the above, and in view of some of the weaknesses of the seafood fishery and industry, I focused research on seven main aspects that will likely further knowledge in this sensitive area of the food sector.

The above abovementioned issues were further grouped into the following two chapters:

CHAPTER I - Emerging Contaminants in Seafood Products

CHAPTER II - Postharvest Technologies and Enhancing the Quality of Seafood

The first chapter deals with the emerging problem of microplastics and other pollutants in the marine environment:

1. A systematic review of "Detection methods of micro and nanoplastics" was published as a chapter in the book "Nano/micro-Plastics Toxicity on Food Quality and Food Safety" edited by Fatih Özogul.

2. This review was followed by a second study on the presence of microplastics in the stomach, liver and muscle of thornback rays (*Raja clavata*), an important commercial Mediterranean elasmobranch that is positioned high in the trophic chain. The results of this study are currently being prepared for publication in an authoritative journal in the field. An abstract regarding this study was accepted and presented at the World Seafood Congress 2023, Portugal. I received the IAFI Peter Howgate Award for participating in this congress.

3. A third study complements the two previous ones and regards other pollutants: results have recently been published in the article «Exploring the Impact of Contaminants of Emerging Concern on Fish and Invertebrates Physiology in the Mediterranean Sea», published in the journal "Biology". This publication explores the impact of prevalent pollutants on the aquatic ecosystem, focusing on the physiological responses of two indicator species: the catshark *Scyliorhinus canicula* and the mussel *Mytilus galloprovincialis*.

Under the heading "Postharvest Technologies and Enhancing the Quality of Seafood" (Chapter II) of this thesis, I address:

1. The effects of storing shrimp in a modified atmosphere (without known chemical additives such as sulphites) on the free amino acid (FAA) composition of the deep water rose shrimp (*Parapenaeus longirostris*). Results were published in Frontiers in Nutrition.

2. The quality of Norway lobster (*Nephrops norvegicus*) products was also investigated. In particular, specimens of this species caught in two Mediterranean fishing grounds were compared with those from the North Sea using gut microbiome analysis. The findings from this study can also be used to control Seafood Fraud.

3. During the last year of my dissertation, I focused my scientific work on seabream, a species of great interest to the aquaculture industry. The aim of this subtask was to compare three batches of farmed sea bream, one fed on a conventional diet (control) and the other two fed on two new experimental probiotic diets (ED1 and ED2).

4. As a final activity in the field of valuable crustaceans, which are important assets for international seafood trade, we recently completed a systematic review on "The impact of the handling process and different storage conditions on shrimp quality", a chapter in the book "Postharvest Technologies and Quality Control of Shrimp" published on October 1, 2024. The chapter describes how these valuable crustaceans are caught today (worldwide), which processing and preservation techniques are used and what can be done to improve the state of the art with a view to the sustainable use of fish resources.

REFERENCES

Alasalvar, C., Shahidi, F., Miyashita, K., & Wanasundara, U. (2010). Handbook of Seafood Quality, Safety and Health Applications. In *Handbook of Seafood Quality, Safety and Health Applications*. https://doi.org/10.1002/9781444325546

Ali, M. E., Razzak, M. A., Hamid, S. B. A., Rahman, M. M., Amin, M. Al, Rashid, N. R. A., & Asing. (2015). Multiplex PCR assay for the detection of five meat species forbidden in Islamic foods. *Food Chemistry*, *177*, 214–224. https://doi.org/10.1016/J.FOODCHEM.2014.12.098

Anagnostopoulos, D. A., Parlapani, F. F., & Boziaris, I. S. (2022). The evolution of knowledge on seafood spoilage microbiota from the 20th to the 21st century: Have we finished or just begun? In *Trends in Food Science and Technology* (Vol. 120). https://doi.org/10.1016/j.tifs.2022.01.004

Anbumani, S., & Kakkar, P. (2018). Ecotoxicological effects of microplastics on biota: a review. *Environmental Science and Pollution Research* 25 (2018): 14373-14396 https://doi.org/10.1007/s11356-018-1999-xAnderson, J. L., Asche, F., & Garlock, T. (2018). Globalization and commoditization: The transformation of the seafood market. *Journal of Commodity Markets*, *12*. https://doi.org/10.1016/j.jcomm.2017.12.004

Arias, A. H., Spetter, C. V., Freije, R. H., & Marcovecchio, J. E. (2009). Polycyclic aromatic hydrocarbons in water, mussels (Brachidontes sp., Tagelus sp.) and fish (Odontesthes sp.) from Bahía Blanca Estuary, Argentina. *Estuarine, Coastal and Shelf Science*, *85*(1). https://doi.org/10.1016/j.ecss.2009.06.008

Bashir, I., Lone, F. A., Bhat, R. A., Mir, S. A., Dar, Z. A., & Dar, S. A. (2020). Concerns and threats of contamination on aquatic ecosystems. In *Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation*. https://doi.org/10.1007/978-3-030-35691-0_1

Bita, S., & Sharifian, S. (2024). Assessment of biogenic amines in commercial tuna fish: Influence of species, capture method, and processing on quality and safety. *Food Chemistry*, *435*. https://doi.org/10.1016/j.foodchem.2023.137576

Bohnes, F. A., Rodriguez, U. P., Nielsen, M., & Laurent, A. (2020). Are aquaculture growth policies in high-income countries due diligence or illusionary dreams? Foreseeing policy implications on seafood production in Singapore. *Food Policy*, *93*. https://doi.org/10.1016/j.foodpol.2020.101885

Boziaris, I. S., & Parlapani, F. F. (2017). Specific Spoilage Organisms (SSOs) in Fish. In *The Microbiological Quality of Food: Foodborne Spoilers*. https://doi.org/10.1016/B978-0-08-100502-6.00006-6

Chai, Z., Wang, C., & Bi, H. (2021). Rapid identification between two fish species using UV-Vis spectroscopy for substitution detection. *Molecules*, *26*(21). https://doi.org/10.3390/molecules26216529

Cooney, R., de Sousa, D. B., Fernández-Ríos, A., Mellett, S., Rowan, N., Morse, A. P., Hayes, M., Laso, J., Regueiro, L., Wan, A. H., & Clifford, E. (2023). A circular economy framework for seafood waste valorisation to meet challenges and opportunities for intensive production and sustainability. In *Journal of Cleaner Production* (Vol. 392). https://doi.org/10.1016/j.jclepro.2023.136283

Denham, F. C., Howieson, J. R., Solah, V. A., & Biswas, W. K. (2015). Environmental supply chain management in the seafood industry: Past, present and future approaches. In *Journal of Cleaner Production* (Vol. 90). https://doi.org/10.1016/j.jclepro.2014.11.079

Department, F. F. and A. (2022). The state of world fisheries and aquaculture 2022. towards blue transformation. *THE STATE OF WORLD FISHERIES AND AQUACULTURE*.

Donlan, C. J., & Luque, G. M. (2019). Exploring the causes of seafood fraud: A meta-analysis on mislabeling and price. *Marine Policy*, *100*. https://doi.org/10.1016/j.marpol.2018.11.022

FAO, Duke University & WorldFish. 2023. *Illuminating Hidden Harvests – The contributions of small-scalefisheries to sustainable development, [Online]*, *12*(May).

FAO. (2020a). FishStatJ - Fishery and Aquaculture Statistical Time Series. *FAO Fisheries and Aquaculture Department [Online]*, *12*(May).

FAO. (2020b). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en. In *INFORM* (Vol. 32, Issue 6).

FAO. (2022a). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Food and Agriculture Organization of the United Nations. In *The State of World Fisheries and Aquaculture 2022*.

FAO. (2022b). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en. In *In Brief to The State of World Fisheries and Aquaculture 2022*.

FAO. (2022c). *World Fisheries and Aquaculture, Towards Blue Transformation*. https://doi.org/https://doi.org/10.4060/cc0461en The

Fishery and Aquaculture Statistics – Yearbook 2021. (2024). In *Fishery and Aquaculture Statistics – Yearbook 2021*. https://doi.org/10.4060/cc9523en

Fox, M., Mitchell, M., Dean, M., Elliott, C., & Campbell, K. (2018). The seafood supply chain from a fraudulent perspective. In *Food Security* (Vol. 10, Issue 4). https://doi.org/10.1007/s12571-018-0826-z

Geng, Lianxin, Kunlun Liu, and Huiyan Zhang. "Lipid oxidation in foods and its implications on proteins." *Frontiers in Nutrition* 10 (2023): 1192199. https://doi.org/10.3389/fnut.2023.1192199

Global Environment Outlook – GEO-6: Healthy Planet, Healthy People. (2019). In *Global Environment Outlook – GEO-6: Healthy Planet, Healthy People*. https://doi.org/10.1017/9781108627146

Gordon, A., & Williams, R. (2020). The role and importance of packaging and labeling in assuring food safety, quality and regulatory compliance of export products II: Packaging & labeling considerations. In *Food Safety and Quality Systems in Developing Countries: Volume III: Technical and Market Considerations*. https://doi.org/10.1016/B978-0-12-814272-1.00007-3

Han, Q. F., Song, C., Sun, X., Zhao, S., & Wang, S. G. (2021). Spatiotemporal distribution, source apportionment and combined pollution of antibiotics in natural waters adjacent to mariculture areas in the Laizhou Bay, Bohai Sea. *Chemosphere*, *279*. https://doi.org/10.1016/j.chemosphere.2021.130381

Hannah Ritchie (2023) - "How much plastic waste ends up in the ocean?" Published online at OurWorldInData.org. Retrieved from: 'https://ourworldindata.org/how-much-plastic-waste-ends-up-in-the-ocean' [Online Resource]

Horreo, J. L., Machado-Schiaffino, G., & García-Vázquez, E. (2017). Forensic assignment to geographic origin, a useful tool in seafood fraud control. *Forensic Science International*, *272*. https://doi.org/10.1016/j.forsciint.2017.01.003

Ibáñez, A. L. (2015). Fish traceability: Guessing the origin of fish from a seafood market using fish scale shape. *Fisheries Research*, *170*. https://doi.org/10.1016/j.fishres.2015.05.016

Jayasekara, C., Mendis, E., & Kim, S. K. (2020). Seafood in the human diet for better nutrition and health. In *Encyclopedia of Marine Biotechnology*. https://doi.org/10.1002/9781119143802.ch131

Kaw, H. Y., & Kannan, N. (2017). A review on polychlorinated biphenyls (PCBs) and Polybrominated diphenyl ethers (PBDEs) in South Asia with a focus on Malaysia. *Reviews of Environmental Contamination and Toxicology*, *242*. https://doi.org/10.1007/398_2016_14

Kurek, M., Barchańska, H., & Turek, M. (2017). Degradation processes of pesticides used in potato cultivations. *Reviews of Environmental Contamination and Toxicology*, *242*. https://doi.org/10.1007/398_2016_13

Lawrence, S., Elliott, C., Huisman, W., Dean, M., & van Ruth, S. (2022). The 11 sins of seafood: Assessing a decade of food fraud reports in the global supply chain. *Comprehensive Reviews in Food Science and Food Safety*, *21*(4). https://doi.org/10.1111/1541-4337.12998

Leroi, F., & Joffraud, J. J. (2011). Microbial degradation of seafood. In *Aquaculture Microbiology and Biotechnology* (Vol. 2). https://doi.org/10.1201/b10923-6

Liu, C., & Ralston, N. V. C. (2021). Seafood and health: What you need to know? In *Advances in Food and Nutrition Research* (Vol. 97). https://doi.org/10.1016/bs.afnr.2021.04.001

McManus, A., & Newton, W. (2011). Seafood, nutrition and human health: A synopsis of the nutritional benefits of consuming seafood. In *Centre of Excellence Science, Seafood & Health* (Issue March 2011).

Microbe Notes, 2023. Microbial Spoilage of Fish & Preservation. Available at:

https://microbenotes.com/microbial-spoilage-of-fish-preservation/ [Accessed 1 June 2024].

Miller, D., Jessel, A., & Mariani, S. (2012). Seafood mislabelling: Comparisons of two western European case studies assist in defining influencing factors, mechanisms and motives. *Fish and Fisheries*, *13*(3). https://doi.org/10.1111/j.1467-2979.2011.00426.x

Nesheim, M. C., & Yaktine, A. L. (2007). Seafood choices: Balancing benefits and risks. In *Seafood Choices: Balancing Benefits and Risks*. https://doi.org/10.17226/11762

Pan, Y., Yan, S. W., Li, R. Z., Hu, Y. W., & Chang, X. X. (2017). Lethal/sublethal responses of Daphnia magna to acute norfloxacin contamination and changes in phytoplankton-zooplankton interactions induced by this antibiotic. *Scientific Reports*, *7*. https://doi.org/10.1038/srep40385

Panebianco, F., Nobile, M., Pasinetti, G., Pattono, D., Panseri, S., & Civera, T. (2024). Cured or fresh? Between fish maturation trends in restaurants and food safety: The case of dry-aged rainbow trout. *Food Control*, *165*, 110612. https://doi.org/10.1016/J.FOODCONT.2024.110612

Reames, E. (2012). Nutritional benefits of seafood. *SRAC Publication - Southern Regional Aquaculture Center*, *7300*.

Regueiro, L., Newton, R., Soula, M., Méndez, D., Kok, B., Little, D. C., Pastres, R., Johansen, J., & Ferreira, M. (2022). Opportunities and limitations for the introduction of circular economy principles in EU aquaculture based on the regulatory framework. *Journal of Industrial Ecology*, *26*(6). https://doi.org/10.1111/jiec.13188

Ryburn, S. J., Ballantine, W. M., Loncan, F. M., Manning, O. G., Alston, M. A., Steinwand, B., & Bruno, J. F. (2022). Public awareness of seafood mislabeling. *PeerJ*, *10*. https://doi.org/10.7717/peerj.13486

Sustainable Fisheries UW, 2023. What Does the World Eat? Available at: https://sustainablefisheries-uw.org/seafood-101/what-does-the-world-eat/#:~=Though%20fish%20compose%20a%20small,of%20the%20world's%20meat%20consumption [Accessed 15 May 2024].

Straume, H. M., Asche, F., Oglend, A., Gaasland, I., Pettersen, I. K., & Sogn-Grundvåg, G. (2024). The structure of Norwegian seafood trade. *Marine Policy*, *159*. https://doi.org/10.1016/j.marpol.2023.105921

Sumaila, U. R., Zeller, D., Hood, L., Palomares, M. L. D., Li, Y., & Pauly, D. (2020). Illicit trade in marine fish catch and its effects on ecosystems and people worldwide. *Science Advances*, *6*(9). https://doi.org/10.1126/sciadv.aaz3801

Thomas, K., Obaidullah, F., & Dorey, C. (2019). Ghost Gear: the Abandoned Fishing Nets Haunting Our Oceans. *Greenpeace Germany*.

UNEP (United Nations Environment Programme). (2021). From pollution to solution: a global assessment of marine litter and plastic pollution. In *New Scientist* (Vol. 237, Issue 3169).

United Nations Environment Programme. (2023). Heavy Metals. Retrieved from https://www.unep.org/cep/heavy-metals

Zhang, Y., Jiao, Y., Li, Z., Tao, Y., & Yang, Y. (2021). Hazards of phthalates (PAEs) exposure: A review of aquatic animal toxicology studies. In *Science of the Total Environment* (Vol. 771). https://doi.org/10.1016/j.scitotenv.2021.145418

Zhang, Y., Lyu, L., Tao, Y., Ju, H., & Chen, J. (2022). Health risks of phthalates: A review of immunotoxicity. In *Environmental Pollution* (Vol. 313). https://doi.org/10.1016/j.envpol.2022.120173

CHAPTER I

Emerging Contaminants in Seafood Products

As marine pollution is increasing uncontrollably, it has become a topical issue. The first of the two chapters that make up my thesis is therefore dedicated to the known recent problem of microplastics and older issue of heavy metals and pesticides, etc. - all pollutants that are ubiquitous in the marine environment and consequently in edible species.

The following are two publications and an ongoing research project focusing on these critical issues.

CHAPTER FIVE

Detection methods of micro and nanoplastics

Abdo Hassoun[a,b,*], Luisa Pasti[c], Tatiana Chenet[c], Polina Rusanova[d,e], Slim Smaoui[f], Abderrahmane Aït-Kaddour[g], and Gioacchino Bono[d,h]

[a]Sustainable AgriFoodtech Innovation & Research (SAFIR), Arras, France
[b]Syrian Academic Expertise (SAE), Gaziantep, Turkey
[c]Department of Environmental and Prevention Sciences, University of Ferrara, Ferrara, Italy
[d]Institute for Biological Resources and Marine Biotechnologies, National Research Council (IRBIM-CNR), Mazara del Vallo, TP, Italy
[e]Department of Biological, Geological and Environmental Sciences (BiGeA) – Marine Biology and Fisheries Laboratory of Fano (PU), University of Bologna (BO), Bologna, Italy
[f]Laboratory of Microbial Biotechnology and Engineering Enzymes (LMBEE), Center of Biotechnology of Sfax (CBS), University of Sfax, Sfax, Tunisia
[g]Université Clermont Auvergne, INRAE, VetAgro Sup, UMRF, Lempdes, France
[h]Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università Di Palermo, Palermo, Italy
*Corresponding author: e-mail address: a.hassoun@saf-ir.com

Contents

Abstract

Plastics and related contaminants (including microplastics; MPs and nanoplastics; NPs) have become a serious global safety issue due to their overuse in many products and applications and their inadequate management, leading to possible leakage into the environment and eventually to the food chain and humans. There is a growing literature reporting on the occurrence of plastics, (MPs and NPs) in both marine and terrestrial organisms, with many indications about the harmful impact of these contaminants on plants and animals, as well as potential human health risks. The presence of MPs and NPs in many foods and beverages including seafood (especially finfish, crustaceans,

Advances in Food and Nutrition Research, Volume 103
ISSN 1043-4526
https://doi.org/10.1016/bs.afnr.2022.08.002

bivalves, and cephalopods), fruits, vegetables, milk, wine and beer, meat, and table salts, has become popular research areas in recent years. Detection, identification, and quantification of MPs and NPs have been widely investigated using a wide range of traditional methods, such as visual and optical methods, scanning electron microscopy, and gas chromatography–mass spectrometry, but these methods are burdened with a number of limitations. In contrast, spectroscopic techniques, especially Fourier-transform infrared spectroscopy and Raman spectroscopy, and other emerging techniques, such as hyperspectral imaging are increasingly being applied due to their potential to enable rapid, non-destructive, and high-throughput analysis. Despite huge research efforts, there is still an overarching need to develop reliable analytical techniques with low cost and high efficiency. Mitigation of plastic pollution requires establishing standard and harmonized methods, adopting holistic approaches, and raising awareness and engaging the public and policymakers. Therefore, this chapter focuses mainly on identification and quantification techniques of MPs and NPs in different food matrices (mostly seafood).

1. Introduction

Nowadays, plastics, including microplastics (MPs) and nanoplastics (NPs) are everywhere. As plastic is not a biodegradable material, its wastes can only be broken down to smaller particles, which accumulate in the environment and could end up in the food chain. MPs are particles with a size smaller than 5 mm, whereas NPs are those within a size ranging from 1 to 1000 nm (Jadhav et al., 2021; van Raamsdonk et al., 2020). According to their sources, MPs can be divided into two classes, namely, primary and secondary MPs. Primary MPs are plastic particles produced for a specific function (e.g., specific personal care and cosmetics products) while secondary MPs result from the breakdown of larger plastic debris (Dehaut et al., 2016; van Raamsdonk et al., 2020). MPs can be classified into different categories according to their different shapes, including fibers, fragments, pellets, flakes, bead, filament, foam, and granules (López-Martínez et al., 2021; Mercogliano et al., 2020).

Plastic materials are used in numerous food-related applications (e.g., agriculture and food packaging) and many other fields, such as automotive industry, building and construction, and even cosmetic and health care products (Chatterjee & Sharma, 2019; Gündogdu et al., 2022). However, improper applications, overuse, and poor management of plastic wastes are transforming earth planet into a "plastic planet" (Chatterjee & Sharma, 2019). Therefore, environmental ubiquity of MPs and NPs has become a critical concern (Gündogdu et al., 2022; Mercogliano et al., 2020). For example,

plastics materials, such as polypropylene (PP), polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC), among others, continue to be among the most used materials in conventional food packaging, despite their potential effect on human health and the environment (Sid et al., 2021; ZabihzadehKhajavi et al., 2019). MPs can be released from packaging materials and interact with human cells, increasing acute inflammation and cell damage (Jadhav et al., 2021).

Over the last few years, a considerable research attention has been paid to the potential risk of plastics and MPs ingested through food for human health and environment, which can be reflected by the huge number of publications, making plastic/MPs/NPs one of the hottest research topics. In fact, according to the data collected using the Scopus database, the number of publications on MPs and NPs in food has recently increased significantly. Fig. 1 shows this trend in the number of articles published during the last decade and the corresponding number of citations.

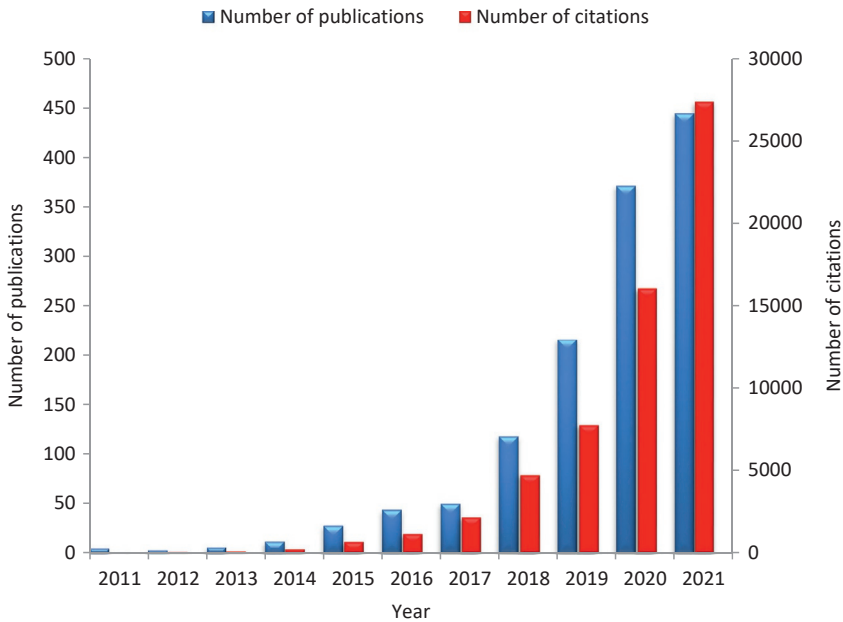

Fig. 1 Number of publications and citations per year on MPs/NPs in food/seafood over the last decade (search query was performed on 3rd May 2022). The following keywords search query was used in Scopus: TITLE-ABS-KEY (Microplastics) OR TITLE-ABS-KEY (Nanoplastics) AND TITLE-ABS-KEY (Food) OR TITLE-ABS-KEY (Seafood).

Many publications have been devoted to this topic, reporting the occurrence of MPs and NPs in different food categories such as fruit and vegetables (Oliveri Conti et al., 2020), packaged meat (Kedzierski et al., 2020), seafood (Dehaut, Hermabessiere, & Duflos, 2019; López-Martínez et al., 2021; Vázquez-Rowe, Ita-Nagy, & Kahhat, 2021), salt (Fadare, Okoffo, & Olasehinde, 2021; Renzi, Grazioli, et al., 2019), honey (Diaz-Basantes, Conesa, & Fullana, 2020), among many other foodstuffs.

Among these food groups, fish and other marine organisms have received a particular attention due to the fact that these foods are one of the most important routes of exposure for humans through the dietary intake (Dawson et al., 2021; Dehaut et al., 2019). For instance, the occurrences of MPs in canned fish (Akhbarizadeh et al., 2020), finfish, predominantly Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and channel catfish (*Ictalurus punctatus*) (Baechler et al., 2020), shellfish, such as mussel, oyster, clam, winkle, and scallop (Daniel et al., 2021; Li et al., 2021a, 2021b), and Mediterranean marine species including anchovy (*Sardina pilchardus*), sea bream (*Sparus aurata*), red mullet (*Mullus surmuletus*), and sole (*Solea solea*) (Ferrante et al., 2022) have been recently reported.

Most MPs studies include three steps: (i) extraction, (ii) detection and quantification, and (iii) identification and characterization. Different methods have been developed to determine and identify the MPs in recent years. The most commonly used techniques are visual and optical microscopy, spectroscopic methods as well as chromatographic techniques, such as gas chromatography/mass spectrometry (GC/MS) (Akoueson et al., 2021; Kwon et al., 2020; Mercogliano et al., 2020; van Raamsdonk et al., 2020; Veerasingam et al., 2021; Yuan, Nag, & Cummins, 2022).

Especially, the application of Fourier-transform infrared spectroscopy (FT-IR) and Raman spectroscopy has gained momentum since 2015, as can be noticed from data collected using the Scopus database (Fig. 2). During the last few years, the FT-IR has been extensively applied for the detection of MPs pollution in many various matrices, including dust/air, water, waste water treatment plants, sediment, biota, and salt (Bai et al., 2022; Veerasingam et al., 2021). FT-IR is often used in combination with optical microscopy to increase the detection performance, specifically for small MPs particles (Dehaut et al., 2019; Veerasingam et al., 2021). The Raman spectroscopy technique has been used in many applications for the analysis of very small microplastics (<20 μm) (Anger et al., 2018; Araujo et al., 2018).

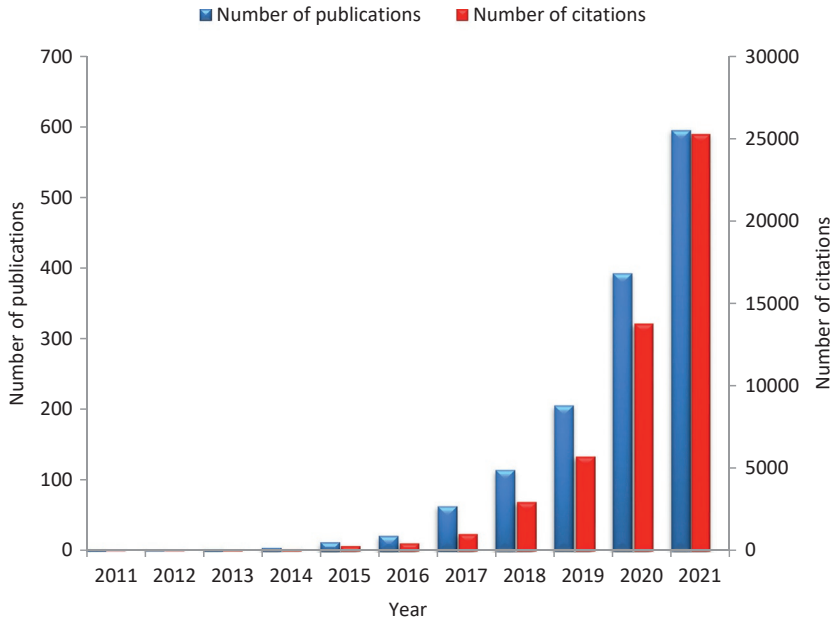

Fig. 2 Number of publications and citations per year on MPs/NPs using Fourier transform infrared spectroscopy or Raman spectroscopy over the last decade (search query was performed on 3rd May 2022). The following keywords search query was used in Scopus: TITLE-ABS-KEY (Fourier AND transform AND infrared) OR TITLE-ABS-KEY (FT-IR) OR TITLE-ABS-KEY (Raman) AND TITLE-ABS-KEY (Microplastics) OR TITLE-ABS-KEY (Nanoplastics).

2. Definition, sources, and types

Plastic debris is found in every environmental sphere, with a great variety of dimensions, shapes, composition, and color. The increased number of studies on the detection and effects of plastic debris in the last years, in particular on MPs, led to the need for a proper classification.

In general, plastic particles detected in the environment are classified according to their size, but there is a lack of a standard classification as many studies use different approaches. The term MPs was introduced in 2004 (Ivleva, 2021) referring to plastic fragments of small dimensions found in marine environment. In numerous studies published in the last 20 years, different upper limits have been considered to define the microplastic class, specifically from 0.5 to 5 mm, with most studies considering as MPs those particles with dimension lower than 5 mm (Hartmann et al., 2019).

In 2016, the UN advisory Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) recommended the use of 5 mm as the cut-off value for MPs to preserve the information of the majority of the published works (Rosal, 2021).

Regarding NPs, many studies define as such those particles with dimensions lower than 0.1 μm (Ferreira et al., 2019; Nguyen et al., 2019; Venâncio et al., 2019), but in other studies, NPs have also been classified considering dimension ranges up to 1, 20 or even 335 μm (Hartmann et al., 2019). Recently, Hartmann et al. (2019) tackled this problem by suggesting a standard method for the classification of plastic fragments based on their size; according to the method proposed, plastic debris are classified as indicated in Table 1.

Among the plastic debris, MPs, and very recently NPs, have attracted the attention of researchers all around the world due to their ubiquity and their dimensions that allow them to enter more easily the food chain and even to penetrate in the organs and tissues of organisms. The presence of MPs and NPs in the ecosystem is due to the widespread use of plastic products in a vast variety of applications. Plastics are produced by polymerization of different monomers and additives, leading to a wide variety of lightweight and inexpensive polymeric materials characterized by their low density, low electrical conductivity, transparency, as well as toughness. The main polymers used, which account for 90% of the total world plastic production, are PET, high-density polyethylene (HDPE), PVC, low-density polyethylene (LDPE), PP, PS, and polyurethane (PU) (Boyle & Örmeci, 2020). Often, particular substances, such as phthalate acid esters, perfluoroalkyl substances, nonylphenol and bisphenol A, are added to the polymer in order to improve its properties; these additives, being of small molecular size, have the potential to leach from the plastic matrix during the degradation processes, and cause damage to the environment and biota (Boyle & Örmeci, 2020).

Table 1 Classification of plastic particles according to dimensions, as proposed by Hartmann et al. (2019).

Classification	Dimension range
Macroplastics	1 cm and larger
Mesoplastics	1 to <10 mm
Microplastics	1 to <1000 μm
Nanoplastics	1 to <1000 nm

Plastics are used in a variety of different applications, such as packaging, which represent the major sector for plastic consumption, building and construction, textile sector to produce clothes and other products, electrical/electronic, industry and machinery, personal care products, marine coating, and others (Ryberg et al., 2019). Due to the wide variety of products containing polymeric materials, the main criterion for identifying plastic particles is based on their chemical composition. A distinction can be made by considering different classes: polymers include all synthetic and semi-synthetic polymers, copolymers are those materials produced from more than one type of monomers, and composites include those materials that contain synthetic polymer as an essential ingredient. All particles that fall into these categories should be considered as plastic debris (Hartmann et al., 2019).

Plastic debris has been detected in every environmental sphere all around the world. Plastic materials find their way to soils, sediments, freshwaters, oceans, and air through different transport pathways; the most significant inputs of plastics in the environment are losses from landfills, wastewater treatment plant (WWTP) effluents and sludge, and mishandled waste. Ryberg et al. (2019) estimated the global losses of plastics to the environment occur along the plastic value chains, identifying the municipal solid waste management as the major source of plastic loss. Rivers represent a major vector for the transportation of plastics to the oceans; it has been estimated that from 70% to 80% of the plastics that reach the oceans are transported through freshwater streams (Alimi et al., 2018).

Usually, MPs found in the environment can be divided according to their origin: primary or secondary MPs. Primary MPs are manufactured to be already of microscopic dimensions for different industrial and domestic applications, such as beads in scrubbers for paint or rust removal, in toothpaste formulations and in cosmetic products. Pollution of water bodies by primary MPs arises from the direct use of these products, while the contamination of agricultural land may occur through the use of fertilizer, or sludge generated by WWTPs, which can act as a sink for MPs during the water treatment processes (Sol et al., 2020). Conversely, secondary MPs are those particles originating from the degradation of bigger plastic debris through various natural processes, such as physical fragmentation, photodegradation, chemical, as well as biological degradation (Dehaut et al., 2016; van Raamsdonk et al., 2020). The same distinction can be applied to NPs: primary NPs are those produced with dimensions lower than 1 μm and used in different applications, whereas secondary NPs originate from the fragmentation of MPs (Gonçalves & Bebianno, 2021).

Once entered the environment, there are many pathways, abiotic or biotic, through which plastic debris can break down leading to the generation and dispersion of MPs and NPs, and different processes may often work synergistically (Alimi et al., 2018). The abiotic pathways by which plastic debris can be broken down into smaller fragments include mechanical breakdown and processes involving degradation through oxidation, hydrolytic, photo and thermal reactions. Mechanical degradation occurs due to weathering (e.g., water turbulence, freezing, thawing and changes in pressures) and it leads to morphological changes, but does not affect the polymeric bonds in the plastic matrix. This is particularly the case of disposable plastic bags, which represent one of the major plastic wastes often found in the environment (Ke et al., 2019).

Photodegradation is one of the main processes that damage the most plastic materials; in particular, the ultraviolet (UV; with wavelengths between 290 and 400 nm) and visible (between 400 and 800 nm) radiation is considered the primary factor in the degradation of polymers in the environment. Usually, the radiation is absorbed by chromophore groups or impurities present in the polymer matrix, leading to the formation of radicals that can react with oxygen generating peroxyl radicals, then hydroperoxides. Further reactions between radicals lead to polymer chain scission, cross-linking, chain branching and formation of groups containing oxygen (Masry et al., 2021). The oxidation reactions, thermal or photo-induced, bring about the introduction of carbonyl (C=O) and/or hydroxyl (OH) groups in the polymer chain, promoting biodegradation (Boyle & Örmeci, 2020).

Biotic degradation processes involve the action of enzymes secreted by microorganisms that, through hydrolytic reactions, break down the polymer chains, the progressive loss of molecular weight of the polymeric matrix triggers further microbial degradation. The continued loss of molecular structure results eventually in the formation of water-soluble oligomers, which in turn can be mineralized and assimilated by microorganisms as carbon and nitrogen (Boyle & Örmeci, 2020).

Another important input of MP/NP particles in the environment, especially in the atmosphere, is from the transport sector. In fact, the wearing of tires on the road over time due to abrasion is a common source of rubber particles, which enter the atmosphere and they can be transported to long distances by wind currents and pollute other ecosystems leading to adverse effects (Šourková, Adamcová, & Vaverková, 2021). Tire wear particles have

been considered as pollutants for many years although they were recognized as MPs only recently (Knight et al., 2020). Järlskog et al. (2020) in their investigation on the occurrence of MPs on urban streets, sweep sand, and wash water found that tire tread wear and bitumen made up the largest proportion of anthropogenic particles detected.

MPs can be found in the environment in a wide variety of dimensions, shapes and colors depending on the initial material from which they originate, and the degradation processes they underwent. In general, when categorized according to their shape, MPs can be described as spheres, fibers, fragments, pellets, beads, films, flakes, and foams (Hartmann et al., 2019). MPs in the form of fibers are commonly found in the ecosystems due to the use of synthetic polymers for the production of textile fibers, and the presence of fibers of microscopic dimensions in the environment is caused by ordinary use of clothing and, especially in water, through the discharges of washing (Dalla Fontana, Mossotti, & Montarsolo, 2020). Fibers can have different compositional nature: they can be composed of exclusively synthetic polymers (e.g., polyesters or polyamides), of semi-synthetic materials (e.g., rayon), or of natural materials (e.g., cellulose). Fibers can pose a threat to organisms since they usually undergo further processes, such as dyeing; once in the environment, the pigments can leach from the fiber and lead to adverse effects toward the biota and the environment (Collard et al., 2018).

The term "fragments" refers to a broad category of plastic debris characterized by an irregular shape. They can originate from the degradation of bigger plastic debris, or they can be produced intentionally with irregular forms, for example, as abrasive particles in personal care products (Sun, Ren, & Ni, 2020), and enter the environment owing to the use of the products in which they are contained. Films and flakes are particles with irregular shapes and with a very thin thickness; they can be found in the environment due to fragmentation of paint, for instance, the coating of boats and ships can break down over time releasing small fragments into the water (Turner, 2021).

Another aspect taken into account when MPs and NPs are considered is their color; the coloration may help the identification of the sources of these particles, but it should be noticed that discoloration might occur due to degradation processes. While the classification of MPs and NPs by color is usually not crucial, it can be helpful in the biological field because colored plastic particles can be mixed with food and ingested by marine

organisms. In fact, MPs have been found in wild aquatic species (Chenet et al., 2021; Mancia et al., 2020; Tsangaris et al., 2020) where the predominant colors of both filaments and fragments were blue, black, green, and red. Reinold et al. (2021) investigated the ingestion of MPs in *Dicentrarchus labrax* cultivated in aquaculture facilities, finding that 65% of the specimen analyzed ingested MPs mainly in the form of fibers with blue and yellow as predominant colors. In their study on the uptake routes of MPs by fishes, Roch, Friedrich, and Brinker (2020) considered, among other factors, the role presented by the color of the particles on the ingestion; they found that MPs with food-like coloration were ingested in higher amounts by the organisms considered.

Density represents another property of MPs and NPs that plays an important role on their distribution and possibility of ingestion by aquatic species (Roch et al., 2020). According to the composition of the matrix, particles, once released in water, can float or sink, becoming more prone to transportation through currents or to deposition in sediments. In the last case, these particles can pose a risk to marine species, which usually feed on organisms present on the seabed. The density of a given particle may change over time due to degradation processes that lead to a variation in the composition of the matrix, but also to the formation of biofilms onto the surface of the particles caused by microorganisms (Borges-Ramírez et al., 2020).

3. Occurrence of MPs and NPs in seafood products

3.1 MPs/NPs in finfish, crustaceans, molluscs, and other seafood products

Some 1300 scientific papers published between 2011 and 2021 (source: Scopus database) reveal how MPs are now ubiquitous in major seafood products (finfish, crustaceans, bivalves, cephalopods, gastropods, etc.) and other marine species (anemones, jellyfish, sea cucumbers, etc.) consumed around the world. Since 2014, 165 papers have also highlighted the threat of NPs (Fig. 3). Overall, the Scopus search revealed 1547 publications pertaining to MPs and NPs in the major seafood products listed above. Publications were grouped into 25 subject areas, 75% of which fall within the three basic areas of Environmental Science, Agricultural and Biological Sciences, and Earth and Planetary Sciences.

Another Scopus search using the combined terms "TITLE-ABS-KEY (microplastics) ORTITLE-ABS-KEY (nanoplastics) AND TITLE-ABS-KEY (crustaceans) OR TITLE-ABS-KEY (bivalves) OR TITLE-ABS-KEY

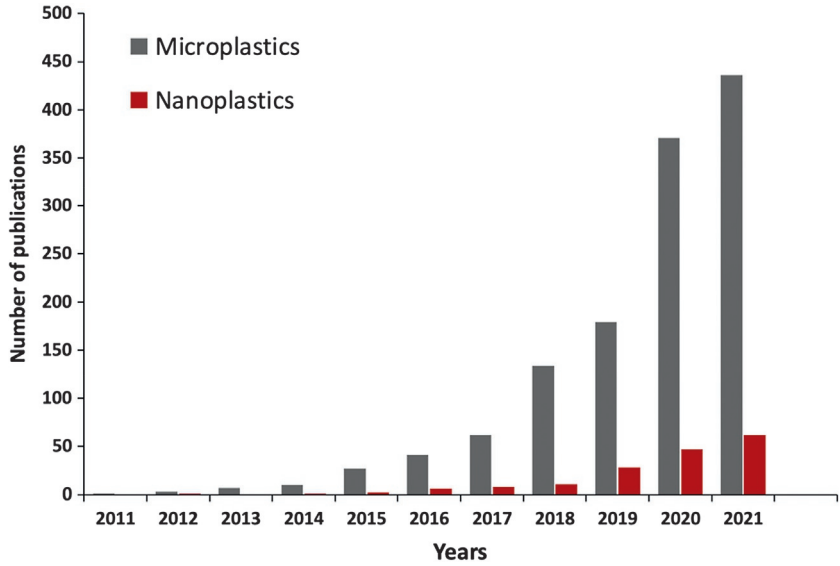

Fig. 3 Publications on microplastics and nanoplastics in fish, crustaceans, bivalves, cephalopods, gastropods, anemones, jellyfish, and sea cucumbers (source: Scopus database—search within "title, abstract and keywords") in the years 2011–2021.

(fish) OR TITLE-ABS-KEY (cephalopods) OR TITLE-ABS-KEY (sea AND cucumber) OR TITLE-ABS-KEY (gastropods) OR TITLE-ABS-KEY (anemones) OR TITLE-ABS-KEY (jellyfish)" was carried out in order to perform a cluster-based VOSviewer analysis.

According to the VOSviewer output, co-occurrence analysis identified 15,002 keywords, of which 557 occurred at least 20 times. As expected, "MPs" was the most frequent keyword identified (the size of the node indicates the frequency of the keyword), followed by "plastic," "animals," "fish," "polystyrene," "nanoplastic," "particle size," "polypropylene," "ingestion" and "oxidative stress" (Fig. 4). Three "clusters" were generated: these define thematic proximity among all considered keywords.

In this scenario, by comparing the huge amount of scientific data produced in just 10 years, it is clear that the millions of tons of plastic litter that has irresponsibly ended up in the oceans in the last half century is now returning to us (Ragusa et al., 2021) through the food web: zooplankton > shellfish > fish > humans.

Not all marine organisms respond in the same way to these emerging pollutants. MP and NP accumulation is strongly influenced by the environment in which organisms live (i.e., pelagic-neritic, demersal habitats), what,

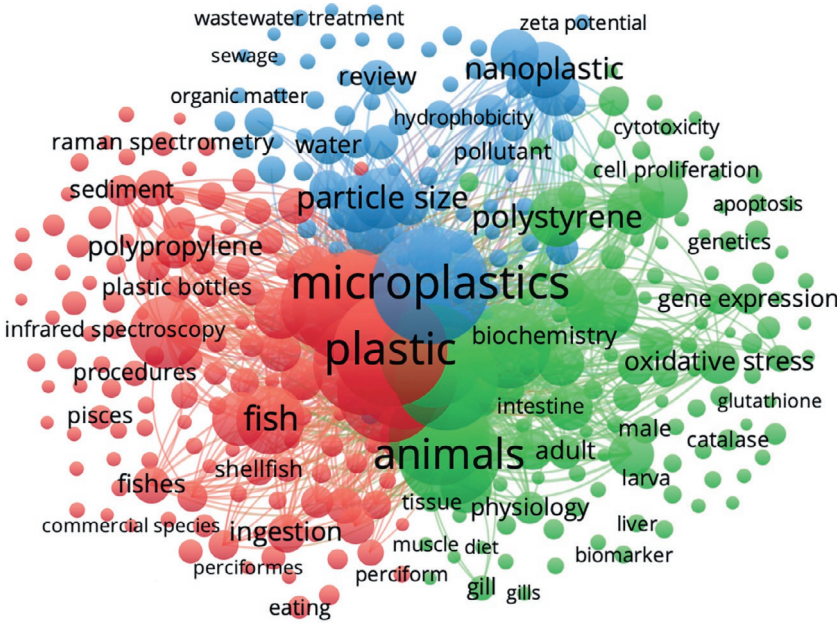

Fig. 4 Network map of 557 keywords (with the minimum occurrence set to 20) identified by VosViewer on the Scopus (CSV) database. The size of nodes indicates the frequency of occurrence, while the colors identify three clusters that define the thematic proximity and indicate research directions.

how and where they feed, as well as their trophic level and their anatomic features (Ribeiro et al., 2020). For example, mussels, clams, oysters and other minor bivalves are more subject to MP and NP contamination and accumulation because they feed by filtering significant quantities of coastal water, which is often more polluted than the open sea environment. As they are eaten whole, they directly transfer to humans a significant portion of MPs and NPs ingested during their life cycle. Other marine organisms, such as small fish, gastropods, cephalopods, and other small invertebrates, raise a similar concern because they are traditionally eaten whole (i.e., including the gastrointestinal tract), both fresh and/or dried, especially in low-income countries.

The risk of transferring these emerging pollutants to the food chain is obviously lower in the case of large marine species consumed after eliminating the gastrointestinal tract. It should be noted that there might be a microbiological risk associated with the consumption of contaminated seafood products. Recent studies have revealed that MPs and NPs are associated with

a higher risk of transmitting pathogen microorganisms, such as viruses, bacteria, and fungi (Gündogdu et al., 2022). Considering that seafood products tend to be packed in a modified atmosphere and/or under vacuum skin packaging, two methods involving a high consumption of plastic [PP, ethylene vinyl alcohol (EVOH), polyamide (PA), PE], further studies are needed to understand how much these techniques, and the massive plastic polymers used, affect the final product in terms of contamination (Sobhani et al., 2020).

Given the growing interest in these new pollutants, Danopoulos et al. (2020) state that the issue of contamination by MPs and NPs should be included in food quality risk analysis and in the HACCP (Hazard Analysis and Critical Control Point) or HARPC (Hazard Analysis and Risk-based Preventive Controls for Human Food) programs for food safety.

From a physiological perspective, MP and NP ingestion could also alter endocrine system functions and the seafood microbiome, reduce the rate of fecundity, and cause DNA and neurological damage in various species (Chenet et al., 2021; Mancia et al., 2020). This is due to their intrinsic nature and to their ability to act as carriers of other pollutants, such as plastic additives (phthalates, triclosan, bisphenols, brominated and flame retardants), other persistent organic pollutants (POPs) and heavy metals dissolved in the aquatic marine environment (Guo & Wang, 2019; Kutralam-Muniasamy et al., 2021; Tourinho et al., 2019), with serious consequences for marine biota. Owing to their high toxicity, it is important to acquire greater insight into the effects of MPs and NPs on consumed marine organisms in terms of seafood safety and human health (Gündogdu et al., 2022; Piyawardhana et al., 2022). According to Gündogdu et al. (2022), another important aspect is the fate of MPs and NPs once they have been ingested by marine organisms. Specifically, how long they remain confined to the gastrointestinal tract before being excreted in feces, or whether they migrate through the circulatory system to other organs such as flesh muscle and gonads, as well as the liver and spleen. Therefore, although it seems that particles smaller than 150 μm in size can cross the intestinal epithelium, only 0.2–0.45% of the ingested MPs and NPs (<1.5 μm in size) can penetrate deeply into other organs (Food Safety Authority, 2016; Hazimah et al., 2021).

3.2 Connection between MPs/NPs and the most consumed wild seafood products

Based on FAO report (2020) "The state of world fisheries and aquaculture," Table 2 shows the most caught marine species in 2018. Starting from this

Table 2 Summary of reported microplastic ingestion by the most caught marine species in 2018 (FAO, 2020).

Common name	Scientific name	Average production in 2018 (million tons/percentage)	Habitat	Studied fishing ground	Presence of MPs (%)	Main recent references
Finfish						
Anchoveta	*Engraulis ringens*	7.045 (10%)	Pelagic-neritic	Pacific Ocean	0.8%	Ory et al. (2018)
Skipjack tuna	*Katsuwonus pelamis*	3.161 (4%)	Pelagic-oceanic	North Maluku Ocean	100%	Ridwan Lessy and Sabar (2021)
				Indian Ocean (Pantai Baron)	100%	Suwartiningsih, Setyowati, and Astuti (2020)
				South Pacific Ocean	23%	Markic et al. (2018)
				Southeast-south coast of Brazil	25.8%	Neto et al. (2020)
				Western equatorial Atlantic Ocean	0.75%	de Mesquita et al. (2021)
				North-East Atlantic	10%	Pereira et al. (2020)
Atlantic herring	*Clupea harengus*	1.820 (3%)	Benthopelagic	English Channel	50%	Collard et al. (2017)
				Baltic Sea	12.7%	Białowąs et al. (2022)
				Baltic Sea	20%	Beer et al. (2018)
Blue Whiting	*Micromesistius poutassou*	1.712 (2%)	Bathypelagic	English Channel	51.9%	Mercogliano et al. (2020)
				North-western Iberian Shelf Sea	0.02%	López-López et al. (2018)
				North Altlantic	0%	Murphy et al. (2017)
				Not reported	29.8%	Walkinshaw et al. (2020)
				Tyrrhenian coast	0%	Pittura et al. (2018)

European pilchard	*Sardina pilchardus*	1.608 (2%)	Pelagic-neritic	Adriatic Sea	96%	Renzi, Specchiulli, et al. (2019)
				Spanish Mediterranean coast	15.2%	Compa et al. (2018)
				Southern Tyrrhenian Sea	52.6%	Savoca et al. (2020)
				Northern Ionian Sea	47.2%	Digka et al. (2018)
				North Adriatic Sea	30%	Mistri et al. (2022)
				Mediterranean Sea	57%	Güven et al. (2017)
				Western Mediterranean Sea	17.39%	Rios-Fuster et al. (2019)
				Western and southern parts of Iberian coast	58%	Lopes et al. (2020)
				Northwestern Iberian continental shelf	87%	Filgueiras et al. (2020)
				South Adriatic Sea	50%	Anastasopoulou et al. (2018)
				Middle Adriatic Sea	37%	
				Ionian Sea (Greece)	47%	
Pacific chub mackerel	*Scomber japonicus*	1.557 (2%)	Pelagic-neritic	Southeast Pacific Ocean	3.3%	Ory et al. (2018)
				South-western Japan	62.5%	Yagi et al. (2022)
				Mediterranean Sea	57%	Güven et al. (2017)
				Mediterranean Sea	50%	Bray et al. (2019)
Yellowfin tuna	*Thunnus albacares*	1.458 (2%)	Pelagic-oceanic	South Pacific Ocean	2% (only mesoplastics >5 mm)	Chagnon et al. (2018)
Scads nei	*Decapterus* spp.	1.336 (2%)	Reef-associated, demersal, pelagic-oceanic benthopelagic	South Pacific	80%	Ory et al. (2017)
				Indonesia	29%	Rochman et al. (2015)

Continued

Table 2 Summary of reported microplastic ingestion by the most caught marine species in 2018 (FAO, 2020).—cont'd

Common name	Scientific name	Average production in 2018 (million tons/percentage)	Habitat	Studied fishing ground	Presence of MPs (%)	Main recent references
Atlantic cod	*Gadus morhua*	1.218 (2%)	Benthopelagic	Fogo Island, Newfoundland and Labrador	1.4%	Saturno et al. (2020)
				Baltic Sea	14.8%	Białowąs et al. (2022)
				Norwegian coast (Bergen City Harbor)	0–27%	Bråte et al. (2016)
				Newfoundland	1.7%	Liboiron et al. (2019)
				North Sea	0–13%	de Vries et al. (2020)
Largehead hairtail	*Trichiuruslepturus*	1.151 (2%)	Benthopelagic	Atlantic Ocean	1.4%	di Beneditto and da Silva Oliveira (2019)
				Oman Sea	14%	Ghattavi, Naji, and Kord (2019)
				South Atlantic Ocean	20%	Pegado et al. (2018)
Atlantic mackerel	*Scomber scombrus*	1.047 (1%)	Pelagic-neritic	Western Mediterranean Sea	49.2%	Palazzo et al. (2021)
				Portuguese coast	100%	Lopes et al. (2020)
				Coast of Portugal	31%	Neves et al. (2015)
Japanese anchovy	*Engraulis japonicus*	957 (1%)	Pelagic-neritic	Seto Inland Sea	90–100%	Ohkubo et al. (2022)
				Pacific Ocean (Tokyo Bay)	77%	Tanaka and Takada (2016)
				Yellow Sea	33%	Sun et al. (2019)

Sardinellas nei	*Sardinella* spp.	887 (1%)	Pelagic-neritic, reef-associated, pelagic	Southwest coast of India	38%	James et al. (2022)
				Pantai Indah Kapuk coast, Indonesia	100%	Hastuti, Lumbanbatu, and Wardiatno (2019)
				Northern Bay of Bengal	100%	Hossain et al. (2019)
				Thoothukudi region (Indian Ocean)	21%	Kalaiselvan et al. (2022)
				Indian Ocean	60–100%	Palermo et al. (2020)
				South eastern Arabian Sea, Indian coasts	5–9.1%	James et al. (2020)
				Central Atlantic Ocean, off the Coast of Ghana	26–41%	Adika et al. (2020)
Crustaceans						
Gazami crab	*Portunus trituberculatus*	493 (8%)	Benthopelagic	Yellow Sea	66.7–100%	Zhang et al. (2021)
Marine crabs nei	*Pachygrapsus transversus* *Carcinus maenas* *Eriocheir sinensis* *Callinectes sapidus*	314 (5%)	Benthic benthic benthic benthopelagic	Ponta Verde Beach, Brazil	47.4%	de Barros, dos Santos Calado, and de Sá Leitão Câmara de Araújo (2020)
				Thames Estuary	71.3%	McGoran et al. (2020)
				Thames Estuary	100%	McGoran et al. (2020)
				Adriatic Sea	83.3%	Renzi et al. (2020)
Blue swimming crab	*Portunuspelagicus*	298 (5%)	Reef-associated	Arabian Sea	13.3%	Daniel et al. (2021)

Continued

Table 2 Summary of reported microplastic ingestion by the most caught marine species in 2018 (FAO, 2020).—cont'd

Common name	Scientific name	Average production in 2018 (million tons/percentage)	Habitat	Studied fishing ground	Presence of MPs (%)	Main recent references
Argentine red shrimp	*Pleoticus muelleri*	256 (4%)	Benthic	Southwestern Atlantic Ocean	90%	Fernández Severini et al. (2020)
Molluscs						
Jumbo flying squid	*Dosidicus gigas*	892 (15%)	Pelagic	South Pacific Ocean	79.2%	Gong et al. (2021)
Marine Molluscs nei	*Mollusca*	664 (11%)	–	Liaohe Estuary (China)	67%	Wang et al. (2021a, 2021b)
Cuttlefish, bobtail squids nei	*Sepiidae, Sepiolidae*	348 (6%)	Benthic	Portugal	100% (cuttlefish)	Oliveira et al. (2020)

baseline, it was added the specific habitat (from pelagic to benthonic) of each taxa/species and what currently known about the presence of MPs and NPs (in the gastrointestinal tract, gills, liver, muscle tissue, gonads, etc.) within each taxa/species on a geographical basis.

In the literature of the last 5 years (2017–April 2022), only one study (Ory et al., 2018) focused on the most captured fish species in the world (*Engraulis ringens)* and yellowfin tuna (*Thunnus albacore*) (Chagnon et al., 2018); MP concentrations reported in these studies were 0.8% and 2%, respectively. No data are available for Alaska pollok (*Gadus chalcogrammus*), which is the second most captured species worldwide. Further studies are needed to discern whether and to what extent this species is affected by MPs and/or NPs. In studies (two to four) on Scad nei (*Decapterus* spp.), the largehead hairtail (*Trichiurus lepturus*), Atlantic mackerel (*Scomber scombrus*) and Japanese anchovy (*Engraulis japonicus*), MP concentrations ranged from 1.4% in largehead hairtail caught in the Atlantic Ocean (di Beneditto & da Silva Oliveira, 2019) to 100% in Japanese anchovy caught in the Seto Inland Sea (western Japan) (Ohkubo et al., 2022).

At least five published studies have reported MPs in the following marine species: In Skipjack tuna (*Katsuwonus pelamis*), MPs ranged from 0.75% in specimens from the western equatorial Atlantic Ocean (de Mesquita et al., 2021) to 100% in those from the Indian Ocean (Suwartiningsih et al., 2020). As for blue whiting (*Micromesistius poutassou*), MPs concentrations ranged from 0% in specimens from the Tyrrhenian Sea (central Mediterranean) (Pittura et al., 2018) to 51.9% in samples from the English Channel (Mercogliano et al., 2020). In the case of European pilchardus (*Sardina pilchardus*), MPs concentrations ranged from 15.24% in specimens from the Mediterranean coast of Spain (western Mediterranean) (Compa et al., 2018) to 96% in specimens from the Adriatic Sea (central Mediterranean) (Renzi, Specchiulli, et al., 2019). MP concentrations in Pacific chub mackerel (*Scomber japonicus*) ranged from 3.3% in specimens from the southeast Pacific Ocean (Ory et al., 2018) to 62.5% in samples from south western Japan (Yagi et al., 2022). The Atlantic cod (*Gadus morhua*) showed MP values ranging from 1.4% in specimens from Fogo Island, Newfoundland, and Labrador (Saturno et al., 2020) to 27% in specimens from Norwegian coast. MP concentrations in Sardinellas nei (*Sardinella* spp.) ranged from 5% in specimens from the South-eastern Arabian Sea, India (James et al., 2020), to 100% in specimens from the Pantai Indah Kapuk coast, Indonesia (Hastuti et al., 2019). When considering crustaceans,

the only available study on the most harvested gazami crab (*Portunustri tuberculats*) reports MP concentrations of 66.7–100% (Zhang et al., 2021), whereas the blue swimming crab (*Portunus pelagicus*) caught in the Arabian Sea is impacted the least, with MP concentrations of up to 13% (Daniel et al., 2021). As for other invertebrates of particular commercial interest, such as cephalopods, jellyfish, and echinoderms, all three of these taxa have quite high MP contents ranging from 75% in the sea urchin nei (*Strongylocentrotus* spp.) of the northern coast of China (Feng et al., 2020) to 100% in cuttlefish (*Sepiidae*) caught from the coast of Portugal (Oliveira et al., 2020). To date, the literature on the incidence of NPs in marine organism, especially in the abovementioned major species/taxa, is still scarce. It is evident that more information is needed concerning the impact of these troubling contaminants on the food web.

3.3 MPs and NPs in marine aquaculture and processed seafood products

Considering the general decrease in global wild fishery captures and the increase in the total aquaculture production (from an average per year of 59.7 million tons in 2015 to 82.1 million tons in 2018) (FAO, 2020), as well as the forecast will reach 40 million tons in 2030, close attention should be paid to analyzing the impact of MPs and NPs on fed and non-fed marine aquaculture products. In 2018, the global production of the top five marine aquaculture species individually was 5.2 million tons for cupper oyster (*Crassostea* spp.), 5 million tons for whiteleg shrimp (*Litoenaeus vannamei*), 4.1 million tons for Japanese carpet shell (*Ruditapes philippinarum*), 2.4 million tons for Atlantic salmon (*Salmo salar*), and 1.9 million tons for scallops (Pectinidae) (FAO, 2020). For cupped oysters, the most aquacultured shellfish species worldwide, Walkinshaw et al. (2020) reported an average MPs value (50–100 μm size range) of 0.18–3.84 items/g (*w*/w) whereas the average value reported for the Japanese carpet shell ranged from 0.9 to 2.5 items/g (w/w).

For *Litopenaeus vannamei* farmed in Southern China, Li et al. (2021a, 2021b) reported a MPs abundance of 4–21 items/individual (mean value 10.87), whereas Valencia-Castañeda et al. (2022) found 7.6 items/individual in the gastrointestinal tract, 6.3 in the gills, and 4.3 in the exoskeleton, with an average of 18.5 MPs items per shrimp (1.06 items/g, wet weight [ww]). As for Atlantic salmon, the literature on the incidence of MPs is lacking in this fish species. Liboiron et al. (2019) found that the frequency of occurrence of plastic in the gastrointestinal tract of this highly consumed species

that is 69 specimens collected in Newfoundland (Canada) was zero. Focusing on MPs abundance in Pectinidae, Ding et al. (2021) detected an average of 0.5–2.9 items/individual (0.4–3.4 items/g) in *Chlamys farreri* specimens collected around Qingdao, China.

Drying is one of the oldest techniques used for preserving foods at all latitudes to dehydrate different marine products. For example, it is traditionally used in the cold northern regions of Europe to produce stockfish (Inderhaug, 2020), and in the Mediterranean region to produce "bottarga," a delicious seafood-derived product based on salting and subsequent air drying of the raw roe of tuna (*Thunnus* spp.) and gray mullet (*Mugil* spp.). In some developing countries of South Asia and sub-Saharan Africa, this low-cost technique is often the only way to reduce water content (moisture) and prevent post-mortem degradation of seafood products. According to Karami, Golieskardi, Bin Ho, et al. (2017), seafood products prepared using these techniques tend to concentrate more MPs and NPs than other seafood products. This is due to post-harvest contamination during the long period spent in direct contact with common air impurities during the drying/salting process, which often occurs in environments that are not particularly healthy. This outcome has been recently confirmed by Piyawardhana et al. (2022), who studied the occurrence of MPs in 12 dried seafood consumed throughout the Asia-Pacific region, where such products are a staple in the diet. They observed that all species samples contained MPs both in the gastrointestinal tract and in the muscle. The authors also detected a positive correlation between the incidence of MPs and the weight of the analyzed specimens. By contrast, very little information is currently available on stockfish and salted fish (the Mediterranean salted sardine and anchovies), or on the abovementioned dried/salted fish roe (bottarga).

Canning is another traditional preservation technique used widely in fish and shellfish processing because it significantly extends the shelf life of highly perishable seafood products. In canned tuna and mackerel products, there is a high risk of contamination by MPs during the product cleaning and canning process (Akhbarizadeh et al., 2020).

4. Presence of MPs and NPs in other foods and beverages

In addition to seafood, the presence of MPs and NPs has been widely reported in other food products, such as fruit and vegetables, honey, salt, sugar, and beer (Table 3). A Scopus search using the combined terms

Table 3 Identification and quantification techniques of microplastics and nanoplastics in different food matrices.

Food	Origin	Type (species)	Type of MPs/NPs	Size	References
Fruits and vegetables	Italy	Apple (*M. domestica)*)	MPs and NPs	2.17 μm	Oliveri Conti et al. (2020)
	Italy	Pear (*P. communis*)	MPs and NPs	1.99 μm	Oliveri Conti et al. (2020)
	Italy	Broccoli (*B. oleracea* italic)	MPs and NPs	2.10 μm	Oliveri Conti et al. (2020)
	Italy	Lettuce (*Lactuca sativa*)	MPs and NPs	2.52 μm	Oliveri Conti et al. (2020)
	Italy	Carrot (*Daucus carota*)	MPs and NPs	1.51 μm	Oliveri Conti et al. (2020)
	Chile	Maize (*Zeamays* L.)	PET microbeads	–	Urbina et al. (2020)
	The Netherlands	Garden cress (*Lepidium sativum* L.)	MPs and NPs	<100 nm <5 mm	Bosker et al. (2019)
	China	Seedling growth of wheat (*Triticum aestivum* L.)	PSNPs	88 nm	Lian et al. (2020)
	China	Lettuce (*Lactuca sativa*)	SPS and LPS	SPS: 100–1000 nm LPS: >10,000 nm	Gao et al. (2021)
	China	Lettuce (*Lactuca sativa* L.)	PVC	PVC- [18 μm −100 nm] PVC-b [18–150 μm]	Lian et al. (2020)
	China	Cucumber (*Cucumis sativus)*	PSNPs	100, 300, 500, and 700 nm	Lian et al. (2020)

Beverages	Ecuador	Skim milk	MPs	2.48–183.37 μm	Diaz-Basantes et al. (2020)
	Ecuador	Refreshing beverage	MPs	5.94–247.54 μm	Diaz-Basantes et al. (2020)
	Ecuador	Industrial Beer	MPs	3.50–202.29 μm	Diaz-Basantes et al. (2020)
	Ecuador	Craft Beer	MPs	6.15–160.345 μm	Diaz-Basantes et al. (2020)
	Mexico	Milk	PES andPSU	100–5000 μm	Kutralam-Muniasamy et al. (2020)
	Germany	Beer	PET	–	Liebezeit and Liebezeit (2014)
	Portugal	Wine	MPs	–	Prata et al. (2020)
	Mexico	Cold Tea	PA, PET, PEA and ABS	100–2000 μm	Shruti et al. (2020)
	Mexico	Soft drink	PA, PET, PEA and ABS	100–3000 μm	Shruti et al. (2020)
	Mexico	Energy drink	PA, PET, PEA and ABS	100–3000 μm	Shruti et al. (2020)
	Mexico	Beers	PA, PET, PEA and ABS	100–3000 μm	Shruti et al. (2020)
Honey	Ecuador	Industrial honey	MPs	5.63–182.96 μm	Diaz-Basantes et al. (2020)
	Ecuador	Craft honey	MPs	5.15–226.01 μm	Diaz-Basantes et al. (2020)
Salts	Eight African countries	Table salt	PVA, PP and PE	3.3–4660 μm	Fadare et al. (2021)
	Turkey	Lake salt	PE, PET, PU, PP, PMMA, PA-6 and PVC	20 μm–5 mm	Gündoğdu (2018)
	Turkey	Rock salt	PE, PET, PU, PP, PMMA, PA-6 and PVC	20 μm–5 mm	Gündoğdu (2018)
	Turkey	Sea salt	PE, PET, PU, PP, PMMA, PA-6 and PVC	20 μm–5 mm	Gündoğdu (2018)

Continued

Table 3 Identification and quantification techniques of microplastics and nanoplastics in different food matrices.—cont'd

Food	Origin	Type (species)	Type of MPs/NPs	Size	References
	Spain	Sea salt	polyethylene terephthalate (PET), polyethylene (PE) and polypropylene (PP)	30 μm–3.5 mm	Iñiguez, Conesa, and Fullana (2017)
	Spain	Well salt	PET, PE and PP	160–980 μm	Iñiguez et al. (2017)
	Republic of Korea	Lake salt	PP, PE, PS, PET	100–5000 μm	Kim et al. (2018)
	Republic of Korea	Rock salt	PP, PE, PS and PET	100–5000 μm	Kim et al. (2018)
	Republic of Korea	Sea salt	PP, PE, PS and PET	100–5000 μm	Kim et al. (2018)
	Italy	Salt	MPs	4–4628 μm	Renzi and Blašković (2018)
	Italy and Croatia	Salt	PET, PVC, PE, and PA,	10–150 μm	Renzi, Grazioli, et al. (2019)
Others	The Netherlands	Growth of sediment-rooted macrophytes	MPs and NPs	20–500 μm 50–190 nm	van Weert et al. (2019)
	China	Chicken meat	PS and PVC	3 μm and 100 μm	Huang et al. (2020)
	France	Packaged chicken breasts, packaged turkey escalopes (Extruded PS tray)	EPS and XPS	300–450 μm	Kedzierski et al. (2020)
	The Netherlands	Chicken gizzard and Chicken crop	MPs and NPs	1000–5000 μm	Lwanga et al. (2017)
	Italy	Terrestrail snail	MPs	100–2500 μm	Panebianco et al. (2019)

PE, Polyethylene; PSNPs, Polystyrene nanoplastics; SPS, Small polystyrene; LPS, Large polystyrene; PVC, Polyvinylchloride; PES, Polyethersulfone; PSU, Polysulfone; PET, Polyethylene-terephthalate; PA, Polyamide; PEA, Polyester-amide; ABS, Acrylonitrile-butadiene-styrene; PVA, Polyvinyl acetate; PP, Polypropylene; PU, Polyurethane; PMMA, Polymethyl-methacrylate; PA-6, Polyamide-6; EPS, Expanded polystyrene; XPS, Extruded polystyrene.

"TITLE-ABS-KEY (microplastics) OR TITLE-ABS-KEY (nanoplastics) AND TITLE-ABS-KEY (fruits) OR TITLE-ABS-KEY (vegetables) OR TITLE-ABS-KEY (salt) OR TITLE-ABS-KEY (sugar) OR TITLE-ABS-KEY (honey) OR TITLE-ABS-KEY (meat) OR TITLE-ABS-KEY (chicken) OR TITLE-ABS-KEY (wine) NOTTITLE-ABS-KEY(marine) AND NOT TITLE-ABS-KEY(sea) AND NOTTITLE-ABS-KEY(fish))" was carried out in order to perform a cluster-based VOSviewer analysis. Overall, the Scopus search revealed 156 publications pertaining to MPs and NPs in the food products listed above.

According to the VOSviewer output, co-occurrence analysis identified 2599 keywords, of which 173 occurred at least 5 times. As expected, "MPs" was the most frequent keyword identified, followed by "water pollutant," "soil," "environmental monitoring," "vegetable," "plant leaf," "fruits" and others (Fig. 5). Four "clusters" were generated: these define thematic proximity among all considered keywords.

Oliveri Conti et al. (2020) conducted a study to identify and quantify MPs in common edible fruit (apples and pears) and vegetables (carrot,

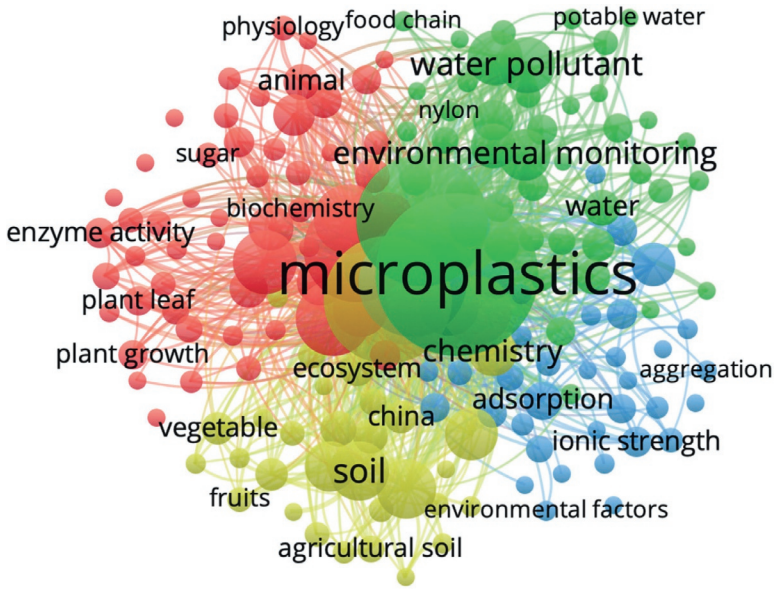

Fig. 5 Network map of 173 keywords (with the minimum occurrence set to 5) identified by VosViewer on the Scopus (CSV) database. The size of nodes indicates the frequency of occurrence, while the colors identify four clusters that define the thematic proximity and indicate research directions.

lettuce, broccoli, and potato). MPs particle sizes extended from 1.51 to 2.52 μm, with the smallest size (1.51 μm) of MPs being found in the carrot samples, while the biggest (2.52 μm) one was found in the lettuce. The authors reported that apple was the most contaminated fruit whereas carrot was the most contaminated vegetable. Depending on vegetable samples, MPs ranged from 52.050 to 233.000 particles/g. The authors supposed that MPs could be absorbed and translocated to the vegetal tissues in the same way as carbon nanomaterials.

Some studies have investigated the impact of plastics and related contaminants on plant growth and development. For example, Bosker et al. (2019) studied the impact of MPs with three different sizes (50, 500, and 4800 nm) on cress (*Lepidium sativum*). The results showed that the exposure to these contaminants could significantly affect germination and root growth, which was explained by a physical blockage of plant pores with plastic particles, inhibiting water uptake. In a similar study, Gao et al. (2021) investigated the harmful effects of small (100–1000 nm) and large (>10,000 nm) MPs on lettuce under dibutyl phthalate (DBP) stress. The findings suggested that the adhesion of plastic particles, especially the small MPs, on the root surface causes the physical clogging of root pores, aggravating the negative effects (decreased lettuce biomass and oxidative damage in lettuce leaves and roots) of DBP on lettuce growth.

In a study conducted by Urbina et al. (2020) in an experimental hydroponic culture of maize, the adsorption and potential uptake, as well as the physiological effects of PE microbeads were assessed. These authors observed that plastic accumulation in the rhizosphere provoked an important decrease in transpiration, nutrient uptake, and the plant growth. In another study, the effects of six different MPs, including polyester fibers, PA beads, PE, polyester terephthalate, PP, and PS, on the growth of spring onion (*Allium fistulosum*) and soil microbial activities, as well as soil–plant interactions, were investigated (De Souza Machado et al., 2019). The findings showed significant consequences of plastic contamination, in particular the contamination by polyester fibers and PA beads, for agro-ecosystems and general terrestrial biodiversity. The impact of biodegradable plastic and PS residues was also reported on wheat (*Triticum aestivum*) growth (Qi et al., 2018). The plastic contamination, notably the biodegradable plastic, showed an impact on the plant mass both above and below ground during the vegetative and reproductive growth.

The aforementioned examples, in addition to many others, reported in recently published review papers (Bai et al., 2022; Oliveri Conti et al., 2020; Silva et al., 2021; Vitali et al., 2022) demonstrate the occurrence and the

negative impact of MPs and NPs on plant growth, biodiversity, and soil ecosystems. However, more research studies are still needed to confirm these findings as some investigations seem to indicate contradicting results. In fact, Lian et al. (2020) studied the influence of PS NPs at level ranges between 0.01 and 10 mg/mL on the germination of seed and growth seedling of wheat. Although the exposure to these NPs extremely altered the metabolic profiles in wheat leaves, the wheat seedling growth seemed to be significantly enhanced.

Some studies have reported the presence of MPs and NPs in sea salt, sugar, honey, milk, wine, beer, and other food and beverages, although little information is still available on MPs occurrence in these products. Kutralam-Muniasamy et al. (2021) tested the occurrence of a variety of MPs with different colors (blue, brown, red, and pink), shapes (fibers and fragments), and sizes (0.1–5 mm) in 23 milk samples in Mexico. The results showed that thermoplastic sulfone polymers (polyethersulfone and polysulfone) were common types of MPs due to their use in membrane materials in dairy processes. Blue fibers of size less than 0.5 mm were found to be prevalent in the studied samples.

The presence of MPs was first reported in Germany by Liebezeit and Liebezeit (2014) who examined 24 German beer brands for the contents of microplastic fibers, fragments, and granular. The results displayed a high variability among individual samples and samples from different production dates, and the counts ranged from 2 to 79/L for the fibers, 12 to 109/L for the fragments, and 2 to 66/L for the granules. Prata et al. (2020) detected MPs in 26 bottles of white wine capped with plastic stoppers. MPs were present in the examined samples with concentrations up to 5857 particles/L, and median dimensions of $26 \times 122\,\mu m$. Shruti et al. (2020) evaluated the MPs occurrence in Mexican soft drinks, cold tea, energy drinks, and they found that beer sample had the highest abundance. PA, poly (ester-amide) (PEA), acrylonitrile-butadiene-styrene (ABS), PET, and blue pigments were the most identified microplastic polymers, while synthetic textiles and packaging were reported as the contamination origins in these beverage products.

MPs contamination in commercial table salts has been monitored and reported in different countries around the world, such as Spain (Iñiguez et al., 2017), Turkey (Gündoğdu, 2018), India (Seth & Shriwastav, 2018), Italy and Croatia (Renzi & Blašković, 2018), and China (Yang et al., 2015). Fadare et al. (2021) analyzed 23 brands of table salts from 8 African countries: Nigeria (4), Cameroon (2), Ghana (3), Malawi (1), Zimbabwe (1), South Africa (6), Kenya (5) and Uganda (1). The highest

concentration levels (0–1.33 particles/kg) were found in South Africa, followed by Nigeria, Cameroun, and Ghana with concentrations ranging between 0 and 0.33 particles/kg. Polyvinyl acetate (PVA), PP, and PE represented being the most prevalent MPs. Interestingly, the samples originated from the other countries (Malawi, Zimbabwe, Kenya and Uganda) had no detectable MPs at 0.3 μm filter pore size. In another study, Yang et al. (2015) reported that China salts are contaminated by MPs, with levels ranging from 550 to 681 particles/kg in sea salts, from 43 to 364 particles/kg in lake salts, and from 7 to 204 particles/kg in rock/well salts. The common MPs were PET, followed by PE and cellophane in sea salts, and 55% of particles were <200 μm. Seth and Shriwastav (2018) have reported several types of MPs, such PE, PET, PA, polyesters, and PS in Indian salt samples. The authors suggested a simple sand filtration to remove more than 90% of the contamination. MPs content of 16 brands of table salts obtained from the Turkish market was evaluated (Gündoğdu, 2018). MPs contents in sea salt, lake salt, and rock salt were in the range of 16–84, 8–102, and 9–16 item/kg, respectively, with PE and PP being the common plastic polymers. Karami, Golieskardi, Choo, et al. (2017) observed MPs in 88% of salt samples analyzed from eight countries (Australia, France, Iran, Japan, Malaysia, New Zeeland, Portugal, and South Africa). The MPs levels ranged from 0 particles (French sea salt) to 10 particles/kg (Portuguese sea salt).

It has been also reported that MPs can be present in chicken meat and meat products (Huang et al., 2020; Kedzierski et al., 2020). For instance, Huang et al. (2020) assessed the level of contamination by PS and PVC in homogenized chicken meat, while Kedzierski et al. (2020) identified and quantified MPs in meat packed in extruded PS trays, reporting a value range of 4.0–18.7 particles/kg. A recent study reported a severe MP contamination, represented by PP, PE, and polyester resin, in livestock and poultry farms in South China, highlighting the importance of understanding the contamination risk of MPs in livestock and poultry manure before its utilization as fertilizers during crop planting (Wu et al., 2021).

5. Identification and quantification of MPs

The presence of MPs in both terrestrial and aquatic ecosystems has become a widespread problem and an issue of great concern in recent years. MPs in marine habitats are those small plastic fragments that can be consumed by different aquatic species including fish, shellfish, marine invertebrates, bivalves, among others, and are ultimately transferred along the

food chain to human beings (Chatterjee & Sharma, 2019; Dawson et al., 2021; López-Martínez et al., 2021; Savoca, McInturf, & Hazen, 2021; van Raamsdonk et al., 2020). To detect, identify and quantify MPs, several approaches have been developed, ranging from the simple visual inspection (by the naked eye or microscopically) to more advanced techniques such as chromatography coupled with mass spectrometry and spectroscopic techniques (Dawson et al., 2021; Lv et al., 2021; Razeghi et al., 2021; Silva et al., 2018; Wang & Wang, 2018). Many review papers have been published on this topic, comparing these different methods, their applications, advantages and limitations, etc. (Akoueson et al., 2021; Gong & Xie, 2020; la Nasa et al., 2020; Peñalver et al., 2020). The optical methods based on the visual inspection or the use of microscopes, scanning electron microscopy (SEM), and gas chromatography–mass spectrometry (GC–MS) have been widely used. The spectroscopic methods, especially the microscopic infrared and Raman spectroscopies remain the most commonly applied techniques (Cowger et al., 2020; Dehaut et al., 2019; Kwon et al., 2020; Li et al., 2019; Primpke et al., 2020; Schwaferts et al., 2019).

5.1 Traditional analysis techniques

5.1.1 Optical methods

A number of studies have demonstrated that MPs of size >100 μm can be easily detected and classified directly through a simple naked-eye observation, based on the examination of size, shape, and color of the microplastic particles. However, most often, this visual inspection is accompanied by optical microscopy (Lv et al., 2021; Silva et al., 2018; Zhu & Wang, 2020). For example, a stereomicroscope was used by Bono et al. (2020) to classify the microplastic particles ingested by Atlantic chub mackerel (*Scomber colias*) according to their size, texture, and shape into fragments, fibers, lines, paint and films.

Visual sorting is simple and cost effective but cannot be used to detect and identify particles of small size (<100 μm). In addition, the human subjectivity related to visual inspection is a challenging factor that can lead to variability in the detection results, and either under or overestimation of MPs can often occur (Cowger et al., 2020; Gong & Xie, 2020; Pinto da Costa et al., 2019; Strungaru et al., 2019; Zhu & Wang, 2020). It has been reported that the misidentifications can reach a high percentage rate (65–67%) for transparent polymers (Lakshmi Kavya, Sundarrajan, & Ramakrishna, 2020). Microscopic methods also proved to be a weak means

to distinguish between the synthetic and natural fibers (e.g., PES vs dyed cotton). To overcome the limitations, several approaches have been developed in recent years. For example, Nile red staining and fluorescent microscopy has been applied in several studies in order to identify MPs in fresh and processed fish samples (Akhbarizadeh et al., 2020; TaghizadehRahmat Abadi et al., 2021).

5.1.2 Scanning electron microscopy (SEM)

SEM uses focused electron beams to investigate size, shape, and other physical parameters of MPs, providing high-resolution surface images under high-magnification overcoming all the limitation of optical microscopy. This technique is often coupled to energy dispersive X-ray spectroscopy (EDS or EDX) to obtain detailed information about the elemental composition of the microplastic particles (Primpke et al., 2020; Silva et al., 2018; Wang & Wang, 2018; Zhu & Wang, 2020) to ensure differentiation between plastics and gives the possibility to distinguish between organic and plastic particles.

Several studies have used SEM-EDS (or SEM-EDX) for the characterization of surface structure and elemental composition in MPs (Akhbarizadeh et al., 2020; Bermúdez-Guzmán et al., 2020; Jonathan et al., 2021; Karbalaei et al., 2019) (Table 4). For instance, in a recent one, the presence of MPs ingested by fishes of Magdalena bay was examined by means of SEM microscope in conjunction with EDS (Jonathan et al., 2021). SEM images showed angular and irregular edges of MPs, revealing the impact of mechanical disintegration of plastic fibers due to probably weathering processes, whereas the majority of the EDX spectra of the fibers presented carbon and oxygen peaks, suggesting them as plastics. Similarly, Martinez-Taveraand coauthors used the same techniques (i.e., SEM and EDX) to study the surface morphology and the elemental composition of MPs in freshwater tilapia (*Oreochromis niloticus*) that caught from a metropolitan reservoir in Central Mexico (Martinez-Tavera et al., 2021). However, these techniques are destructive and can be time-consuming and quite expensive and thus only few samples are usually examined in order to confirm the width and length of the fibers.

5.1.3 Gas chromatography-mass spectrometry (GC–MS)

Chromatographic techniques coupled with mass spectrometry have attracted increasing attention, and they have been widely used in the microplastic research to enable quantitative analysis. Two GC–MS-based approaches have been commonly applied: pyrolysis gas chromatography

Table 4 Examples of analytical methods used for the detection and identification of microplastics in fish/seafood and other food products.

Products category	Species/products	Analytical approach	Main conclusions	References
Fish and other seafood	Norway lobster	SEM, μ-Raman	83% of the examined samples contained plastic filaments	Murray and Cowie (2011)
	Laboratory fish (Medaka), Pelagic prey fish (Myctophid)	Optical microscopy, SEM/EDS, μ-FT-IR, μ-Raman	Optical microscopy is not enough to identify MPs, especially if the particles are of various morphologies	Wang et al. (2017)
	Pelagic and demersal fish	Py-GC–MS	The technique was able to identify the chemical composition of plastic polymers by analyzing its characteristic thermal degradation products and comparing with pyrolysis reference maps of known pure polymers	Fischer and Scholz-Böttcher (2017)
	Demersal and pelagic fish	Microscopic analyses	828 pieces of MPs were detected, most of them were in the form of fibers	Abbasi et al. (2018)
	Commercial marine fish from Malaysia	SEM-EDS Raman	MPs were detected in 9 out of 11 samples, and polyethylene was the most abundant plastic polymer	Karbalaei et al. (2019)
	Mussels and cockles	TED-GC–MS, μ-Raman	Up to 58% of the samples were contaminated by MPs. 13 plastic additives and 27 hydrophobic organic compounds were quantified in bivalves' flesh	Hermabessiere et al. (2019)
	Herring *Opisthonema* sp.	Optical microscopy, SEM-EDX, FT-IR-ATR	MPs were detected in all the sampled fishes; 20.5% were classified as particles and 79.5% as fibers	Bermúdez-Guzmán et al. (2020)

Continued

Table 4 Examples of analytical methods used for the detection and identification of microplastics in fish/seafood and other food products.—cont'd

Products category	Species/products	Analytical approach	Main conclusions	References
	Four fish species (*Clarias gariepinus*, *Cyprinus carpio*, *Carassius carassius*, and *Oreochromis niloticus*) from the Lake Ziway	ATR-FT-IR	Polypropylene, polyethylene, and alkyd-varnish were found to be the dominant polymers	Merga et al. (2020)
	Penaeid shrimp	μ-FT-IR	Polyamide-6 and rayon polymers were the most dominant polymers detected in the samples	Hossain et al. (2020)
	Sea bass	Fluorescence microscopy	Feeding juveniles of fish with a diet containing fluorescent microplastic particles showed that the entrance of MPs to lymphatic and/or vascular systems through the gastrointestinal tract was the most likely pathway for translocation of MPs	Zeytin et al. (2020)
	Commercial fish from Southeast coast of the Bay of Bengal	SEM FT-IR	20 plastic particulates (of polyethylene, polyamide and polyester types) were isolated from the gastrointestinal tract of 17 fish	Karuppasamy et al. (2020)
	Tuna and mackerel	SEM-EDX, fluorescence analysis, μ-Raman	At least one microplastic particle was found in 80% of the investigated samples, and polyethylene terephthalate, polystyrene, and polypropylene were the most abundant polymers	Akhbarizadeh et al. (2020)
	24 fish species from Beibu Gulf	μ-FT-IR	Polyester and nylon were the predominant polymer types and the fibers were the dominant form of particles	Koongolla et al. (2020)

Shrimp *Pleoticus muelleri*	SEM/EDS, μ-Raman	Complete studies of MPs require both physical and chemical analysis using microscopy (to measure color, size, shape) and spectroscopy (to identify polymer types), respectively	Fernández Severini et al. (2020)
Commercial fish from the Arabian Gulf	FT-IR	5.71%, of the examined fish was contaminated with MPs (especially polyethylene and polypropylene)	Baalkhuyur et al. (2020)
Commercial fish from Tunisian coasts	μ-Raman	Small MPs ($\leq$3 μm) were found in the in the gastrointestinal tracts and muscle of the examined fish	Zitouni et al. (2020)
Blue panchax fish (*Aplocheilus* sp.)	FT-IR	75% of the samples were contaminated by various types of microplastic shapes and sizes	Cordova, Riani, and Shiomoto (2020)
Oysters	Optical microscopy, μ-ATR-FT-IR	About 83% of MPs in samples were identified as plastic materials, including polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyethylene terephthalate, rayon, and nylon	Wang et al. (2021a)
Kutum fish	SEM-EDS, fluorescent microscopy	80% of investigated fish contained MPs, and fibers were the dominant type followed by fragments and synthetic microbeads	TaghizadehRahmat Abadi et al. (2021)
Various seafood (fish, crustaceans, and molluscs)	Py-GC–MS, fluorescence microscopy, SEM, ATR-FT-IR, μ-Raman	Digestion efficiency was more than 98% in most of the analyzed seafood. Optimization of a protocol for the isolation of MPs present in the edible part of seafood	Süssmann et al. (2021)

Continued

Table 4 Examples of analytical methods used for the detection and identification of microplastics in fish/seafood and other food products.—cont'd

Products category	Species/products	Analytical approach	Main conclusions	References
Fruit and vegetables	Apples, pears, broccoli, lettuce and carrots	Scanning Electron Microscopy—SEM-EDX	Apples were the most contaminated fruit samples, whereas carrot was the most contaminated vegetable	Oliveri Conti et al. (2020)
	Maize	Isotope analysis (δ13C)	About 30% of the carbon in the rhizosphere of microplastic-exposed plants was derived from PE	Urbina et al. (2020)
	Carrots, lettuces, broccoli, potato apples and pears	SEM-EDX	Apple was the most contaminated samples, while carrots were the most contaminated vegetable. The smallest MPs size was found in carrots (1.51 μm) and the biggest ones in lettuce (2.52 μm)	Oliveri Conti et al. (2020)
Milk and dairy products	Milk	Visual identification and enumeration, SEM-EDS, μ-Raman	MPs exhibited variety of colors (blue, brown, red and pink), shapes (fibers and fragments) and sizes (0.1–5 mm) Blue colored fibers (<0.5 mm) were predominant. The most common microplastic particles were polyethersulfone and polysulfone	Kutralam-Muniasamy et al. (2020)
	Farm milk, reconstituted milk powder and skimmed milk	SEM–EDX, μ-Raman	PE, PES, PP, PTFE and PS were the most common MPs. Smaller quantities of PA, PU, PSU and PVA were detected. The concentration of MPs ranged varied between 204 and 1004 MPs per 100 mL of milk	da Costa Filho et al. (2021)
Meat and meat products	Homogenized chicken meat	ATR-MIR	PVC (particle size: 3 μm, 100 μm and 2–4 mm) and PS (particle size: 100 μm) can be detected and quantified by ATR-MIR at a concentration between 1% and 10%	Huang et al. (2020)

SEM, Scanning electron microscopy; EDS, Energy dispersive spectroscopy; EDX, Energy dispersive X-ray spectroscopy; GC–MS, Gas chromatography-mass spectrometry; Py-GC–MS, Pyrolysis-gas chromatography–mass spectrometry; FTIR, Fourier-transform infrared spectroscopy; ATR, Attenuated total reflection.

mass spectrometry (Py-GC–MS) and thermal extraction desorption gas chromatography mass spectrometry (TED-GC–MS). However, these techniques have been mainly used to analyze plastic and MPs in environmental samples (e.g., water or sediment) while few studies have been conducted on seafood (Dehaut et al., 2019; la Nasa et al., 2020; Peñalver et al., 2020; Yakovenko, Carvalho, & ter Halle, 2020). For instance, a Py-GC/MS approach was applied for polymer identification of microplastic ingested by benthivore fish from the Texas Gulf Coast (Peters et al., 2018). The results showed that PVC and PET were the most common microplastic polymers collected from the stomach content of the fish. In another study, Dehaut and coauthors investigated the potential of Py-GC–MS to identify polymers in mussel, crab, and fish tissues using different digestion protocols (Dehaut et al., 2016). The effect of the different approaches was tested on a set of 15 different plastic polymers, and the authors found that alkaline digestion procedures (KOH 10%) gave the best results, enabling a correct identification of all the polymer types, except in the case of cellulose acetate. The same protocol was later applied to characterize MPs in mussels and cockles (Hermabessiere et al., 2018). In a recent study, Ribeiro and coworkers succeeded in developing a Py-GC/MS methodology for the identification and quantification of MPs isolated from edible portions of oysters, prawns, squid, crabs, and sardines (Ribeiro et al., 2020). Five different plastics, including PS, PE, PVC, PP, and methyl methacrylate were appropriately identified and quantified. Nevertheless, it should be stressed that the technique is destructive and may be used only as a complementary technique to spectroscopic methods.

5.2 Spectroscopic methods

Spectroscopic techniques, especially vibrational spectroscopy (i.e., total reflection FT-IR and μFT-IR, Raman or stimulated Raman scattering; SRS) are tools that have been found to be useful for MPs identification and characterization.

5.2.1 Infrared (IR) spectroscopy

FT-IR spectroscopy is certainly the most popular technique for MPs quantification and characterization. This is probably due to the sensitivity of FT-IR to functional groups (C-H, N-H, O-H) providing a spectral fingerprint (distinct absorption bands) of the analyzed polymers (Cowger et al., 2020; Veerasingam et al., 2021). The identification of the polymer is also an easy task when using this technique due to a possible comparison to commercially

available polymers library. However, the identification is generally complex because there is no standardized method defining different parameters, such as hit quality index (HQI) threshold or spectral pre-treatment. In addition, different forms (with irregular shapes), colors, chemical additives can be found in MPs, making their identification complex. Moreover, their affinity to chemical pollutants from seawater, denaturation by sun light, temperature variation, and biodegradation add complexity to this task (Kwon et al., 2020; Lv et al., 2021; Renner et al., 2019; Renner, Schmidt, & Schram, 2018; Veerasingam et al., 2021). Therefore, a consolidation of databases seems necessary.

FT-IR spectroscopy can be used in attenuated total reflection (ATR), transmission or reflection modes, noticing that each mode has some advantages and disadvantages (Gong & Xie, 2020; Renner et al., 2019). For instance, the ATR and reflectance modes are more suitable for thick and opaque samples compared to the transmission mode and needs no or little sample preparation. Furthermore, the use of ATR provides a stable and reliable spectral line data, even with surfaces presenting a rough texture (Lakshmi Kavya et al., 2020). An intimate contact must be established between the sample and the ATR crystal. This can be obtained by pressing the sample to the crystal; however, this can damage the fragile particles or alter polymers and cause tiny particles to stick to the crystal due to electrostatic forces (Shim, Hong, & Eo, 2017). A recent study conducted by Daniel et al. (2021) proved that this method is suitable for studying MP contaminants in various seafoods. They identified the presence of MPs in the size range of 100 μm–5 mm in four species of shellfishes (two species of shrimp, one species of crab, and one species of squid). Three common polymers (including PP, PE, and PS) were isolated from these shellfishes and identified.

However, it is well known that FT-IR measurements are limited to a size of MPs of about 20 μm. This drawback can be overcome by using FT-IR microscopy (μ-FT-IR). This technique combines FT-IR with microscopic imaging and provides both high spatial resolution and spectral information of the analyzed sample. Nevertheless, μ-FT-IR can be time-consuming (Pinto da Costa et al., 2019). There are essentially three FT-IR microscopy configurations: (1) point detector analysis in a confocal arrangement; (2) FT-IR mapping, performed by integrating the signal from successive locations of the specimen surface; and (3) FT-IR imaging, performed with bi-dimensional arrays, e.g., focal plane array (FPA) detectors. A wide range of studies using μ-FT-IR for MPs analysis has been reported and proved the effectiveness of this technique for polymer characterization and identification. For instance, μ-FT-IR was used to describe the distribution of MPs

in gut, gills, and muscle of fish and the possibility of bioaccumulation in their tissues (Su et al., 2019). The μ-FT-IR was also used to analyze MPs in four species of finfish samples as well as water and sediment (Saha et al., 2021). This study proved the reliability of the μ-FT-IR to analyze a huge variety of MPs polymers, up to 37. ATR-FT-IR was also suitably used to differentiate between low density polyethylene (LDPE) and high density polyethylene (HDPE) in ingested plastic by sea turtles (Jung et al., 2018).

Despite the many advantages of the different techniques presented above, one of the biggest drawbacks is the need to apply robust methodologies to separate plastic products from organic-rich intestinal tract content. The separation and preparation of sample are often time-consuming. This is generally performed by density separation and visual inspection before positioning the sample on a filter. In recent years, Vis-NIR (Visible Near Infrared) hyperspectral imaging (HSI) has been investigated as a new approach for rapid and non-invasive, and online determination and identification of MPs (Huang et al., 2020; Zhang et al., 2019). Vis-NIR HSI allows to obtain 3D images(data cube) in the 400–1000 nm range. The data cube is characterized by two spatial axis and one wavelength axis. Different configurations exist; point scanning, line scanning and area scanning. Zhang and coauthors proposed this technique to separate, identify, and characterize MPs without prior separation from the intestinal tract content of crucian carps (*Carassius carassius*) (Zhang et al., 2019). The sample analysis was performed in a short time (sample preparation, image acquisition, and data analysis were performed in only 36 min). The analysis of data by a machine learning algorithm (support vector machine: SVM) allowed to obtain recall and precision factors higher than 96.22% for five MPs (PE, PS, PET, PP and PC). This method was capable of identifying only plastics with a size higher than 0.2 mm that is larger than the particle size sensitivity of the μ-FT-IR. This limit of detection was also reported for seawater study (Shan et al., 2019). However, it is admitted that the general MPs size found in fish is >0.1 mm (Garnier et al., 2019; Yuan et al., 2019; Zhu et al., 2019) meaning that this technique could lead to underestimation of MPs contamination in biomass.

5.2.2 Raman spectroscopy

Raman spectroscopy is another vibrational spectroscopic technique that has been frequently used to identify MPs in different environmental samples with high reliability. A Raman spectrum is obtained after irradiation with a monochromatic laser source, generally 455, 633, 532, 785, and 1064 nm (Anger et al., 2018; Araujo et al., 2018; Käppler et al., 2016). After molecule

irradiation, a unique backscatter is recorded (Löder & Gerdts, 2015). Compared to FT-IR, Raman spectroscopy has several advantages, such as detection of smaller size of microplastic particles, higher spatial resolution, less interference of water, narrower spectral bands with wider spectral range. In addition, the technique provides detailed compositions of the polymers (Gong & Xie, 2020; Lv et al., 2021; Wang & Wang, 2018). Like FT-IR spectroscopy, Raman spectroscopy and its variants (surface-enhanced Raman, FT-Raman, tip-enhanced Raman, confocal Raman imaging, and others) have become a common technique to study MPs.

As with IR spectroscopy, Raman spectroscopy can be associated with microscopy (μ-Raman), making it possible to identify plastic particles of various and small sizes (below to 1 μm in size) (Anger et al., 2018; Lv et al., 2021). For instance, by using μ-Raman coupled with other techniques, Akhbarizadeh and coauthors showed that PET was the most abundant polymer in canned fish samples, followed by PS and PP (Akhbarizadeh et al., 2020). However, the long time required to acquire Raman spectra and the interference of fluorescence, as well as overlapping of signals due to the presence of additives or other contaminants and the high costs of instrumentation limit its applications for identification of microplastic polymers (Araujo et al., 2018; Peñalver et al., 2020; Zhu & Wang, 2020). To overcome these limitations, several studies have suggested the combined use of FT-IR and Raman for a reliable and complete chemical characterization of MPs, especially in the case of colored particles (Käppler et al., 2016; Xu et al., 2019). One example is a recent study conducted by Vinay Kumar and coauthors, who combined μ-Raman with μ-FT-IR to identify a broad size range (from 3 to 5000 μm) of MPs in commercial mussels (Vinay Kumar et al., 2021).

Recently, a new trend has been emerging in the field of analytical techniques: the development of portable devices and miniaturized systems that are suitable for in situ accurate detection and monitoring of MPs and NPs (Asamoah et al., 2021).

6. Conclusions

There is ample evidence that plastics, MPs, and NPs are everywhere. They can interact with vegetable and animal organisms, causing acute inflammation and cell damage, among other health concerns. Therefore, the environmental ubiquity of these emerging pollutants, in both aquatic and terrestrial environments, has become a critical concern, especially in the food sector. In addition, the COVID-19 pandemic has increased our

utilization of plastics, specifically single-use plastics (Vanapalli et al., 2021), emphasizing the need for urgent solutions.

The majority of seafood, fruit, vegetables, meat, and beverages used today can be seriously impacted by MPs and other pollutants that use this microscopic material as a carrier. An intensive research interest has been recently devoted to this topic with a special focus being put on the occurrence of plastic materials in marine aquaculture and processed seafood products.

MPs and NPs ingestion by marine organisms could alter endocrine system functions and the seafood microbiome, reduce the rate of fecundity, and cause DNA and neurological damage in various species. Moreover, plastic materials could act as carriers of other pollutants, such as plastic additives and other persistent organic pollutants, including heavy metals, dissolved in the aquatic marine environment, with serious consequences for marine biota.

It has been reported that marine products of particular commercial interest, such as finfish, crustaceans, molluscs, jellyfish, and echinoderms, have quite high microplastic contents. Specifically, MPs accumulation in seafood products is strongly influenced by the environment in which organisms live, what, how and where they feed, as well as their trophic levels and anatomic features. In particular, close attention should be paid to some of them such as mussels, clams and oysters that seem more subject to MP and NP contamination and accumulation because they feed by filtering significant quantities of coastal water, which is often more polluted than the open sea environment. Moreover, drying, canning, salting and other traditional methods used to produce special seafood products seem to concentrate MPs and NPs. This is due to post-harvest contamination during their processing that often occurs in environments that are not particularly healthy.

The occurrence of MPs and NPs in terrestrial food (crops and livestock) has received less attention, although contaminations have been recently documented in fruit and vegetables, beer and wine, soft drinks, sugar, table salt, chicken and other meat products. Some publications reported that plastic particles could cause mechanical blocking of the pores in contaminated plants, thus reducing water and nutrient uptake and impairing growth.

To detect, identify, and quantify plastics, MPs, and NPs, several approaches, particularly optical methods and chromatography coupled with mass spectrometry have been developed. The optical detection, based on manual or microscopic counting, chromatographic-based methods and scanning electron microscopy have been widely used, but cannot be considered as standard methods due to their multiple disadvantages. Spectroscopic

methods, specifically, Fourier-transform infrared spectroscopy and Raman spectroscopy, have been extensively suggested over the past few years as alternative techniques to achieve rapid and non-destructive measurements. However, spectroscopic techniques would not be a silver bullet as they still suffer from some limitations; e.g., the size of plastic particles must be higher than 10–20 μm and 1 μm to be detected and identified using Fourier-transform infrared spectroscopy and Raman spectroscopy, respectively (Silva et al., 2018). Therefore, more efforts are still needed to develop efficient, sensitive, and reliable detection, identification, and quantification methods. The ongoing development in hyperspectral imaging and the most recent trend of miniaturization and portability are promising strategies in this direction.

There is also a need for national and international authorities to provide stricter regulations so that plastic litter's impact on the environment and the food web could be better studied and managed. For instance, it can be recommended to include this new threat in food quality risk analysis and in the HACCP (Hazard Analysis and Critical Control Point) or HARPC (Hazard Analysis and Risk-based Preventive Controls for Human Food) programs for food safety. Promoting social awareness to reduce the consumption of single use plastics and increase the use of bio-plastics is one of the actions that should be encouraged. It is important to acquire greater insight into the MPs and NPs detection and their effects on food chain, both in terms of food safety and human health. Additionally, holistic approaches are needed and common standards and operating procedures for routine analysis should be implemented to address this global complex issue.

Finally, it is crucial to explore new technologies and innovative solutions that could contribute to solving the problem of plastics. Recently, a study published in Nature has opened up a new promising avenue, reporting on the possibility of breaking down plastics in days (instead of years), using an enzyme named FAST-PETase, acronymic for "functional, active, stable, and tolerant PETase" (Lu et al., 2022). This study is one example among others that show the significance of research and innovation to help solve the plastic dilemma.

References

Abbasi, S., et al. (2018). Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. *Chemosphere*, *205*, 80–87. https://doi.org/10.1016/j.chemosphere.2018.04.076.

Adika, S. A., et al. (2020). Microplastic ingestion by pelagic and demersal fish species from the Eastern Central Atlantic Ocean, off the Coast of Ghana. *Marine Pollution Bulletin*, *153*, 110998. https://doi.org/10.1016/J.MARPOLBUL.2020.110998.

Akhbarizadeh, R., et al. (2020). Abundance, composition, and potential intake of microplastics in canned fish. *Marine Pollution Bulletin*, *160*. https://doi.org/10.1016/j.marpolbul.2020.111633.

Akoueson, F., et al. (2021). Identification and quantification of plastic additives using pyrolysis-GC/MS: A review. *Science of the Total Environment*, *773*, 145073. https://doi.org/10.1016/j.scitotenv.2021.145073.

Alimi, O. S., et al. (2018). Microplastics and nanoplastics in aquatic environments: Aggregation, deposition, and enhanced contaminant transport. *Environmental Science & Technology*, *52*, 1704–1724. https://doi.org/10.1021/acs.est.7b05559.

Anastasopoulou, A., et al. (2018). Assessment on marine litter ingested by fish in the Adriatic and NE Ionian Sea macro-region (Mediterranean). *Marine Pollution Bulletin*, *133*, 841–851. https://doi.org/10.1016/J.MARPOLBUL.2018.06.050.

Anger, P. M., et al. (2018). Raman microspectroscopy as a tool for microplastic particle analysis. *TrAC Trends in Analytical Chemistry*, *109*, 214–226. https://doi.org/10.1016/j.trac.2018.10.010.

Araujo, C. F., et al. (2018). Identification of microplastics using Raman spectroscopy: Latest developments and future prospects. *Water Research*, *142*, 426–440. https://doi.org/10.1016/j.watres.2018.05.060.

Asamoah, B. O., et al. (2021). Towards the development of portable and in situ optical devices for detection of micro- and nanoplastics in water: A review on the current status. *Polymers*, *13*(5), 730. https://doi.org/10.3390/POLYM13050730.

Baalkhuyur, F. M., et al. (2020). Microplastics in fishes of commercial and ecological importance from the Western Arabian Gulf. *Marine Pollution Bulletin*, *152*, 110920. https://doi.org/10.1016/j.marpolbul.2020.110920.

Baechler, B. R., et al. (2020). Microplastic occurrence and effects in commercially harvested North American finfish and shellfish: Current knowledge and future directions. *Limnology and Oceanography Letters*, *5*(1), 113–136. https://doi.org/10.1002/lol2.10122.

Bai, C. L., et al. (2022). Microplastics: A review of analytical methods, occurrence and characteristics in food, and potential toxicities to biota. *Science of the Total Environment*, *806*, 150263. https://doi.org/10.1016/J.SCITOTENV.2021.150263.

Beer, S., et al. (2018). No increase in marine microplastic concentration over the last three decades—A case study from the Baltic Sea. *Science of the Total Environment*, *621*, 1272–1279. https://doi.org/10.1016/J.SCITOTENV.2017.10.101.

Bermúdez-Guzmán, L., et al. (2020). Microplastic ingestion by a herring *Opisthonema* sp. in the Pacific coast of Costa Rica. *Regional Studies in Marine Science*, *38*, 101367. https://doi.org/10.1016/j.rsma.2020.101367.

Białowąs, M., et al. (2022). Plastic in digestive tracts and gills of cod and herring from the Baltic Sea. *Science of the Total Environment*, *822*, 153333. https://doi.org/10.1016/J.SCITOTENV.2022.153333.

Bono, G., et al. (2020). Microplastics and alien black particles as contaminants of deep-water rose shrimp (*Parapenaeus longistroris* Lucas, 1846) in the Central Mediterranean Sea. *Journal of Advanced Biotechnology and Bioengineering*, *2*(8), 23–28.

Borges-Ramírez, M. M., et al. (2020). Plastic density as a key factor in the presence of microplastic in the gastrointestinal tract of commercial fishes from Campeche Bay, Mexico. *Environmental Pollution*, *267*, 115659. https://doi.org/10.1016/J.ENVPOL.2020.115659.

Bosker, T., et al. (2019). Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant *Lepidium sativum*. *Chemosphere*, *226*, 774–781. https://doi.org/10.1016/J.CHEMOSPHERE.2019.03.163.

Boyle, K., & Örmeci, B. (2020). Microplastics and nanoplastics in the freshwater and terrestrial environment: A review. *Water*. https://doi.org/10.3390/w12092633.

Bråte, I. L. N., et al. (2016). Plastic ingestion by Atlantic cod (*Gadus morhua*) from the Norwegian coast. *Marine Pollution Bulletin*, *112*(1–2), 105–110. https://doi.org/10.1016/J.MARPOLBUL.2016.08.034.

Bray, L., et al. (2019). Determining suitable fish to monitor plastic ingestion trends in the Mediterranean Sea. *Environmental Pollution*, *247*, 1071–1077. https://doi.org/10.1016/J.ENVPOL.2019.01.100.

Chagnon, C., et al. (2018). Plastic ingestion and trophic transfer between Easter Island flying fish (*Cheilopogon rapanouiensis*) and yellowfin tuna (*Thunnus albacares*) from Rapa Nui (Easter Island). *Environmental Pollution*, *243*, 127–133. https://doi.org/10.1016/J.ENVPOL.2018.08.042.

Chatterjee, S., & Sharma, S. (2019). Microplastics in our oceans and marine health. *Field ACTions Science Reports*, 54–61.

Chenet, T., et al. (2021). Plastic ingestion by Atlantic horse mackerel (*Trachurus trachurus*) from Central Mediterranean Sea: A potential cause for endocrine disruption. *Environmental Pollution*, *284*, 117449. https://doi.org/10.1016/J.ENVPOL.2021.117449.

Collard, F., et al. (2017). Morphology of the filtration apparatus of three planktivorous fishes and relation with ingested anthropogenic particles. *Marine Pollution Bulletin*, *116*(1–2), 182–191. https://doi.org/10.1016/J.MARPOLBUL.2016.12.067.

Collard, F., et al. (2018). Anthropogenic particles in the stomach contents and liver of the freshwater fish *Squalius cephalus*. *Science of the Total Environment*, *643*, 1257–1264. https://doi.org/10.1016/J.SCITOTENV.2018.06.313.

Compa, M., et al. (2018). Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. *Marine Pollution Bulletin*, *128*, 89–96. https://doi.org/10.1016/J.MARPOLBUL.2018.01.009.

Cordova, M. R., Riani, E., & Shiomoto, A. (2020). Microplastics ingestion by blue panchax fish (*Aplocheilus sp.*) from Ciliwung Estuary, Jakarta, Indonesia. *Marine Pollution Bulletin*, *161*, 111763. https://doi.org/10.1016/j.marpolbul.2020.111763.

Cowger, W., et al. (2020). Critical review of processing and classification techniques for images and spectra in microplastic research. *Applied Spectroscopy*, *74*(9), 989–1010. https://doi.org/10.1177/0003702820929064.

da Costa Filho, P. A., et al. (2021). Detection and characterization of small-sized microplastics (≥5 μm) in milk products. *Scientific Reports*, *11*, 24046. https://doi.org/10.1038/s41598-021-03458-7.

Dalla Fontana, G., Mossotti, R., & Montarsolo, A. (2020). Assessment of microplastics release from polyester fabrics: The impact of different washing conditions. *Environmental Pollution*, *264*, 113960. https://doi.org/10.1016/J.ENVPOL.2020.113960.

Daniel, D. B., et al. (2021). Microplastics in the edible tissues of shellfishes sold for human consumption. *Chemosphere*, *264*, 128554. https://doi.org/10.1016/j.chemosphere.2020.128554.

Danopoulos, E., et al. (2020). Microplastic contamination of salt intended for human consumption: A systematic review and meta-analysis. *SN Applied Sciences*. https://doi.org/10.1007/s42452-020-03749-0. (Preprint).

Dawson, A. L., et al. (2021). Relevance and reliability of evidence for microplastic contamination in seafood: A critical review using Australian consumption patterns as a case study. *Environmental Pollution*, *276*, 116684. https://doi.org/10.1016/j.envpol.2021.116684.

de Barros, M. S. F., dos Santos Calado, T. C., & de Sá Leitão Câmara de Araújo, M. (2020). Plastic ingestion lead to reduced body condition and modified diet patterns in the rocky shore crab *Pachygrapsus transversus* (Gibbes, 1850) (Brachyura: Grapsidae). *Marine Pollution Bulletin*, *156*, 111249. https://doi.org/10.1016/J.MARPOLBUL.2020.111249.

de Mesquita, G. C., et al. (2021). Feeding strategy of pelagic fishes caught in aggregated schools and vulnerability to ingesting anthropogenic items in the western equatorial Atlantic Ocean. *Environmental Pollution*, *282*, 117021. https://doi.org/10.1016/J.ENVPOL.2021.117021.

De Souza Machado, A. A., et al. (2019). Microplastics can change soil properties and affect plant performance. *Environmental Science and Technology*, *53*(10), 6044–6052. https://doi.org/10.1021/ACS.EST.9B01339/SUPPL_FILE/ES9B01339_SI_003.PDF.

de Vries, A. N., et al. (2020). Microplastic ingestion by fish: Body size, condition factor and gut fullness are not related to the amount of plastics consumed. *Marine Pollution Bulletin*, *151*, 110827. https://doi.org/10.1016/J.MARPOLBUL.2019.110827.

Dehaut, A., Hermabessiere, L., & Duflos, G. (2019). Current frontiers and recommendations for the study of microplastics in seafood. *TrAC Trends in Analytical Chemistry*, *116*, 346–359. https://doi.org/10.1016/j.trac.2018.11.011.

Dehaut, A., et al. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, *215*, 223–233. https://doi.org/10.1016/j.envpol.2016.05.018.

di Beneditto, A. P. M., & da Silva Oliveira, A. (2019). Debris ingestion by carnivorous consumers: Does the position in the water column truly matter? *Marine Pollution Bulletin*, *144*, 134–139. https://doi.org/10.1016/J.MARPOLBUL.2019.04.074.

Diaz-Basantes, M. F., Conesa, J. A., & Fullana, A. (2020). Microplastics in honey, beer, milk and refreshments in Ecuador as emerging contaminants. *Sustainability*, *12*(14), 5514. https://doi.org/10.3390/su12145514.

Digka, N., et al. (2018). Microplastics in mussels and fish from the Northern Ionian Sea. *Marine Pollution Bulletin*, *135*, 30–40. https://doi.org/10.1016/J.MARPOLBUL.2018.06.063.

Ding, J., et al. (2021). Microplastics in four bivalve species and basis for using bivalves as bioindicators of microplastic pollution. *Science of the Total Environment*, *782*, 146830. https://doi.org/10.1016/J.SCITOTENV.2021.146830.

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2016). Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA Journal*. https://doi.org/10.2903/j.efsa.2016.4501.

Fadare, O. O., Okoffo, E. D., & Olasehinde, E. F. (2021). Microparticles and microplastics contamination in African table salts. *Marine Pollution Bulletin*, *164*, 112006. https://doi.org/10.1016/J.MARPOLBUL.2021.112006.

FAO. (2020). *The state of world fisheries and aquaculture 2020*. FAO. https://doi.org/10.4060/ca9229en.

Feng, Z., et al. (2020). Microplastics in specific tissues of wild sea urchins along the coastal areas of northern China. *Science of the Total Environment*, *728*, 138660. https://doi.org/10.1016/j.scitotenv.2020.138660.

Fernández Severini, M. D., et al. (2020). Chemical composition and abundance of microplastics in the muscle of commercial shrimp *Pleoticus muelleri* at an impacted coastal environment (Southwestern Atlantic). *Marine Pollution Bulletin*, *161*, 111700. https://doi.org/10.1016/j.marpolbul.2020.111700.

Ferrante, M., et al. (2022). Microplastics in fillets of Mediterranean seafood. A risk assessment study. *Environmental Research*, *204*, 112247. https://doi.org/10.1016/J.ENVRES.2021.112247.

Ferreira, I., et al. (2019). Nanoplastics and marine organisms: What has been studied? *Environmental Toxicology and Pharmacology*, *67*, 1–7. https://doi.org/10.1016/J.ETAP.2019.01.006.

Filgueiras, A. V., et al. (2020). Microplastic ingestion by pelagic and benthic fish and diet composition: A case study in the NW Iberian shelf. *Marine Pollution Bulletin*, *160*, 111623. https://doi.org/10.1016/J.MARPOLBUL.2020.111623.

Fischer, M., & Scholz-Böttcher, B. M. (2017). Simultaneous trace identification and quantification of common types of microplastics in environmental samples by pyrolysis-gas chromatography–mass spectrometry. *Environmental Science & Technology*, *51*(9), 5052–5060. https://doi.org/10.1021/acs.est.6b06362.

Gao, M., et al. (2021). Effect of polystyrene on di-butyl phthalate (DBP) bioavailability and DBP-induced phytotoxicity in lettuce. *Environmental Pollution*, *268*, 115870. https://doi.org/10.1016/J.ENVPOL.2020.115870.

Garnier, Y., et al. (2019). Evaluation of microplastic ingestion by tropical fish from Moorea Island, French Polynesia. *Marine Pollution Bulletin*, *140*, 165–170. https://doi.org/10.1016/j.marpolbul.2019.01.038.

Ghattavi, K., Naji, A., & Kord, S. (2019). Investigation of microplastic contamination in the gastrointestinal tract of some species of caught fish from Oman Sea. *Iranian Journal of Health and Environment*, *12*(1), 141–150.

Gonçalves, J. M., & Bebianno, M. J. (2021). Nanoplastics impact on marine biota: A review. *Environmental Pollution*, *273*, 116426. https://doi.org/10.1016/J.ENVPOL.2021.116426.

Gong, J., & Xie, P. (2020). Research progress in sources, analytical methods, eco-environmental effects, and control measures of microplastics. *Chemosphere*, *254*, 126790. https://doi.org/10.1016/j.chemosphere.2020.126790.

Gong, Y., et al. (2021). Microplastics in different tissues of a pelagic squid (*Dosidicus gigas*) in the northern Humboldt current ecosystem. *Marine Pollution Bulletin*, *169*, 112509. https://doi.org/10.1016/J.MARPOLBUL.2021.112509.

Gündoğdu, S. (2018). Contamination of table salts from Turkey with microplastics. *Food Additives & Contaminants: Part A*, *35*(5), 1006–1014. https://doi.org/10.1080/19440049.2018.1447694.

Gündogdu, S., et al. (2022). The impact of nano/micro-plastics toxicity on seafood quality and human health: Facts and gaps. *Critical Reviews in Food Science and Nutrition*, 1–19. https://doi.org/10.1080/10408398.2022.2033684.

Guo, X., & Wang, J. (2019). Sorption of antibiotics onto aged microplastics in freshwater and seawater. *Marine Pollution Bulletin*, *149*, 110511. https://doi.org/10.1016/J.MARPOLBUL.2019.110511.

Güven, O., et al. (2017). Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. *Environmental Pollution*, *223*, 286–294. https://doi.org/10.1016/J.ENVPOL.2017.01.025.

Hartmann, N. B., et al. (2019). Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. *Environmental Science & Technology*, *53*(3), 1039–1047. https://doi.org/10.1021/acs.est.8b05297.

Hastuti, A. R., Lumbanbatu, D. T., & Wardiatno, Y. (2019). The presence of microplastics in the digestive tract of commercial fishes off Pantai Indah Kapuk coast, Jakarta, Indonesia. *Biodiversitas Journal of Biological Diversity*, *20*(5). https://doi.org/10.13057/biodiv/d200513.

Hazimah, N., et al. (2021). Lifetime accumulation of microplastic in children and adults. *Environmental Science & Technology*, *55*, 5096. https://doi.org/10.1021/acs.est.0c07384.

Hermabessiere, L., et al. (2018). Optimization, performance, and application of a pyrolysis-GC/MS method for the identification of microplastics. *Analytical and Bioanalytical Chemistry*, *410*(25), 6663–6676. https://doi.org/10.1007/s00216-018-1279-0.

Hermabessiere, L., et al. (2019). Microplastic contamination and pollutant levels in mussels and cockles collected along the channel coasts. *Environmental Pollution*, *250*, 807–819. https://doi.org/10.1016/j.envpol.2019.04.051.

Hossain, M. S., et al. (2019). Microplastics in fishes from the Northern Bay of Bengal. *Science of the Total Environment*, *690*, 821–830. https://doi.org/10.1016/J.SCITOTENV.2019.07.065.

Hossain, M. S., et al. (2020). Microplastic contamination in Penaeid shrimp from the Northern Bay of Bengal. *Chemosphere*, *238*, 124688. https://doi.org/10.1016/j.chemosphere.2019.124688.

Huang, Y., et al. (2020). Rapid measurement of microplastic contamination in chicken meat by mid infrared spectroscopy and chemometrics: A feasibility study. *Food Control*, *113*, 107187. https://doi.org/10.1016/J.FOODCONT.2020.107187.

Inderhaug, T. (2020). Stockfish production, cultural and culinary values. *Food Ethics*, *5*(1–2), 6. https://doi.org/10.1007/s41055-019-00060-6.

Iñiguez, M. E., Conesa, J. A., & Fullana, A. (2017). Microplastics in Spanish table salt OPEN. *Scientific Reports*, *7*(1), 1–7. https://doi.org/10.1038/s41598-017-09128-x.

Ivleva, N. P. (2021). Chemical analysis of microplastics and nanoplastics: Challenges, advanced methods, and perspectives. *Chemical Reviews*, *121*(19), 11886–11936. https://doi.org/10.1021/acs.chemrev.1c00178.

Jadhav, E. B., et al. (2021). Microplastics from food packaging: An overview of human consumption, health threats, and alternative solutions. *Environmental Nanotechnology, Monitoring & Management*, *16*, 100608. https://doi.org/10.1016/j.enmm.2021.100608.

James, K., et al. (2020). An assessment of microplastics in the ecosystem and selected commercially important fishes off Kochi, south eastern Arabian Sea, India. *Marine Pollution Bulletin*, *154*, 111027. https://doi.org/10.1016/J.MARPOLBUL.2020.111027.

James, K., et al. (2022). Microplastics in the environment and in commercially significant fishes of mud banks, an ephemeral ecosystem formed along the southwest coast of India. *Environmental Research*, *204*, 112351. https://doi.org/10.1016/J.ENVRES.2021.112351.

Järlskog, I., et al. (2020). Occurrence of tire and bitumen wear microplastics on urban streets and in sweeps and washwater. *Science of the Total Environment*, *729*, 138950. https://doi.org/10.1016/J.SCITOTENV.2020.138950.

Jonathan, M. P., et al. (2021). Evidences of microplastics in diverse fish species off the Western Coast of Pacific Ocean, Mexico. *Ocean and Coastal Management*, *204*, 105544. https://doi.org/10.1016/j.ocecoaman.2021.105544.

Jung, M. R., et al. (2018). Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Marine Pollution Bulletin*, *127*, 704–716. https://doi.org/10.1016/j.marpolbul.2017.12.061.

Kalaiselvan, K., et al. (2022). Environmental science and pollution research occurrence of microplastics in gastrointestinal tracts of planktivorous fish from the Thoothukudi region. *Environmental Science and Pollution Research*, *1*, 1–9. https://doi.org/10.1007/s11356-022-19033-0.

Käppler, A., et al. (2016). Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Analytical and Bioanalytical Chemistry*, *408*(29), 8377–8391. https://doi.org/10.1007/s00216-016-9956-3.

Karami, A., Golieskardi, A., Bin Ho, Y., et al. (2017). Microplastics in eviscerated flesh and excised organs of dried fish. *Scientific Reports*, 7(1), 5473. https://doi.org/10.1038/s41598-017-05828-6.

Karami, A., Golieskardi, A., Choo, C. K., et al. (2017). The presence of microplastics in commercial salts from different countries. *Scientific Reports*, 7(1), 1–11. https://doi.org/10.1038/srep46173.

Karbalaei, S., et al. (2019). Abundance and characteristics of microplastics in commercial marine fish from Malaysia. *Marine Pollution Bulletin*, *148*, 5–15. https://doi.org/10.1016/j.marpolbul.2019.07.072.

Karuppasamy, P. K., et al. (2020). Baseline survey of micro and mesoplastics in the gastro-intestinal tract of commercial fish from Southeast coast of the Bay of Bengal. *Marine Pollution Bulletin*, *153*, 110974. https://doi.org/10.1016/j.marpolbul.2020.110974.

Ke, A. Y., et al. (2019). Impacts of leachates from single-use polyethylene plastic bags on the early development of clam *Meretrix meretrix* (Bivalvia: Veneridae). *Marine Pollution Bulletin*, *142*, 54–57. https://doi.org/10.1016/J.MARPOLBUL.2019.03.029.

Kedzierski, M., et al. (2020). Microplastic contamination of packaged meat: Occurrence and associated risks. *Food Packaging and Shelf Life*, *24*, 100489. https://doi.org/10.1016/J.FPSL.2020.100489.

Kim, J.-S., et al. (2018). Global pattern of microplastics (MPs) in commercial food-grade salts: Sea salt as an indicator of seawater MP pollution. *Environmental Science & Technology*, *52*(21), 12819–12828. https://doi.org/10.1021/acs.est.8b04180.

Knight, L. J., et al. (2020). Tyre wear particles: An abundant yet widely unreported microplastic? *Environmental Science and Pollution Research*, *27*, 18345–18354. https://doi.org/10.1007/s11356-020-08187-4.

Koongolla, J. B., et al. (2020). Occurrence of microplastics in gastrointestinal tracts and gills of fish from Beibu Gulf, South China Sea. *Environmental Pollution*, *258*, 113734. https://doi.org/10.1016/j.envpol.2019.113734.

Kutralam-Muniasamy, G., et al. (2020). Branded milks—Are they immune from microplastics contamination? *Science of the Total Environment*, *714*, 136823. https://doi.org/10.1016/J.SCITOTENV.2020.136823.

Kutralam-Muniasamy, G., et al. (2021). Overview of microplastics pollution with heavy metals: Analytical methods, occurrence, transfer risks and call for standardization. *Journal of Hazardous Materials*, *415*, 125755. https://doi.org/10.1016/J.JHAZMAT.2021.125755.

Kwon, J.-H., et al. (2020). Microplastics in food: A review on analytical methods and challenges. *International Journal of Environmental Research and Public Health*, *17*(18), 6710. https://doi.org/10.3390/ijerph17186710.

la Nasa, J., et al. (2020). A review on challenges and developments of analytical pyrolysis and other thermoanalytical techniques for the quali-quantitative determination of microplastics. *Journal of Analytical and Applied Pyrolysis*, *149*, 104841. https://doi.org/10.1016/j.jaap.2020.104841.

Lakshmi Kavya, A. N. V., Sundarrajan, S., & Ramakrishna, S. (2020). Identification and characterization of micro-plastics in the marine environment: A mini review. *Marine Pollution Bulletin*, *160*, 111704. https://doi.org/10.1016/j.marpolbul.2020.111704.

Li, J., et al. (2019). Using mussel as a global bioindicator of coastal microplastic pollution. *Environmental Pollution*, *244*, 522–533. https://doi.org/10.1016/j.envpol.2018.10.032.

Li, Q., et al. (2021a). Microplastics in shellfish and implications for food safety. *Current Opinion in Food Science*, *40*, 192–197. https://doi.org/10.1016/j.cofs.2021.04.017.

Li, Y., et al. (2021b). Microplastics environmental effect and risk assessment on the aquaculture systems from South China. *International Journal of Environmental Research and Public Health*, *18*(4), 1869. https://doi.org/10.3390/ijerph18041869.

Lian, J., et al. (2020). Impact of polystyrene nanoplastics (PSNPs) on seed germination and seedling growth of wheat (*Triticum aestivum L.*). *Journal of Hazardous Materials*, *385*, 121620. https://doi.org/10.1016/J.JHAZMAT.2019.121620.

Liboiron, M., et al. (2019). Low incidence of plastic ingestion among three fish species significant for human consumption on the island of Newfoundland, Canada. *Marine Pollution Bulletin*, *141*, 244–248. https://doi.org/10.1016/J.MARPOLBUL.2019.02.057.

Liebezeit, G., & Liebezeit, E. (2014). Synthetic particles as contaminants in German beers. *Food Additives & Contaminants: Part A*, *31*(9), 1574–1578. https://doi.org/10.1080/19440049.2014.945099.

Löder, M. G. J., & Gerdts, G. (2015). Methodology used for the detection and identification of microplastics—A critical appraisal. In *Marine anthropogenic litter* (pp. 201–227). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-16510-3_8.

Lopes, C., et al. (2020). Microplastic ingestion and diet composition of planktivorous fish. *Limnology and Oceanography Letters*, *5*, 103–112. https://doi.org/10.1002/lol2.10144.

López-López, L., et al. (2018). Incidental ingestion of meso- and macro-plastic debris by benthic and demersal fish. *Food Webs*, *14*, 1–4. https://doi.org/10.1016/J.FOOWEB.2017.12.002.

López-Martínez, S., et al. (2021). Overview of global status of plastic presence in marine vertebrates. *Global Change Biology*, *27*(4), 728–737. https://doi.org/10.1111/gcb.15416.
Lu, H., et al. (2022). Machine learning-aided engineering of hydrolases for PET depolymerization. *Nature*, *604*(7907), 662–667. https://doi.org/10.1038/s41586-022-04599-z.
Lv, L., et al. (2021). Challenge for the detection of microplastics in the environment. *Water Environment Research*, *93*(1), 5–15. https://doi.org/10.1002/wer.1281.
Lwanga, E. H., et al. (2017). Field evidence for transfer of plastic debris along a terrestrial food chain. *Scientific Reports*, *7*(1), 1–7. https://doi.org/10.1038/s41598-017-14588-2.
Mancia, A., et al. (2020). Adverse effects of plastic ingestion on the Mediterranean small-spotted catshark (*Scyliorhinus canicula*). *Marine Environmental Research*, *155*. https://doi.org/10.1016/j.marenvres.2020.104876.
Markic, A., et al. (2018). Double trouble in the South Pacific subtropical gyre: Increased plastic ingestion by fish in the oceanic accumulation zone. *Marine Pollution Bulletin*, *136*, 547–564. https://doi.org/10.1016/J.MARPOLBUL.2018.09.031.
Martinez-Tavera, E., et al. (2021). Microplastics and metal burdens in freshwater Tilapia (*Oreochromis niloticus*) of a metropolitan reservoir in Central Mexico: Potential threats for human health. *Chemosphere*, *266*, 128968. https://doi.org/10.1016/j.chemosphere.2020.128968.
Masry, M., et al. (2021). Characteristics, fate, and impact of marine plastic debris exposed to sunlight: A review. *Marine Pollution Bulletin*, *171*, 112701. https://doi.org/10.1016/J.MARPOLBUL.2021.112701.
McGoran, A. R., et al. (2020). High prevalence of plastic ingestion by *Eriocheir sinensis* and *Carcinus maenas* (Crustacea: Decapoda: Brachyura) in the Thames Estuary. *Environmental Pollution*, *265*, 114972. https://doi.org/10.1016/J.ENVPOL.2020.114972.
Mercogliano, R., et al. (2020). Occurrence of microplastics in commercial seafood under the perspective of the human food chain. A review. *Journal of Agricultural and Food Chemistry*, *68*(19), 5296–5301. https://doi.org/10.1021/acs.jafc.0c01209.
Merga, L. B., et al. (2020). Distribution of microplastic and small macroplastic particles across four fish species and sediment in an African lake. *Science of the Total Environment*, *741*, 140527. https://doi.org/10.1016/j.scitotenv.2020.140527.
Mistri, M., et al. (2022). Microplastic accumulation in commercial fish from the Adriatic Sea. *Marine Pollution Bulletin*, *174*, 113279. https://doi.org/10.1016/J.MARPOLBUL.2021.113279.
Murphy, F., et al. (2017). The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland. *Marine Pollution Bulletin*, *122*(1–2), 353–359. https://doi.org/10.1016/J.MARPOLBUL.2017.06.073.
Murray, F., & Cowie, P. R. (2011). Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). *Marine Pollution Bulletin*, *62*(6), 1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032.
Neto, J. G. B., et al. (2020). Ingestion of plastic debris by commercially important marine fish in southeast-south Brazil. *Environmental Pollution*, *267*, 115508. https://doi.org/10.1016/J.ENVPOL.2020.115508.
Neves, D., et al. (2015). Ingestion of microplastics by commercial fish off the Portuguese coast. *Marine Pollution Bulletin*, *101*(1), 119–126. https://doi.org/10.1016/J.MARPOLBUL.2015.11.008.
Nguyen, B., et al. (2019). Separation and analysis of microplastics and nanoplastics in complex environmental samples. *Accounts of Chemical Research*, *52*(4), 858–866. https://doi.org/10.1021/acs.accounts.8b00602.
Ohkubo, N., et al. (2022). Microplastic uptake and gut retention time in Japanese anchovy (*Engraulis japonicus*) under laboratory conditions. *Marine Pollution Bulletin*, *176*, 113433. https://doi.org/10.1016/J.MARPOLBUL.2022.113433.

Oliveira, A. R., et al. (2020). Microplastics presence in cultured and wild-caught cuttlefish, *Sepia officinalis*. *Marine Pollution Bulletin*, *160*, 111553. https://doi.org/10.1016/J.MARPOLBUL.2020.111553.

Oliveri Conti, G., et al. (2020). Micro- and nano-plastics in edible fruit and vegetables. The first diet risks assessment for the general population. *Environmental Research*, *187*, 109677. https://doi.org/10.1016/j.envres.2020.109677.

Ory, N. C., et al. (2017). Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. *Science of the Total Environment*, *586*, 430–437. https://doi.org/10.1016/J.SCITOTENV.2017.01.175.

Ory, N., et al. (2018). Low prevalence of microplastic contamination in planktivorous fish species from the southeast Pacific Ocean. *Marine Pollution Bulletin*, *127*, 211–216. https://doi.org/10.1016/J.MARPOLBUL.2017.12.016.

Palazzo, L., et al. (2021). A novel approach based on multiple fish species and water column compartments in assessing vertical microlitter distribution and composition. *Environmental Pollution*, *272*, 116419. https://doi.org/10.1016/J.ENVPOL.2020.116419.

Palermo, J. D. H., et al. (2020). Susceptibility of Sardinella lemuru to emerging marine microplastic pollution. *Global Journal of Environmental Science and Management*, *6*(3), 373–384.

Panebianco, A., et al. (2019). First discoveries of microplastics in terrestrial snails. *Food Control*, *106*, 106722. https://doi.org/10.1016/J.FOODCONT.2019.106722.

Pegado, T., et al. (2018). First evidence of microplastic ingestion by fishes from the Amazon River estuary. *Marine Pollution Bulletin*, *133*, 814–821. https://doi.org/10.1016/J.MARPOLBUL.2018.06.035.

Peñalver, R., et al. (2020). An overview of microplastics characterization by thermal analysis. *Chemosphere*, *242*, 125170. https://doi.org/10.1016/j.chemosphere.2019.125170.

Pereira, J. M., et al. (2020). Microplastic in the stomachs of open-ocean and deep-sea fishes of the North-East Atlantic. *Environmental Pollution*, *265*, 115060. https://doi.org/10.1016/J.ENVPOL.2020.115060.

Peters, C. A., et al. (2018). Pyr-GC/MS analysis of microplastics extracted from the stomach content of benthivore fish from the Texas Gulf Coast. *Marine Pollution Bulletin*, *137*, 91–95. https://doi.org/10.1016/j.marpolbul.2018.09.049.

Pinto da Costa, J., et al. (2019). Micro (nano)plastics—Analytical challenges towards risk evaluation. *TrAC Trends in Analytical Chemistry*, *111*, 173–184. https://doi.org/10.1016/j.trac.2018.12.013.

Pittura, L. *et al.* (2018) Less Plastic More Mediterranean, 2017 Campaign on board the Rainbow Warrior Greenpeace Ship.

Piyawardhana, N., et al. (2022). Occurrence of microplastics in commercial marine dried fish in Asian countries. *Journal of Hazardous Materials*, *423*, 127093. https://doi.org/10.1016/J.JHAZMAT.2021.127093.

Prata, J. C., et al. (2020). Identification of microplastics in white wines capped with polyethylene stoppers using micro-Raman spectroscopy. *Food Chemistry*, *331*, 127323. https://doi.org/10.1016/J.FOODCHEM.2020.127323.

Primpke, S., et al. (2020). Critical assessment of analytical methods for the harmonized and cost-efficient analysis of microplastics. *Applied Spectroscopy*, *74*(9), 1012–1047. https://doi.org/10.1177/0003702820921465.

Qi, Y., et al. (2018). Macro- and microplastics in soil-plant system: Effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth. *Science of the Total Environment*, *645*, 1048–1056. https://doi.org/10.1016/J.SCITOTENV.2018.07.229.

Ragusa, A., et al. (2021). Plasticenta: First evidence of microplastics in human placenta. *Environment International*, *146*, 106274. https://doi.org/10.1016/J.ENVINT.2020.106274.

Razeghi, N., et al. (2021). Scientific studies on microplastics pollution in Iran: An in-depth review of the published articles. *Marine Pollution Bulletin*, *162*, 111901. https://doi.org/10.1016/j.marpolbul.2020.111901.

Reinold, S., et al. (2021). Evidence of microplastic ingestion by cultured European sea bass (*Dicentrarchus labrax*). *Marine Pollution Bulletin*, *168*, 112450. https://doi.org/10.1016/J.MARPOLBUL.2021.112450.

Renner, G., Schmidt, T. C., & Schram, J. (2018). Analytical methodologies for monitoring micro (nano)plastics: Which are fit for purpose? *Current Opinion in Environmental Science & Health*, *1*, 55–61. https://doi.org/10.1016/J.COESH.2017.11.001.

Renner, G., et al. (2019). Data preprocessing & evaluation used in the microplastics identification process: A critical review & practical guide. *TrAC Trends in Analytical Chemistry*, *111*, 229–238. https://doi.org/10.1016/j.trac.2018.12.004.

Renzi, M., & Blašković, A. (2018). Litter & microplastics features in table salts from marine origin: Italian versus Croatian brands. *Marine Pollution Bulletin*, *135*, 62–68. https://doi.org/10.1016/J.MARPOLBUL.2018.06.065.

Renzi, M., Grazioli, E., et al. (2019). Microparticles in table salt: Levels and chemical composition of the smallest dimensional fraction. *Journal of Marine Science and Engineering*, *7*(9), 310. https://doi.org/10.3390/jmse7090310.

Renzi, M., Specchiulli, A., et al. (2019). Marine litter in stomach content of small pelagic fishes from the Adriatic Sea: sardines (*Sardina pilchardus*) and anchovies (*Engraulis encrasicolus*). *Environmental Science and Pollution Research International*, *26*(3), 2771–2781. https://doi.org/10.1007/s11356-018-3762-8.

Renzi, M., et al. (2020). Litter in alien species of possible commercial interest: The blue crab (*Callinectes sapidus* Rathbun, 1896) as case study. *Marine Pollution Bulletin*, *157*, 111300. https://doi.org/10.1016/J.MARPOLBUL.2020.111300.

Ribeiro, F., et al. (2020). Quantitative analysis of selected plastics in high-commercial-value Australian seafood by pyrolysis gas chromatography mass spectrometry. *Environmental Science & Technology*, *54*, 9408–9417. https://doi.org/10.1021/acs.est.0c02337.

Ridwan Lessy, M., & Sabar, M. (2021). Microplastics ingestion by Skipjack tuna (*Katsuwonus pelamis*) in ternate, North Maluku-Indonesia. In *IOP Conference Series: Materials Science and Engineering*. https://doi.org/10.1088/1757-899X/1125/1/012085. (Preprint).

Rios-Fuster, B., et al. (2019). Anthropogenic particles ingestion in fish species from two areas of the western Mediterranean Sea. *Marine Pollution Bulletin*, *144*, 325–333. https://doi.org/10.1016/J.MARPOLBUL.2019.04.064.

Roch, S., Friedrich, C., & Brinker, A. (2020). Uptake routes of microplastics in fishes: Practical and theoretical approaches to test existing theories. *Scientific Reports*, *10*, 3896. https://doi.org/10.1038/s41598-020-60630-1.

Rochman, C. M., et al. (2015). Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Scientific Reports*, *5*, 14340. https://doi.org/10.1038/srep14340.

Rosal, R. (2021). Morphological description of microplastic particles for environmental fate studies. *Marine Pollution Bulletin*, *171*, 112716. https://doi.org/10.1016/J.MARPOLBUL.2021.112716.

Ryberg, M. W., et al. (2019). Global environmental losses of plastics across their value chains. *Resources, Conservation and Recycling*, *151*, 104459. https://doi.org/10.1016/J.RESCONREC.2019.104459.

Saha, M., et al. (2021). Microplastics in seafood as an emerging threat to marine environment: A case study in Goa, west coast of India. *Chemosphere*, *270*, 129359. https://doi.org/10.1016/j.chemosphere.2020.129359.

Saturno, J., et al. (2020). Occurrence of plastics ingested by Atlantic cod (*Gadus morhua*) destined for human consumption (Fogo Island, Newfoundland and Labrador). *Marine Pollution Bulletin*, *153*, 110993. https://doi.org/10.1016/J.MARPOLBUL.2020.110993.

Savoca, M. S., McInturf, A. G., & Hazen, E. L. (2021). Plastic ingestion by marine fish is widespread and increasing. *Global Change Biology*, *27*(10), 2188–2199. https://doi.org/10.1111/gcb.15533.

Savoca, S., et al. (2020). Plastics occurrence in juveniles of *Engraulis encrasicolus* and *Sardina pilchardus* in the Southern Tyrrhenian Sea. *Science of the Total Environment*, *718*, 137457. https://doi.org/10.1016/J.SCITOTENV.2020.137457.

Schwaferts, C., et al. (2019). Methods for the analysis of submicrometer- and nanoplastic particles in the environment. *TrAC Trends in Analytical Chemistry*, *112*, 52–65. https://doi.org/10.1016/j.trac.2018.12.014.

Seth, C. K., & Shriwastav, A. (2018). Contamination of Indian sea salts with microplastics and a potential prevention strategy. *Environmental Science and Pollution Research*, *25*(30), 30122–30131. https://doi.org/10.1007/S11356-018-3028-5.

Shan, J., et al. (2019). Simple and rapid detection of microplastics in seawater using hyperspectral imaging technology. *Analytica Chimica Acta*, *1050*, 161–168. https://doi.org/10.1016/j.aca.2018.11.008.

Shim, W. J., Hong, S. H., & Eo, S. E. (2017). Identification methods in microplastic analysis: A review. *Analytical Methods*, *9*(9), 1384–1391. https://doi.org/10.1039/C6AY02558G.

Shruti, V. C., et al. (2020). First study of its kind on the microplastic contamination of soft drinks, cold tea and energy drinks—Future research and environmental considerations. *Science of the Total Environment*, *726*, 138580. https://doi.org/10.1016/J.SCITOTENV.2020.138580.

Sid, S., et al. (2021). Bio-sourced polymers as alternatives to conventional food packaging materials: A review. *Trends in Food Science & Technology*, *115*, 87–104. https://doi.org/10.1016/j.tifs.2021.06.026.

Silva, A. B., et al. (2018). Microplastics in the environment: Challenges in analytical chemistry—A review. *Analytica Chimica Acta*, *1017*, 1–19. https://doi.org/10.1016/j.aca.2018.02.043.

Silva, G. C., et al. (2021). Microplastics and their effect in horticultural crops: Food safety and plant stress. *Agronomy*, *11*(8), 1528. https://doi.org/10.3390/agronomy11081528.

Sobhani, Z., et al. (2020). Microplastics generated when opening plastic packaging. *Scientific Reports*, *10*(1), 1–7. https://doi.org/10.1038/s41598-020-61146-4.

Sol, D., et al. (2020). Approaching the environmental problem of microplastics: Importance of WWTP treatments. *Science of the Total Environment*, *740*, 140016. https://doi.org/10.1016/J.SCITOTENV.2020.140016.

Šourková, M., Adamcová, D., & Vaverková, M. D. (2021). The influence of microplastics from ground Tyres on the acute, subchronical toxicity and microbial respiration of soil. *Environments*, *8*(11), 128. https://doi.org/10.3390/environments8110128.

Strungaru, S.-A., et al. (2019). Micro- (nano) plastics in freshwater ecosystems: Abundance, toxicological impact and quantification methodology. *TrAC Trends in Analytical Chemistry*, *110*, 116–128. https://doi.org/10.1016/j.trac.2018.10.025.

Su, L., et al. (2019). The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of East China. *Journal of Hazardous Materials*, *365*, 716–724. https://doi.org/10.1016/j.jhazmat.2018.11.024.

Sun, Q., Ren, S. Y., & Ni, H. G. (2020). Incidence of microplastics in personal care products: An appreciable part of plastic pollution. *Science of the Total Environment*, *742*, 140218. https://doi.org/10.1016/J.SCITOTENV.2020.140218.

Sun, X., et al. (2019). Characteristics and retention of microplastics in the digestive tracts of fish from the Yellow Sea. *Environmental Pollution*, *249*, 878–885. https://doi.org/10.1016/J.ENVPOL.2019.01.110.

Süssmann, J., et al. (2021). Evaluation and optimisation of sample preparation protocols suitable for the analysis of plastic particles present in seafood. *Food Control*, *125*, 107969. https://doi.org/10.1016/j.foodcont.2021.107969.

Suwartiningsih, N., Setyowati, I., & Astuti, R. (2020). Microplastics in pelagic and demersal fishes of Pantai Baron, Yogyakarta, Indonesia. *JurnalBiodjati*, *5*(1), 33–49.

TaghizadehRahmat Abadi, Z., et al. (2021). Microplastic content of Kutum fish, *Rutilus frisii kutum* in the southern Caspian Sea. *Science of the Total Environment*, *752*, 141542. https://doi.org/10.1016/j.scitotenv.2020.141542.

Tanaka, K., & Takada, H. (2016). Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. *Scientific Reports*. https://doi.org/10.1038/srep34351.

Tourinho, P. S., et al. (2019). Partitioning of chemical contaminants to microplastics: Sorption mechanisms, environmental distribution and effects on toxicity and bioaccumulation. *Environmental Pollution*, *252*, 1246–1256. https://doi.org/10.1016/J.ENVPOL.2019.06.030.

Tsangaris, C., et al. (2020). Using *Boops boops* (osteichthyes) to assess microplastic ingestion in the Mediterranean Sea. *Marine Pollution Bulletin*, *158*, 111397. https://doi.org/10.1016/J.MARPOLBUL.2020.111397.

Turner, A. (2021). Paint particles in the marine environment: An overlooked component of microplastics. *Water Research X*, *12*, 100110. https://doi.org/10.1016/J.WROA.2021.100110.

Urbina, M. A., et al. (2020). Adsorption of polyethylene microbeads and physiological effects on hydroponic maize. *Science of the Total Environment*, *741*, 140216. https://doi.org/10.1016/J.SCITOTENV.2020.140216.

Valencia-Castañeda, G., et al. (2022). Microplastics in the tissues of commercial semi-intensive shrimp pond-farmed *Litopenaeus vannamei* from the Gulf of California ecoregion. *Chemosphere*, *297*, 134194. https://doi.org/10.1016/J.CHEMOSPHERE.2022.134194.

van Raamsdonk, L. W. D., et al. (2020). Current insights into monitoring, bioaccumulation, and potential health effects of microplastics present in the food chain. *Food*, *9*(1), 72. https://doi.org/10.3390/foods9010072.

van Weert, S., et al. (2019). Effects of nanoplastics and microplastics on the growth of sediment-rooted macrophytes. *Science of the Total Environment*, *654*, 1040–1047. https://doi.org/10.1016/J.SCITOTENV.2018.11.183.

Vanapalli, K. R., et al. (2021). Challenges and strategies for effective plastic waste management during and post COVID-19 pandemic. *Science of the Total Environment*, *750*, 141514. https://doi.org/10.1016/j.scitotenv.2020.141514.

Vázquez-Rowe, I., Ita-Nagy, D., & Kahhat, R. (2021). Microplastics in fisheries and aquaculture: Implications to food sustainability and safety. *Current Opinion in Green and Sustainable Chemistry*, *29*, 100464. https://doi.org/10.1016/j.cogsc.2021.100464.

Veerasingam, S., et al. (2021). Contributions of Fourier transform infrared spectroscopy in microplastic pollution research: A review. *Critical Reviews in Environmental Science and Technology*, *51*(22), 2681–2743. https://doi.org/10.1080/10643389.2020.1807450.

Venâncio, C., et al. (2019). The effects of nanoplastics on marine plankton: A case study with polymethylmethacrylate. *Ecotoxicology and Environmental Safety*, *184*, 109632. https://doi.org/10.1016/J.ECOENV.2019.109632.

Vinay Kumar, B. N., et al. (2021). Analysis of microplastics of a broad size range in commercially important mussels by combining FTIR and Raman spectroscopy approaches. *Environmental Pollution*, *269*, 116147. https://doi.org/10.1016/j.envpol.2020.116147.

Vitali, C., et al. (2022). Microplastics and nanoplastics in food, water, and beverages; part I. Occurrence. *TrAC Trends in Analytical Chemistry*, 116670. https://doi.org/10.1016/J.TRAC.2022.116670.

Walkinshaw, C., et al. (2020). Microplastics and seafood: Lower trophic organisms at highest risk of contamination. *Ecotoxicology and Environmental Safety*, *190*, 110066. https://doi.org/10.1016/J.ECOENV.2019.110066.

Wang, W., & Wang, J. (2018). Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis. *TrAC Trends in Analytical Chemistry*, *108*, 195–202. https://doi.org/10.1016/j.trac.2018.08.026.

Wang, Z.-M., et al. (2017). SEM/EDS and optical microscopy analyses of microplastics in ocean trawl and fish guts. *Science of the Total Environment*, *603–604*, 616–626. https://doi.org/10.1016/j.scitotenv.2017.06.047.

Wang, D., et al. (2021a). Quantitative and qualitative determination of microplastics in oyster, seawater and sediment from the coastal areas in Zhuhai, China. *Marine Pollution Bulletin*, *164*, 112000. https://doi.org/10.1016/j.marpolbul.2021.112000.

Wang, F., et al. (2021b). Microplastic characteristics in organisms of different trophic levels from Liaohe Estuary, China. *Science of the Total Environment*, *789*, 148027. https://doi.org/10.1016/J.SCITOTENV.2021.148027.

Wu, R. T., et al. (2021). Occurrence of microplastic in livestock and poultry manure in South China. *Environmental Pollution*, *277*, 116790. https://doi.org/10.1016/J.ENVPOL.2021.116790.

Xu, J.-L., et al. (2019). FTIR and Raman imaging for microplastics analysis: State of the art, challenges and prospects. *TrAC Trends in Analytical Chemistry*, *119*, 115629. https://doi.org/10.1016/j.trac.2019.115629.

Yagi, M., et al. (2022). Microplastic pollution of commercial fishes from coastal and offshore waters in southwestern Japan. *Marine Pollution Bulletin*, *174*, 113304. https://doi.org/10.1016/J.MARPOLBUL.2021.113304.

Yakovenko, N., Carvalho, A., & ter Halle, A. (2020). Emerging use thermo-analytical method coupled with mass spectrometry for the quantification of micro (nano)plastics in environmental samples. *TrAC Trends in Analytical Chemistry*, *131*, 115979. https://doi.org/10.1016/j.trac.2020.115979.

Yang, D., et al. (2015). Microplastic pollution in table salts from China. *Environmental Science and Technology*, *49*(22), 13622–13627. https://doi.org/10.1021/ACS.EST.5B03163/SUPPL_FILE/ES5B03163_SI_001.PDF.

Yuan, Z., Nag, R., & Cummins, E. (2022). Human health concerns regarding microplastics in the aquatic environment—From marine to food systems. *Science of the Total Environment*, *823*, 153730. https://doi.org/10.1016/j.scitotenv.2022.153730.

Yuan, W., et al. (2019). Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. *Ecotoxicology and Environmental Safety*, *170*, 180–187. https://doi.org/10.1016/j.ecoenv.2018.11.126.

ZabihzadehKhajavi, M., et al. (2019). Strategies for controlling release of plastic compounds into foodstuffs based on application of nanoparticles and its potential health issues. *Trends in Food Science & Technology*, *90*, 1–12. https://doi.org/10.1016/j.tifs.2019.05.009.

Zeytin, S., et al. (2020). Quantifying microplastic translocation from feed to the fillet in European sea bass *Dicentrarchus labrax*. *Marine Pollution Bulletin*, *156*, 111210. https://doi.org/10.1016/j.marpolbul.2020.111210.

Zhang, Y., et al. (2019). Hyperspectral imaging based method for rapid detection of microplastics in the intestinal tracts of fish. *Environmental Science & Technology*, *53*(9), 5151–5158. https://doi.org/10.1021/acs.est.8b07321.

Zhang, T., et al. (2021). Microplastics in different tissues of wild crabs at three important fishing grounds in China. *Chemosphere*, *271*, 129479. https://doi.org/10.1016/J.CHEMOSPHERE.2020.129479.

Zhu, J., et al. (2019). Microplastic pollution in the Maowei Sea, a typical mariculture bay of China. *Science of the Total Environment*, *658*, 62–68. https://doi.org/10.1016/j.scitotenv.2018.12.192.

Zhu, J., & Wang, C. (2020). Recent advances in the analysis methodologies for microplastics in aquatic organisms: Current knowledge and research challenges. *Analytical Methods*, *12*(23), 2944–2957. https://doi.org/10.1039/D0AY00143K.

Zitouni, N., et al. (2020). First report on the presence of small microplastics ($\leq$3 μm) in tissue of the commercial fish *Serranus scriba* (Linnaeus. 1758) from Tunisian coasts and associated cellular alterations. *Environmental Pollution*, *263*, 114576. https://doi.org/10.1016/j.envpol.2020.114576.

Investigating the presence of microplastics in the *Raja clavata* species

"The study described below has been completed and a manuscript is currently being prepared for possible scientific publication"

1. Laboratory activity

Laboratory cleaning procedures

Before starting work, laboratory air was purified using a HEPA-H14 filter. Air filtration took place during all activities (Fig. 1.1).

Figure 1.1 – Air Purifier with an H14 HEPA filter

Pure water (filtered to 0,01 micron) was used to clean all laboratory surfaces and instruments and to rinse specimens and laboratory tools (Fig.1.2).

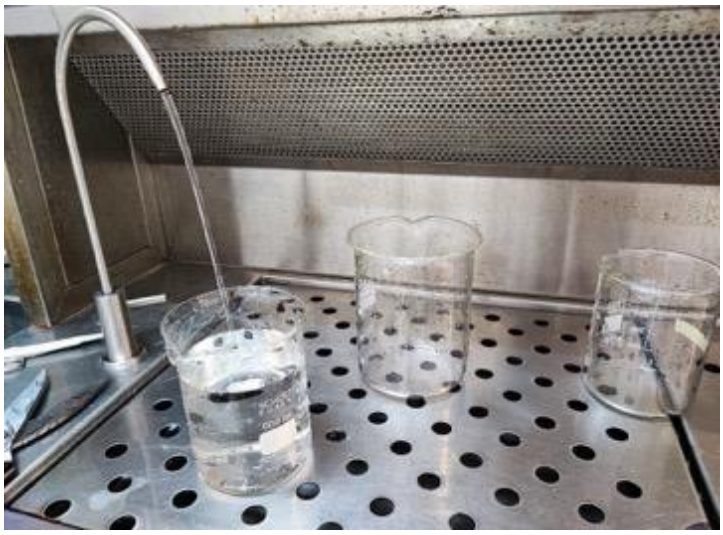

Figure 1.2 – Water filter

Although an air filtration system was used, microplastic fallout in the lab environment was assessed and sampled during laboratory activities. Every 5 specimens, environmental fallout

was caught in a Becher with 250 ml of pure water. The sample was then placed in a glass bottle capped with a pure cork stopper (Fig. 1.3).

Figure 1.3 – Water control samples

Specimen processing

Biometric and biological data (total length, disc width, sex, gonadal maturity, total body weight, total liver weight, total gastrointestinal tract (GIT) weight, total gonad weight, muscle weight, catch, etc.) were collected (Figs. 1.4-1.8). All data were then summarized in a table for further use (Fig. 1.9).

Figure 1.4 –*Raja clavata* specimens

(with number of haul)

Each specimen was rinsed with pure water to remove any contaminants (Fig.1.5).

Figure 1.5 – Rinsing samples with pure water.

The total length, disc width and total weight of each specimen was recorded. Each specimen was dissected along the abdomen (Fig.1.6).

Figure 1.6 – Dissecting the abdomen

The liver (Fig. 1.7), gastrointestinal tract (GIT) and muscle were removed from each specimen (Fig. 1.8). Each part was rinsed with pure water before weighing.

Figure 1.7 – Removing the liver from the *Raja Clavata* specimen and measuring its weight

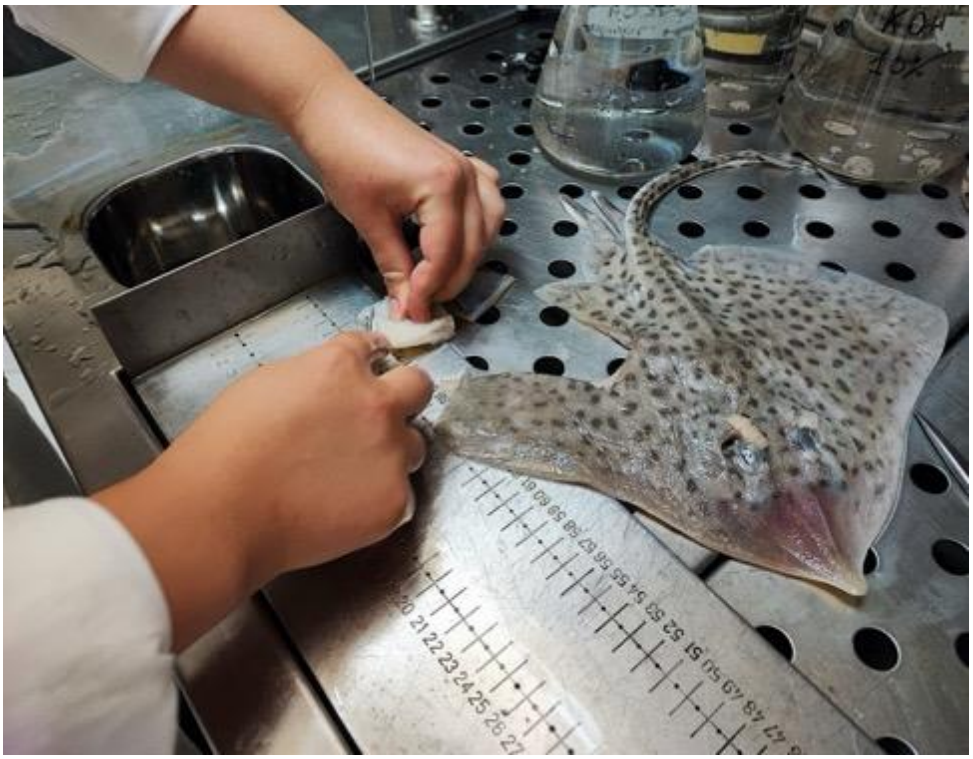

Figure 1.8 –Removing the muscle

SPECIES: ***Raja clavata***

GIT: gastrointestinal tract; GON: Oviger capules-gonads; MUS: Muscles; LIV : Liver

ID number of specimen	Total Length (cm)	Disc Length (cm)	SEX	MAT	Total Weight (g)	Total Weigth of liver (g)	Total Weigth of gastro-intestinal tract (g)	Total Weight of gonads or egg capsules (g)	Weight of muscle (g)	Date	fishing haul	Note
1	60,5	40	M	MAT	1228	56,7	32	4,2	17,4	20.6.2022	21	
2	59	40	M	MAT	1049	31,6	44,8	1,6	14,2	20.6.2022	21	
3	52,5	35	F	NOT MAT	737	24,3	33,5	-	25,5	20.6.2022	21	
4	49,5	30	M	MAT	411	14,9	15,5	1	13,9	20.6.2022	21	
5	44	28	M	NOT MAT	317	9,2	16,6	-	14,3	20.6.2022	21	CONTROL 1 H2O
6	32,5	20,5	M	NOT MAT	130	2,9	6,3	-	2,1	20.6.2022	21	
7	40,5	27	F	NOT MAT	263	5	10,2	-	7,4	20.6.2022	21	

Figure 1.9 – Collection of biometric and biological data

Lastly, each sample was quickly packed in aluminum foil to minimize plastic contamination. It was then labelled and stored at -80 °C until spectroscopic analysis at the NORCE Institute (Norway).

2. Laboratory activity at the NORCE INSTITUTE, Norway

Analyses focused on removing as much organic material as possible from the sample (Fig. 2.1). Special care was taken in the laboratory to avoid the loss of any microplastic contaminants in the sample. To this end, an ultrasonic cleaner was used after each filtration.

Figure 2.1 – Accumulation of organic material during the first filtration

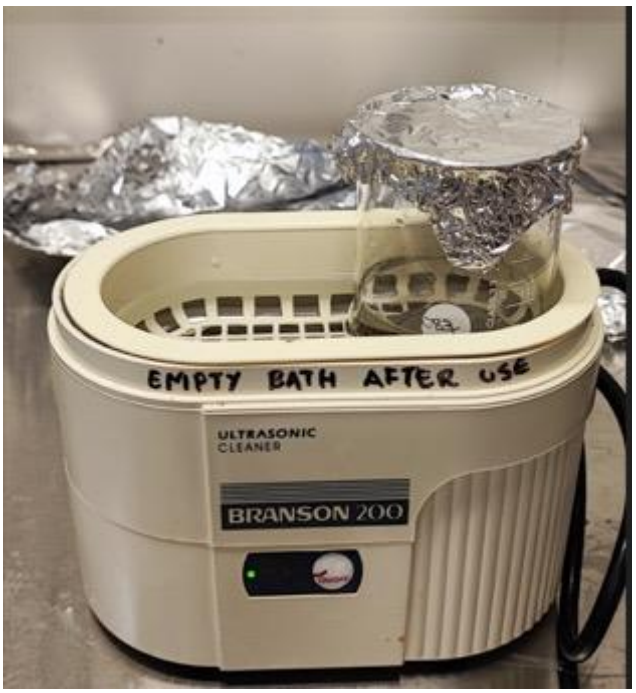

Figure 2.2 – Ultrasonic cleaning

First filtration

Addition of sodium dodecyl sulfonate (SDS) to cover samples, which were placed in the oven at +50°C for 24-48h + filtering of samples + ultrasonic cleaning (Fig. 2.3).

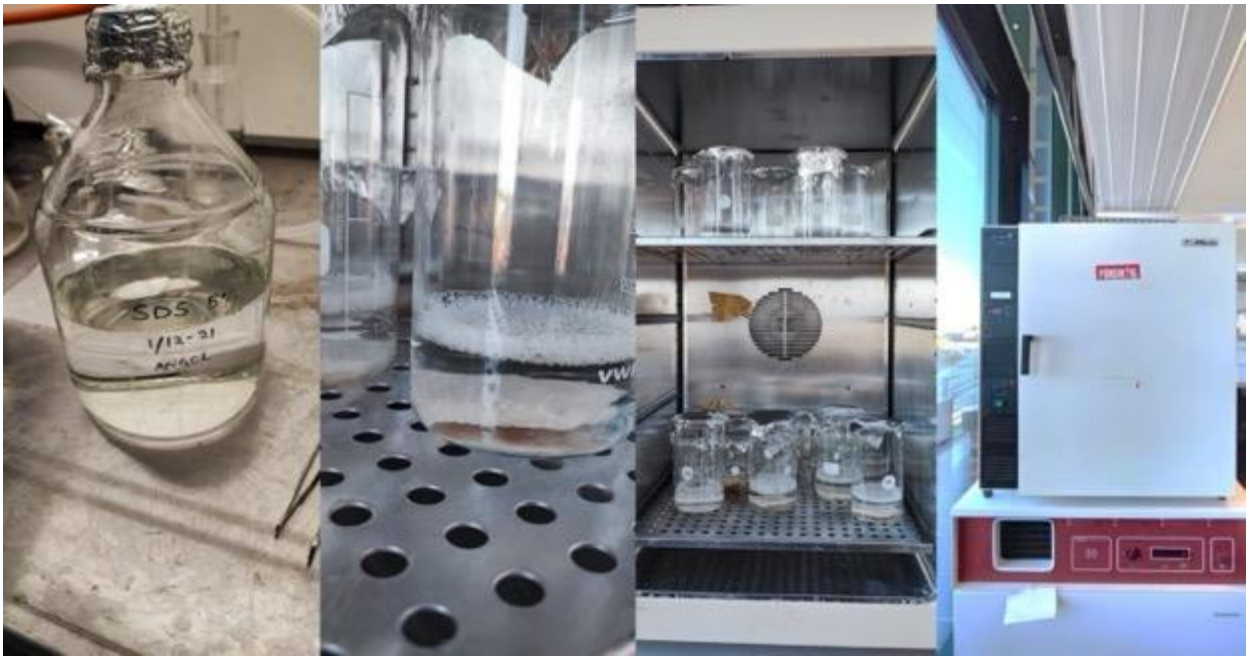

Figure 2.3 – Preparing the first filtration

Second filtration

Addition of glycine buffer (glycine 7,5g, sodium hydroxide 3,7g, Milli-Q Water to 1 liter, pH-9 using 5ml of hydrochloric acid) + 1-5 ml protein enzyme (depending on the sample size) and storage in the oven at +50°C for 24-48h + filtering of samples + ultrasonic cleaning (Fig. 2.4):

Figure 2.4 – Preparing the second filtration

Third filtration

Addition of 100ml of hydrogen peroxide and storage in the oven at +50°C for 24-48h + filtering of samples + ultrasonic cleaning.

Fourth filtration

Addition of ethanol + filtering of samples + vaporing of samples + packing of samples (Fig. 2.5).

Figure 2.5 – Evaporation process and final packing of sample

Fifth filtration

Lastly, the filtration system shown in Figure 2.6 was used to collect microplastics in a zinc oxide

filter: these were then subjected to spectroscopic analysis.

Figure 2.6 – Filter system & filter

Spectroscopy analyses (via µFTIR)

Spectroscopic analyses were performed using a Thermo Scientific™ Nicolet™ iN10 IR microscope. This infrared (IR) microscopy system provides chemical images of heterogeneous samples. The system includes tools to capture images and determine the form factors (shape and size) of each particle. For higher precision, an N2-cooled 64 × 64 line array mapping detector and a quantum MCT detector were used, providing detection in the mid-IR range of 4,000-850 cm^{-1} with a spectral resolution of 4 cm^{-1}. This configuration allows for the detection of particles down to 6.25 µm, although particles smaller than 10 µm may not be fully quantified due to the steel filter mesh used during pre-treatment. The system also enables ultra-fast mapping (10 steps/second) with a resolution of 16 cm^{-1}, covering 1.2 × 1.2 mm in 4.5 minutes. In this process, each pixel in the mapped area contains an IR spectrum, allowing for comprehensive chemical mapping across the entire sample. To identify the polymer types, the system's software compares the spectra of particles with a reference library (SiMPle, v1.3.1β). Particles with a spectral match of 70% or higher are automatically identified, whereas 65%-70% matches are reviewed manually. The software also calculates the mass of each particle by multiplying its volume (derived from dimensions) by the polymer's density. For shape classification, fibers are defined as having a length-to-width ratio greater than 3, while fragments have a ratio of up to 3. This classification system, along with the precise chemical identification and particle size measurements, allowed for detailed analysis of microplastics in samples (Fig.2.7) (Haave et al., 2024; Primpke et al., 2018).

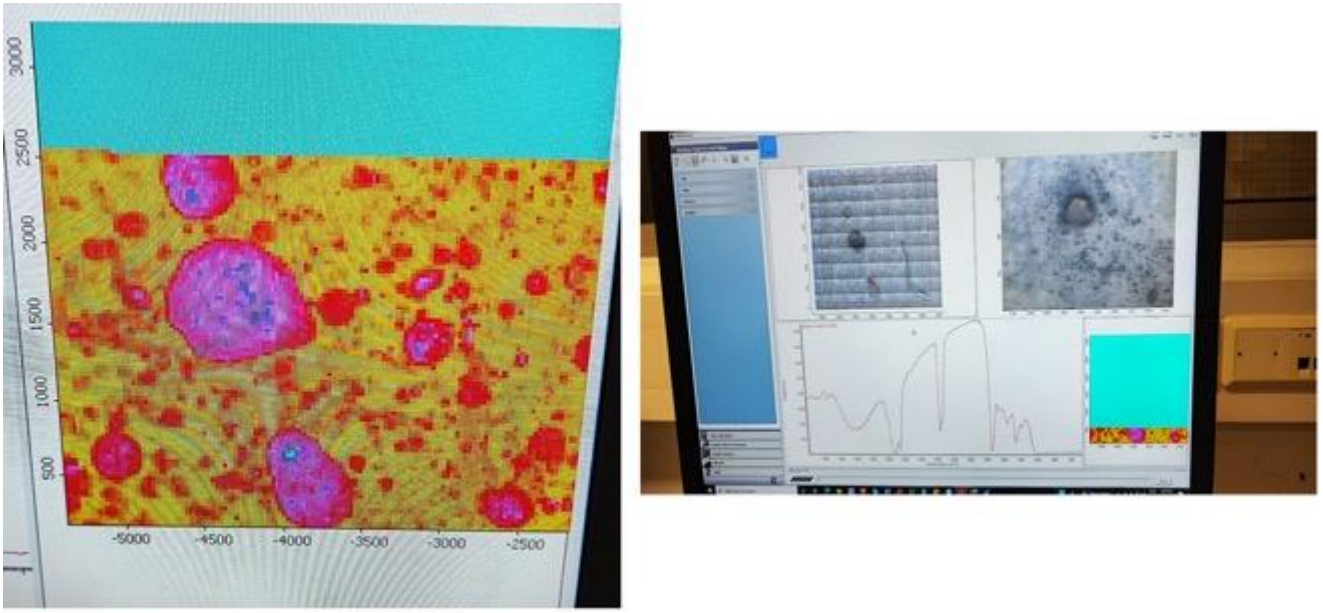

Figure 2.7 – Identifying the type of plastic

Preliminary results for *Raja clavata* (Norce Institute)

Spectroscopy analysis revealed the following types of Microplastics (MPs), both particles and fibers: polypropylene (PP), polyethylene (PE-LDPE, LLDPE), polystyrene (PS), polyvinyl chloride (PVC), polyamide or nylon (PA), polyester, ethylenevinilacetate, polycaprolactone, polyethylene terephthalate, rubber, polyurethane, polycarbonate (PC).

A total of 127 MPs were collected in the gastrointestinal tracts (mean: 4,3±2,8 MPs/ GIT of specimens) and PE was the predominant material (27 particles and 11 fibers).

A total of 87 MPs were detected in the liver (mean: 3±1,9 MPs/liver): polyamide was the predominant polymer (9 particles and 6 fibers), followed by polyester (8 particles and 7 fibers).

The microplastics count in the different tissues of *Raja clavata* includes approximately 3-5% fallout contamination.

Figure 2.11 shows details of MP particles and fibers detected in *R. clavata*.

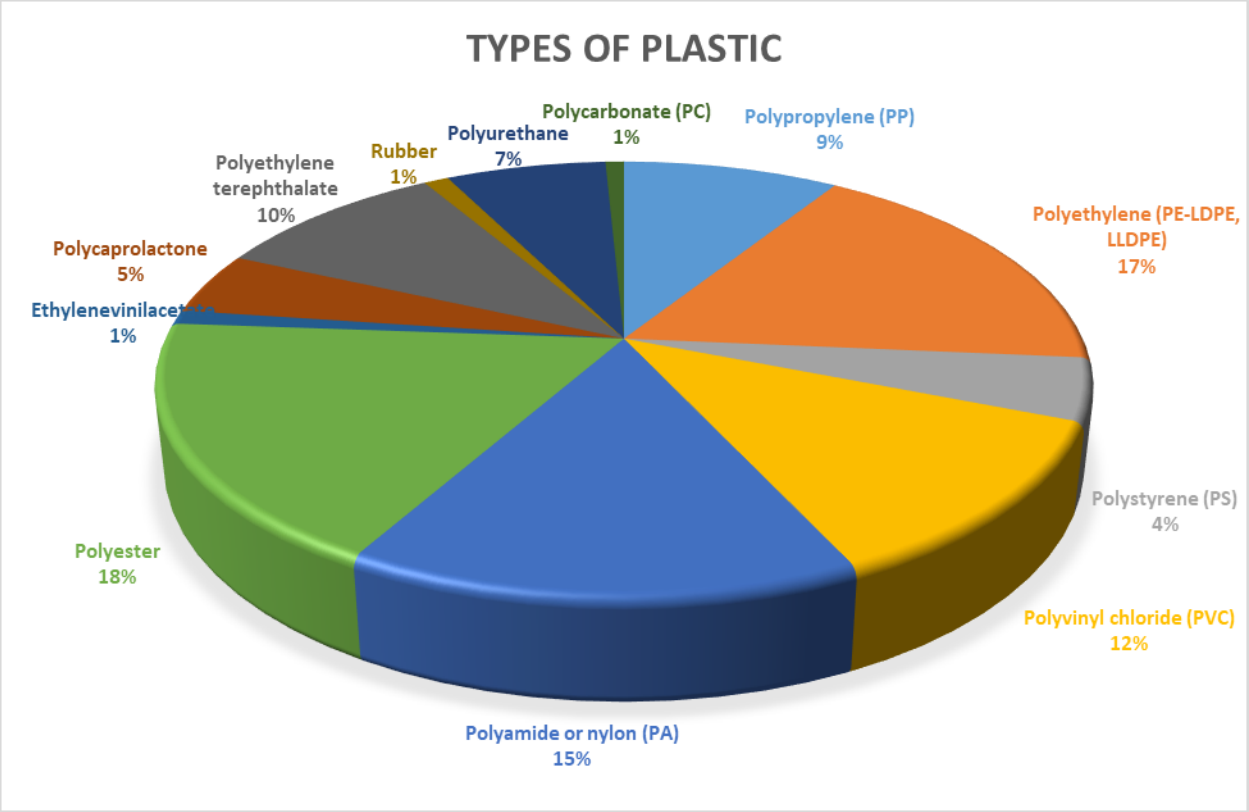

Figure 2.11 – Percentage of different plastic contaminants

MP concentrations did not vary significantly between males and females, nor did they vary with the size of specimens (Fig.2.12) or the depth of catch (Fig. 2.13)

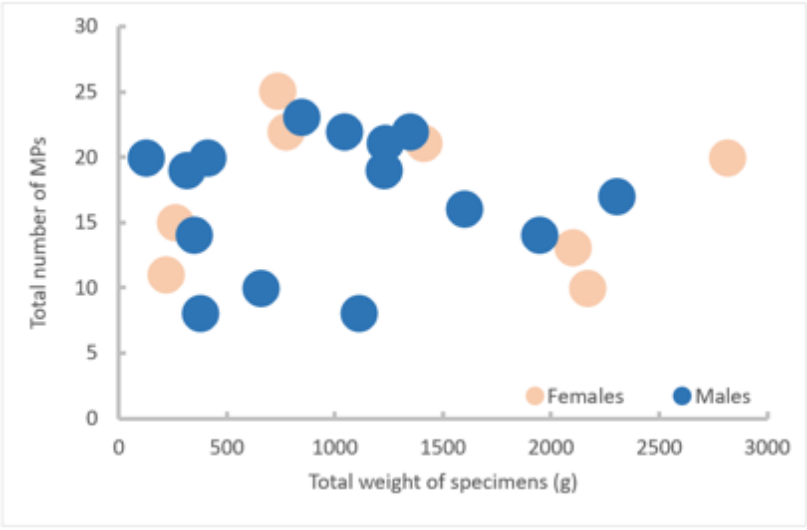

Figure 2.12 – Distribution of plastic contaminants in samples with different total weights

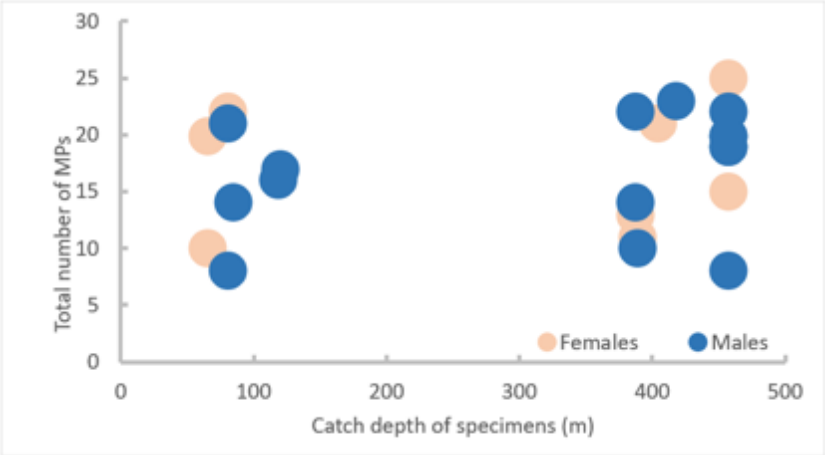

Figure 2.13 – Distribution of plastic contaminants in samples from different catch depths

The aim of this study was to evaluate the ingestion of plastic by thornback rays caught during the MEDITS 2021 survey in southern Sicily [according to the FAO General Fisheries Commission for the Mediterranean (GFCM), Geographical Sub-Area 16]. The study also aimed to investigate possible differences in relation to sex, size and depth of capture and the effects of MPs on the body condition of specimens.

Results indicate that all specimens examined had ingested plastic objects. Counts are higher than, for example, those reported for another Selachian species (*Scyliorhinus canicula*), where up to 71% of specimens had ingested MPs (Mancia et al., 2020). Findings are similar to those of Mancuso et al. (2022), who found plastic particles in all *Scyliorhinus canicula* specimens caught within the same fishing area. In agreement with the works cited above, the high percentage of plastic particles in the GIT of thornback rays can be attributed both to the behaviour of the species and to the state of pollution of the area studied, which is subject to heavy commercial maritime traffic, especially along the Gibraltar-Suez Canal route (Bono et al. 2020). It is also one of the most important fishing areas in the Mediterranean, with one of the largest fishing fleets (Mazara del Vallo) (Fiorentino et al., 2024; Mancuso et al., 2022). All these anthropogenic and environmental processes have led to a high abundance and density of marine litter in southern Sicily, as highlighted by another study on the distribution of macro-litter on this seabed (Garofalo et al., 2020).

References

Bono Gioacchino, Fabio Falsone, Francesca Falco, Federico Di Maio, Marta Gabriele, Vita Gancitano, Michele Luca Geraci, Danilo Scannella, Maria Mancuso, Charles Odilichukwu R. Okpala and Pasti Luisa (2020). Microplastics and Alien Black Particles as Contaminants of Deep-Water Rose Shrimp (Parapenaeus longistroris Lucas, 1846) in the Central Mediterranean Sea. Journal of Advanced Biotechnology and Bioengineering, pp 23-28. DOI: https://doi.org/10.12970/2311-1755.2020.08.04

Fiorentino, F., Garofalo, G., Bono, G., & Vitale, S. (2024). Learning from the history of red shrimp fisheries in the Mediterranean to improve sustainability of deep-water bottom trawling. ICES Journal of Marine Science, 81(4), 652-664.

Garofalo, G., Quattrocchi, F., Bono, G., Di Lorenzo, M., Di Maio, F., Falsone, F., Gancitano, V., Geraci, M.L., Lauria, V., Massi, D., Scannella, D. Titone, A., Fiorentino, F. (2020). What is in our seas? Assessing anthropogenic litter on the seafloor of the central Mediterranean Sea. Environmental Pollution, 115213.

Haave, M., Hæggernes, E., & Gomiero, A. (2024). Absence of microplastic bioaccumulation in cod fillets from plastic-polluted western Norwegian waters. Water Emerging Contaminants and Nanoplastics, 3(3). https://doi.org/10.20517/wecn.2024.07

Mancia A., Chenet T., Bono G., Geraci M.L., Vaccaro C., Munari C., Mistri M., Cavazzini M., Pasti L. (2020). Adverse Effects of Plastic Ingestion on the Mediterranean Small-Spotted Catshark (Scyliorhinus canicula). Marine Environmental Research, 155, 104876.

Monique Mancuso, Giuseppe Panarello, Francesca Falco, Di Paola Davide, Capillo Gioele, Teresa Romeo, Giovanni Presti, Eleonora Gullotta, Nunziacarla Spanò, Gioacchino Bono, Salvatore Giuliano, Teresa Bottari (2022). Investigating the effects of microplastic ingestion in Scyliorhinus canicula from the South of Sicily. Science of the Total Environment, 850: 157875.

Primpke, S., Wirth, M., Lorenz, C., & Gerdts, G. (2018). Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy. Analytical and Bioanalytical Chemistry, 410(21). https://doi.org/10.1007/s00216-018-1156-x

biology

MDPI

Review

Exploring the Impact of Contaminants of Emerging Concern on Fish and Invertebrates Physiology in the Mediterranean Sea

Federica Impellitteri [1], Cristiana Roberta Multisanti [2], Polina Rusanova [3,4], Giuseppe Piccione [1], Francesca Falco [4,*] and Caterina Faggio [2,*]

[1] Department of Veterinary Science, University of Messina, Viale Giovanni Palatucci snc, 98168 Messina, Italy; federica.impellitteri@studenti.unime.it (F.I.); gpiccione@unime.it (G.P.)
[2] Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina, Viale Ferdinando Stagno D'Alcontres, 31, 98166 Messina, Italy; cristiana.multisanti@studenti.unime.it
[3] Department of Biological, Geological and Environmental Sciences (BiGeA)—Marine Biology and Fisheries Laboratory of Fano (PU), University of Bologna, 61032 Bologna, Italy; polina.rusanova@irbim.cnr.it
[4] Institute for Marine Biological Resources and Biotechnology (IRBIM)—CNR, L. Vaccara, 91026 Mazara del Vallo, Italy
* Correspondence: francesca.falco@cnr.it (F.F.); cfaggio@unime.it (C.F.)

Simple Summary: Consumption and excessive use of substances or items that are taken for granted in our daily lives such as personal care products, plastic objects, and medicines inevitably lead to their release into marine and freshwater systems with, unfortunately, potentially devastating consequences over time. For this reason, it is of crucial importance that the scientific community, as well as society, is aware of the environmental problems linked mainly to the release of contaminants into the sea. In this context, the Mediterranean Sea is a special biodiversity hotspot of marine fauna and flora. Nevertheless, it is becoming a source of concern due to the presence of emerging pollutants. In the present paper, the specific focus on the catshark *Scyliorhinus canicula*, as a vertebrate species, and on the Mediterranean mussel *Mytilus galloprovincialis*, as an invertebrate, lies in their ability to provide appropriate information about the health conditions of their surrounding environment. The studies reported on this topic show that it is rather evident that entire aquatic ecosystems suffer from anthropogenic pollution. Therefore, this review aims to collect and demonstrate its effects on the environment and organisms' health.

Abstract: In this historical context, the Mediterranean Sea faces an increasing threat from emerging pollutants such as pharmaceuticals, personal care products, heavy metals, pesticides and microplastics, which pose a serious risk to the environment and human health. In this regard, aquatic invertebrates and fish are particularly vulnerable to the toxic effects of these pollutants, and several species have been identified as bio-indicators for their detection. Among these, bivalve molluscs and elasmobranchs are now widely used as bio-indicators to accurately assess the effects of contaminants. The study focuses on the catshark *Scyliorhinus canicular* and on the Mediterranean mussel *Mytilus galloprovincialis*. The first one is a useful indicator of localised contamination levels due to its exposure to pollutants that accumulate on the seabed. Moreover, it has a high trophic position and plays an important role in the Mediterranean Sea ecosystem. The bivalve mollusc *Mytilus galloprovincialis*, on the other hand, being a filter-feeding organism, can acquire and bioaccumulate foreign particles present in its environment. Additionally, because it is also a species of commercial interest, it has a direct impact on human health. In conclusion, the increasing presence of emerging pollutants in the Mediterranean Sea is a serious issue that requires immediate attention. Bivalve molluscs and elasmobranchs are two examples of bio-indicators that must be used to precisely determine the effects of these pollutants on the marine ecosystem and human health.

Keywords: bioindicators; emerging contaminants; environmental toxicity; Mediterranean Sea

Citation: Impellitteri, F.; Multisanti, C.R.; Rusanova, P.; Piccione, G.; Falco, F.; Faggio, C. Exploring the Impact of Contaminants of Emerging Concern on Fish and Invertebrates Physiology in the Mediterranean Sea. *Biology* **2023**, *12*, 767. https://doi.org/10.3390/biology12060767

Academic Editor: Alberto Teodorico Correia

Received: 4 May 2023
Revised: 18 May 2023
Accepted: 23 May 2023
Published: 24 May 2023

1. Introduction

Environmental quality standards and emerging contaminants are linked. Data on a novel drug's environmental chemistry, ecotoxicology, human toxicity, and epidemiology grow as it begins to rise concerns. Finally, this prompts the creation of environmental standards or criteria by the governments to ensure proper protection. Directly measures on invertebrate models and fish are the best way to study the toxic effects of some emerging contaminants on marine organisms. Additionally, by utilising some models, we can better appreciate the difficult effects on the environment and conservation.

The Mediterranean Sea is a unique and diverse marine ecosystem, home to a variety of invertebrates, fish, and other aquatic species. Recently, however, the Mediterranean Sea has become a source of concern due to the presence of emerging pollutants, including pharmaceuticals and personal care products, heavy metals, pesticides, and microplastics [1–7].

Many of these substances reach coastal and marine waters through direct discharge or river transport due to human activities, including the use of pharmaceuticals on farms, the discharge of untreated wastewater, and the release of heavy metals and pesticides by industry. All these voluntary or involuntary patterns of release of toxic substances into the environment can threaten aquatic ecosystems [8].

Generally, all these pollutants are highly toxic to marine life, through studies showing that they are able to lead to physiological changes, reproductive issues, and even mortality. In the Mediterranean, numerous species are indicated as bio-indicators for the detection of toxic substances in the environment [9,10]. Most of them belong to the invertebrates, such as the mollusc *Mytilus galloprovincialis*, which, as a benthic filter-feeding organism, is reported to be one of the most appropriate bio-indicators. Indeed, they have been widely used to learn about the effects related to pharmaceuticals and personal care products. The effects of two toxicants, i.e., Acetylsalicylic acid (a drug commonly used as an analgesic) and Quaternium-15 (a surfactant easily found in soaps) were evaluated by considering the alteration on the digestive gland cells functionality of *Mytilus galloprovincialis* from the Ionian coast of the Strait of Messina [11]. Particularly, these studies showed a decrease in the normal cellular capacity on the Regulatory Volume Decrease (RVD) test. Cell volume regulation is essential for maintaining the physiology of cell metabolism steady and is involved in specific vital functions such as maintaining the correct cellular pH and ensuring the correct conditions for membrane transport [12]. Particularly, cell volume regulation involves the process of regulatory volume decrease (RVD) response, which entails the efflux of ions and osmolytes from the cell to reduce its volume. However, exposure to pollutants and other environmental stressors can result in damage to the cell membrane and its associated proteins, leading to a loss of the RVD response. When this occurs, cells are unable to return to their original volume, which can disrupt normal cellular processes and contribute to physiological alteration progression. This mechanism, via the analysis of this capacity, reflects the cellular physiological state of the animal and is particularly valued as a method for testing model organisms such as *Mytilus galloprovincialis* for the toxicity of numerous contaminants [13].

Mediterranean Sea invertebrates and fish are particularly vulnerable to the effects of pesticides, as demonstrated through a study on the haematological parameters of the freshwater catfish *Mystus keletius* [14].

In order to measure the impact of these emerging pollutants on invertebrates and fish in the Mediterranean Sea, researchers used a range of methods to assess the levels of pollutants in the water and the body of studied species in the Mediterranean region. These include chemical water analysis and monitoring the presence of pollutants in the tissues of invertebrates and fish. In addition, researchers also examined the reproductive success of Mediterranean Sea invertebrates and fish, and their ability to survive in polluted water. The results of these studies have been mixed, with some species showing signs of physiological stress, while others appear to be relatively unaffected by the presence of emerging pollutants [15].

However, it is clear that the presence of these pollutants is having a significant impact on the health of the fish and invertebrates in the Mediterranean Sea, and further research is required to understand the full extent of the damage caused. It is important to understand the long-term effects of the pollutants on species' health, as well as the potential bioaccumulation of the pollutants in the tissue of fish. For instance, two commercial fish from the Mediterranean Sea were used in a comparison of the bioaccumulation of total aliphatic hydrocarbons (TAH), and it was discovered that each fish had a unique capacity for bioaccumulation that was correlated with the number of fatty acids in the liver [16]. In conclusion, researchers should prioritize studying the effects of emerging pollutants on Mediterranean Sea invertebrates and fish due to their reported association with a variety of adverse health effects in these organisms.

2. Materials and Methods

The data presented in this review were obtained through a careful search of several search engines commonly used for scientific research, including Google Scholar https://scholar.google.it (accessed on 10 March 2023); Pubmed, https://pubmed.ncbi.nlm.Nih.gov (accessed on 11 March 2023); Web of Science, https://clarivate.com/webofsciencegroup/solutions/web-of-science/ (accessed on 13 March 2023); and Scopus, https://www.scopus.com/home.uri (accessed on 13 March 2023).

The search criteria included Boolean structure using "and" or "+" to obtain better results. During the search, the following keywords were used: "pollutant", "emerging pollutants", "impact", "*Scyliorhinus canicula*", "biomarker", "daily care", "shark", "fish", "plastic", "aquatic invertebrates", "*Mytilus galloprovincialis*", and the articles were selected based on the presence of the keywords in the title or abstract. The search also followed a temporal order in which the most recent papers were favoured.

The information has been classified into two tables. The first concerns the direct influence on organisms. The second concerns the different emerging contaminants and their presence in different species, with emphasis on the species *Mytilus galloprovincialis* as an invertebrate model, and *Scyliorhinus canicula*, as a vertebrate species.

3. Bivalve Molluscs and Elasmobranchs as Suitable Indicators of Emerging Pollutants

3.1. Bivalve Molluscs

Among model organisms commonly used in experimental research, invertebrates play a crucial role, due to their intermediate position in food chains [17]. Among bivalve mollusc species, *M. galloprovincialis* is highly valued for its crucial role in environmental monitoring programmes as an early indicator of pollution [18,19]. These mussels have become a cornerstone of such programmes [20,21] due to their unique ability to accumulate and reflect the presence of contaminants in their tissues. As filter feeders, they actively extract water from their surroundings, inadvertently ingesting various substances, including pollutants. Consequently, contaminant levels detected in mussel tissues provide valuable information on the quality of the surrounding aquatic environment [22]. Furthermore, the use of mussels as bio-indicators offers several advantages. Their stationary nature allows for convenient and economical sampling, making them suitable for long-term monitoring. In addition, the ability of mussels to integrate the effects of multiple contaminants over time provides a comprehensive perspective on the overall state of environmental pollution.

Due to their capillary distribution, high filtration rate and long-life span, mussels serve as effective sentinels to assess the health of marine and freshwater ecosystems [23]. Their regular monitoring allows researchers and environmental agencies to detect and assess the presence of emerging pollutants, such as heavy metals, pesticides, and pharmaceutical residues, which may pose a risk to human and ecosystem health [24].

3.2. Elasmobranchs

In recent years, researchers have become increasingly interested in studying the impact of various types of environmental pollutants such as heavy metals, Dichlorodiphenyl-

trichloroethane (DDT), polychlorinated biphenyls (PCBs), microplastics, and personal care products on marine organisms, especially Elasmobranchs. In addition, one of the interesting aspects, because more attention has been focused on other vertebrate groups than the Elasmobranchs group, is that many Elasmobranch species already have strong anthropogenic influence, such as unrestricted illegal fishing activity [25]. As a result of this non-ecological-friendly way of using biological recourse, one in four of these Elasmobranch species (with special attention on the largest sharks and rays) are threatened with extinction [26].

Indeed, to confirm this, Falco et al. [27] in their studies conducted on the blood biomarkers of the catshark *Scylyorinus canicular* and suggested that over-exploitation can be detrimental to the physiological system of the aforementioned species. Moreover, Elasmobranchs have a unique physiology that can make them particularly vulnerable to environmental pollutants due to their slow growth, low reproductive rate, long lifespan, and occupancy of top trophic positions. These factors make them more susceptible to the accumulation of pollutants over time, which can have negative impacts on their health, overall survival and in general the population. For our research, we chose one of the most common species of subclass Elasmobranchii, which includes sharks, rays, and skates [28].

Our focus is on the shark species *Scyliorhinus canicula*. Our choice is explained by a few reasons. Firstly, *S. canicula* is a relatively small, non-migratory species that spends much of its life on the seafloor. This means that it is exposed to pollutants that accumulate on the seafloor, making it a useful indicator of localized pollution levels [29,30]. Secondly, the small-spotted catshark—*S. canicula* has a high trophic position and an important role within marine ecosystems and in the Mediterranean Sea. *S. canicula* is a mesopredator, which impacts the dynamics and stability of marine systems, by connecting different food webs and trophic levels in aquatic ecosystems. The diet of *S. canicula* consists of several other species, including crustaceans, molluscs, and other fish. The latter could account for the bioaccumulation of pollutants in its tissues over time, making it a useful bio-monitor for longer-term exposure to sea pollutants. One of the important parameters for choosing *S. canicula* as an indicator of pollutants [31–33] is that it is a hardy and resilient species that can tolerate a range of different environmental conditions. This means that it can be found in a range of different habitats and is less likely to be affected by natural fluctuations in the environment, making it easier to identify changes in pollutant levels [34].

4. An Overview of Emerging Contaminants Toxicity

The topic of emerging contaminants and their toxicity is of great significance and continues to attract the attention of the scientific community. In recent years, a significant number of articles have been published on this subject in different scientific journals. According to our search on Google Scholar, it has been found that approximately 20,900 research papers have been published on emerging contaminants in water over the last ten years, indicating the significant attention and research dedicated to this topic over the past decade.

As shown in Table 1, different emerging contaminants may have different effects depending on their type and chemical composition, although the results of numerous studies conducted on their toxicity represent them as harmful substances to both ecosystems and organisms.

In this section, the effects of the relevant contaminants on the model organisms described will be addressed, in order to understand their mode of action within organisms, as well as their consequences at population and ecosystem levels. Moreover, Table 2 contains many of the emerging contaminants reported in this review also highlighting the specific part of the Mediterranean Sea to which each study refers.

Table 1. Effects of emerging contaminants on several species identified as model organisms.

Type of Pollutant	Impact on Aquatic Organisms	References
DDTs and toxic evaluation of polychlorinated biphenyls (PCBs)	Accumulation profile analysis of individual PCB congeners and the levels of DDT and its metabolites in the liver of *S. canicula*, from the Mediterranean Sea.	Storelli et al. [35]
Microplastic particles and textile microfibers	Occurrence of MPs in Adriatic food webs in different species (several fish and invertebrates including *M. galloprovincialis*)	Avio et al. [36]
MPs	MPs influence on marine organisms	Baldwin et al. [37]
MPs	Plastic impact on sharks and rays.	Lipej et al. [38]
MPs	Analysis of the different influence of water depth, feeding habits and diets on microplastics accumulation.	Aiguo et al. [39]
MPs and NPs	Toxic effects and bioaccumulation on several aquatic species identified as suitable bioindicators of microplastic pollution.	Multisanti et al. [22]
MPs in cosmetics	Statistical data on MPs in cosmetics.	Guerranti et al. [40]
Pharmaceuticals and Personal care products	Deep explanation about different chemical compositions: pharmaceuticals and personal care products.	Salimi et al. [41]
Pharmaceuticals	Analysis on the impact of tricyclic antidepressants on non-target organisms showed hearth, brain, and cranial and caudal kidney damage; oxidative damage of lipids and also a significant increase in mortality.	Sehonova et al. [42]
Pesticide (fungicide)	Study of the potential risks of a fungicide in the model organism *M. galloprovincialis*, mainly through its bioaccumulation, and the alteration of fundamental physiological processes.	Tresnakova et al. [13]
Pesticide (insecticide)	Evaluation of toxicity due to NeemAzal T/S exposure on early-life stages of common carp (*Cyprinus carpio* L.) showed gills histophatological changes linked to a significant increase in glutathione oxidase, glutathione S-transferase activity and also an increase in oxidised lipids. Moreover, the exposure also induced slow hatching and an increase in mortality.	Chromcova et al. [43]

Table 2. Occurrence and effects of emerging contaminants in relation to samples of species from different geographical areas of the Mediterranean Sea.

Geographical Location	Kind of Pollutant	Species	Influence	References
Strait of Messina, central Mediterranean Sea	Polystyrene microplastics	*Mytilus galloprovincialis*	Insights into early mechanisms of toxicity of polystyrene MPs in mussels. Disorders in osmoregulation, energy and protein metabolism, and oxidative stress were detected.	Cappello et al. [44]
North Adriatic Sea	3 µm polystyrene microplastics	*Mytilus galloprovincialis* (larvae)	Gene expression of genes involved in growth and adaptation mechanisms, due to exposure to polystyrene MPs, was altered.	Capolupo et al. [45]
Black Sea, Aegean, and the Marmara Sea	Microplastic particles	*Mytilus galloprovincialis*	Occurrence of MPs in all the samples from each geographic area analysed.	Gedik and Eryaşar [46]
Southern region of the central Mediterranean Sea.	Plastics injection	*Scyliorhinus canicula*	The presence of plastics, especially macroplastics in the gastrointestinal (GI) tract, was correlated with an increase in the hepatosomatic index and an increased expression of 3 essential immune system genes. It is hypothesized that these effects are induced by additives that are leaching from the ingested plastics. Moreover, plastic particles also act as endocrine disruptors.	Mancia et al. [47]

Table 2. *Cont.*

Geographical Location	Kind of Pollutant	Species	Influence	References
Portuguese coast	Plastic injection/ particles and fibers	*Scyliorhinus canicula*	Depending on the size of the animal, ingested plastic fragments can be small enough to be expelled from the organism through faeces, but larger fragments may be retained in GI tract, causing a false sense of satiety. This paper showed that pelagic species ingest more particles, whereas benthic species ingest more fibres (in relationship to the presence of high quantities of fibres on the seabed).	Neves et al. [48]
Tyrrhenian Sea	Plastic injection	*Scyliorhinus canicula*	MPs occurrence in sharks' GI tract. The main part of particles detected were dark-colored fibers (blue or black) in the size range of 100 µm–330 µm and in high percentages recorded in all three examined species, in high percentages.	Valente et al. [49]
Bay Biskay	Plastic injection	*Scyliorhinus canicula*	High percentage of MPs occurrence.	López-López et al. [50]
Spanish Atlantic and Mediterranean coasts	Plastic injection	*Scyliorhinus canicula*	17% of sharks analysed were found to contain MPs in their stomach.	Bellas et al. [51]
The west coast of Mallorca and in the Mallorca Channel	Plastic injection	*Scyliorhinus canicula*	This paper demonstrated that increasing plastic pollution is related to the increase in water depth, which possibly indicates that plastic pollution is more dependent on depth than spatial coverage.	Alomar et al. [52]
Scotland	Concentration and biomagnification of PCBs and PBDEs	*Scyliorhinus canicula*	All marine mammals, demersal, and pelagic fish had detectable PCBs in their tissues.	Madgett et al. [53]
Tipaza, Algeria	Mixture of Cd, Zn, Cu	*Mytilus galloprovincialis*	Short-term sublethal effects of cadmium (Cd), zinc (Zn), and copper (Cu), carried out via the bioaccumulation. The results revealed a high mortality in mussels exposed to the lowest concentrations of Cu. However, Cd and Zn exposure did not induce a high mortality.	Boudjema et al. [54]
NW Mediterranean Sea & N.E Atlantic Ocean	Metals	*Scyliorhinus canicula*	The shark *S. canicula* had the highest Zn concentrations, and this aspect has a connection because bioaccumulation capacity of Zn from seawater was particularly pronounced in this species. Thus, the peculiar metabolism of *S. canicula* regarding Zn may explain the high values measured excluding local contamination.	Mille et al. [55]
NW Mediterranean Sea&NE Atlantic Ocean	Metals	*Scyliorhinus canicula*	Analysis of Hg concentrations in sharks. Particularly, *S. canicula* presents the highest Hg concentrations	Chouvelon et al. [56]
Portugal	Metals	*Scyliorhinus canicula*	Atlantic lesser-spotted dogfish accumulate high levels of As, Zn, Fe, and Al, which were found to accumulate more in the skin than in sharks' muscles. As levels in muscle reveal this fish unfit for feed production in the EU. Indeed, guideline limits for human consumption were overcome for Hg and As. Risk assessment of meHg and iAs levels indicate a potential risk for human health.	Marques et al. [57]

Table 2. *Cont.*

Geographical Location	Kind of Pollutant	Species	Influence	References
Great Sole Bank and the Atlantic coast of the Iberian Peninsula	Metals	*Scyliorhinus canicula*	Analysis of pollutant levels in discarded fish species by coast of the Iberian Peninsula trawlers showed Hg, Cd, and Pb concentrations in different sampled tissues.	Antelo et al. [58]
NW French Mediterranean	Trace elements	*Scyliorhinus canicula*	*S. canicula* presents the highest Al, As, Cd, Co, and Hg concentrations.	Bouchoucha et al. [59]
Mediterranean	Pollutant Pb burden	*Centroscymnus coelolepis*	Pb content of Mediterranean deep-sea *C. coelolepis* is among the lowest encountered in sharks from various habitats. Pb isotope imprints reveal that Pb is mainly from anthropogenic origin in *C. coelolepis* tissue	Veron et al. [60]
Nigeria	Pollutant from a solid waste dumpsite	*Clarias gariepinus*	These pollutants were found to, possibly, induce endocrine disruption.	Ibor et al. [61]

4.1. Microplastics

The production, use, and mass consumption and disposal of plastic objects are causes of environmental pollution, especially in aquatic habitats. Once plastic wastes are released into the environment, they are subjected to mechanical forces and sunny radiations resulting in the formation of microplastics [62–65]. Indeed, microplastics are small plastic particles less than 5 mm in diameter [66–68] that enter the ecosystem as a result of the breakdown of large plastic particles or the direct release of small plastic particles by climate and human activities [69]. Due to size, shape, and composition, microplastics can be confused with food from aquatic animals actively feeding, whereas they are naturally internalized by filtering-feeding organisms [70]. For example, nanoplastics are even smaller plastic particles than microplastic (which measure less than 100 nanometers in size) and because of their tiny size, have the potential to enter living organisms at the cellular level. Nanoparticles possess distinctive physicochemical properties that can lead to unpredictable interactions with cells and tissues [71]. Due to their comparable size, fabricated nanoparticles may directly interact with cellular molecular organelles and macromolecules, revealing potential nano-bio interface effects [22]. For these reasons, microplastics and nanoplastic can easily reach the top of trophic chains through bioaccumulation and biomagnification processes [62,72].

The Mediterranean Sea accumulates an estimated annual amount of 150 to 610 thousand tonnes of plastics (with an average of 229 thousand tonnes), of which 94% consists of microplastic debris and 6% are microplastics, as stated by IUCN [73]. Plastic particles with a density lower than seawater (which includes most synthetic polymers) typically float on the water's surface, whereas those with higher density sink and accumulate on the seafloor. However, buoyant particles may also sink due to biofouling and particle adherence, and this can significantly impact the distribution of plastic pollution in the ocean. The impacts of microplastics by ingestion, smothering, or entanglement have been well documented for a variety of marine species, which has led to the consideration of plastics as hazardous materials [33,48].

For example, in a study conducted by Álvarez-Ruiz et al. [24]., the bioaccumulation of 20 emerging contaminants, including pharmaceuticals, pesticides, and perfluoroalkyl substances (PFAS), in the mussel *Mytilus galloprovincialis* with or without the presence of microplastics was assessed. The results revealed that some contaminants accumulated in the visceral mass and haemolymph of the mussels and that the presence of microplastics facilitated higher bioconcentration factors and slower elimination rates. Another study conducted by the research group of Trestrail et al. [74] examined the impact of spherical microplastics ingested by the mussel *M. galloprovincialis* on digestive enzyme activities.

This resulted in alterations that may affect the mussels' acquisition of energy from food and deplete their energy reserves.

In addition to the mentioned studies, another research conducted by Mancia et al. [47] assessed plastic ingestion by the catshark *Scyliorhinus canicula* in the Mediterranean Sea and analyzed the expression levels of immune-related genes. The findings revealed that microplastics were widely ingested, and macroplastics were present in approximately 18% of the specimens. Moreover, specimens with macroplastic ingestion exhibited a significant increase in the expression of immune genes, indicating that plastic pollution represents an emerging threat to catsharks and the Mediterranean food web.

4.2. Personal Care Products

The current society has become accustomed to using a wide range of personal care products (PCPs) [75–79]. The class of PCPs includes a variety of products such as body creams and soaps, sunscreens, exfoliants, shampoos, detergents, perfumes, cosmetics, and toothpaste [80,81], according to the US Environmental Protection Agency (US EPA). Nevertheless, the removal of PCPs from wastewater is not always successful, and their concentrations, in both marine and freshwater environments, have been found to be in the range of ng/L–µg/L, which may not seem that high, but still biologically relevant [81]. For this reason, PCP-related substances can lead to potential risk as a source of stress for marine species and ecosystems, affecting different biological levels from cellular interactions to the whole ecosystem, as shown in Table 1. An investigation of the toxicity of sodium lauryl sulphate (SLS), an anionic surfactant used as an emulsifying detergent, conducted on the Mediterranean mussel, *M. galloprovincialis*, and reported by Freitas et al. [82] revealed that the substance had a wide range of effects on the organism. There was a noticeable reduction in respiration rate, which led to a loss in filtration capacity and had negative consequences on the physiological development of organisms. Furthermore, SLS bioaccumulation in mussels affected their metabolic performance and a reduction in the efficiency of natural antioxidant mechanisms. SLS toxicity was also evaluated on the antioxidant system in primary hepatocyte cultures of Van fish (from Lake Van, Turchia). Again, the results showed alterations in the enzymatic activity of the antioxidant defense system [83]. Briefly, changes in the antioxidant defense system, lead to an imbalance between the free radicals produced by cellular metabolic reactions (known as cellular respiration) and the substances used by the body to counteract their effects. This imbalance in turn leads to the expression of oxidative stress. Lastly, it has been observed that modifications brought about by PCPs at the cellular and sub-cellular levels can have far-reaching consequences, which may culminate in effects on populations, including entire communities and thus marine ecosystems [84]. Little is currently known about how personal hygiene items affect elasmobranchs, in particular, *S. canicula*.

A study on the Brazilian guitarfish, *Pseudobatos horkelii* [85] showed how PCPs, particularly legacy pollutants, can influence maternal load and transmit it to the offspring.

Indeed, they hypothesized that the highest transfer rates of methylparaben to the offspring were 6%, due to the presence of this pollutant in the maternal uterus samples. Additionally, a prior study by Martins et al. [86] on the subject of the aforementioned species, as well as other guitarfishes revealed that it is possible for PCPs contaminants to be transferred from mothers to offspring during vitellogenesis when yolk precursors are transferred to ovarian follicles. As a result, the contaminated yolk may be a significant source of contamination for these organisms.

A recent study [87] conducted by the same group investigated the effects of prenatal exposure to contaminants including PPCs in embryos of Brazilian guitarfish. The results suggest that prenatal exposure to contaminants may impact redox status and lead to oxidative damage in embryos.

4.3. Pharmaceuticals

Nowadays, drug consumption is also increasing due to the growing demand for various applications related to chronic diseases, but also, for example, to ageing or always much-increasing demand for farmed food without neglecting or forgetting all agricultural activities [88,89]. The consumption of pharmaceuticals by humans leads to the excretion of their metabolites, which are subsequently released into the environment [42]. Pharmaceuticals, as well as some personal care products, can act as endocrine disruptors. In other words, they can activate or inhibit specific signalling pathways that are downstream of hormonal activity, acting as "hormone mimics" [90]. This kind of interference could lead to damage to the immune system, reducing its effectiveness, hormonal imbalances, and other adverse effects in organisms exposed to these compounds. In this context, according to Pagano et al. [11], the toxicity of anti-inflammatory drugs, i.e., acetylsalicylic acid (ASA) on *M. galloprovincialis* caused alterations in hepatocytes volume regulation, which also resulted in inflammation at the histological level. The gills were also damaged by exposure to ASA and showed numerous alterations such as infiltration of haemocytes, which are responsible for the first defense of the organism against pathogens and xenobiotics. The toxicity due to waterborne antidepressant presence in aquatic environments on non-target organisms living in surface waters was also assessed. Indeed, as stated by Sehonova et al. [42], the investigation conducted into the effects of selective serotonin and selective serotonin-noradrenalin reuptake inhibitors on invertebrates, amphibians, and fish showed that the first tested drug negatively interfered with the behaviour, reproduction, and development of both invertebrates and fish. However, concerning the second antidepressant tested, it was shown not only to affect fish behaviour but also lead to an increase in fish mortality associated with developmental delay, and morpho-pathological alterations in the brain, heart, and cephalic and caudal kidney. Finally, alterations in the natural antioxidant system and an increase in lipid peroxidation were also detected, indicating the high toxicity of the drugs even at the lowest concentrations.

Sharks, compared to bony fish, have received little attention with regard to drug exposure. Information on the effect of drugs on elasmobranch species is very scarce, and we would like to note the gap on this point. For this reason, we chose to consider reporting in this review the literature studies on elasmobranchs conducted in recent years. The main works on the effect of drugs have been conducted on river elasmobranchs, such as those on the absorption of any active pharmaceutical ingredient of human drugs (e.g., citalopram, fluoxetine, fluvoxamine, paroxetine, sertraline, venlafaxine) that were examined and compared in the plasma of neonatal bull sharks (*Carcharhinus leucas*) residing in pristine tributaries (Myakka River) and in tributaries with sewage (Calohosaatchee River) of the Charlotte Harbor estuary in Florida. Gelsleichter and Szabo [91] showed that in the latter case, the drugs can accumulate and put the *C. laucas* population at risk, although the effect remained undiscovered. Instead, Martins et al. [85] demonstrated that the transfer of diclofenac from a maternal uterus to the offspring can occur at a rate of 27%.

4.4. Pesticides

The growing society's demand for plant products has also led to an increase in the use of useful products to safeguard crops from pathogens and predators, i.e., pesticides [92–94]. Within the class of pesticides, insecticides, fungicides, and herbicides, which may have a specific or broad-spectrum mode of action, occupy a key position. These compounds are easily able to reach aquatic ecosystems through several pathways, including wastewater and the atmosphere, interacting with the environment and the organisms that inhabit it [95].

In this context, the question that naturally arises is: why are aquatic invertebrates so sensitive, for example, to insecticides? The answer lies in their proximity to insects, as they share not only the same neurological and respiratory mechanisms but also the same detoxification system, which in both cases is not enough efficient in degrading pesticides [96].

Particularly relevant is a study conducted by Bado-Nilles et al. [97], in which the effects of a mix of 14 pesticides on the oyster *Crassostrea gigas* haemocytes were investigated. In addition, Matozzo et al. [98] also observed that *Ruditapes philippinarum* exposure to different concentrations of glyphosate (a non-selective herbicide) had a significant impact on its haemocytes. Indeed, the results showed that after 7 days of exposure to the pollutant, the total number of haemocytes decreased, and their volume increased significantly in the specimens exposed to 100 and 1000 µg/L of glyphosate. Belonging to the class of neonicotinoids is thiacloprid, a neuroactive insecticide whose chemical structure resembles that of nicotine. As neonicotinoids are competitors of acetylcholine, their action mechanism involves disrupting signal transmission. An investigation of thiacloprid toxicity on haemocytes was conducted by long-term exposure of *M. galloprovincialis* to different concentrations of the compound. The results showed that both concentrations of 1.5 and 10 µg L^{-1} caused a significant decrease in Cl^- and K^+ and a significant increase in glucose content [42].

Some pollutants belonging to the class of pesticides are also known as endocrine disruptors and have been shown to alter the expression of hormones such as 17β oestradiol, progesterone, testosterone, and vitellogenin. The effects of nonylphenol (NP), an environmental pollutant similar to oestrogen, have been evaluated on the synthesis of vitellogenin of adult male cartilaginous fish "*Torpedo marmorata*" [99]; the latter studies showed that injecting *T. marmolata* males with nonylphenol in the liver and kidney resulted in the presence of VTG; this is an extraordinary factor given that this lipophosphoglycoprotein is physiologically induced by oestrogens only in females of oviparous and ovoviviparous vertebrates.

Marsili et al. [100] proposed skin biopsies to assess the toxicological effects of this organochlorine (OC) and polycyclic aromatic (IPA) pollutants in *Carcharodon carcharias* from the South African coast. These studies showed that high concentrations of OC and IPA caused significant loading responses of cytochrome P4501A (CYP1A) in the animal; in addition, vitellogenin and radiated zone proteins were also affected by these contaminants. These latter protein biomarkers were found in the gonads of immature males and females.

4.5. Heavy Metals

Heavy metal pollution by anthropogenic activities is a major concern due to its impact on the environment and aquatic organisms [101,102]. Anthropogenic sources of metals include wastewater, traffic emissions, coal and oil combustion, industrial production, and many others [103]. Aquatic organisms counteract continuously with heavy metal ions also ingesting them from their diet, and their removal from tissues is not always constant as several factors come into play, such as time of exposure to the metal, temperature, metabolic activity, and metal chemistry. At low concentrations, most of these metals are essential for life, as they contribute to bone and shell or muscle development, as well as being cofactors in many biochemical reactions within the body. In the case of invertebrate organisms, the accumulation of heavy metals can directly impact the central nervous system and disrupt homeostasis. Mussels, being filter feeders, are particularly prone to accumulating heavy metals in their tissues due to their feeding habits and prolonged exposure to contaminated water. These effects can manifest in malformations, impaired growth, and reproduction, weakened immune responses, and even mortality [104–106].

In addition, sharks showed to be particularly sensitive to heavy metals, such as mercury, lead, and cadmium. Typically, heavy metals tend to accumulate in the liver, kidneys, muscle tissue, and blood of fish [107] as well as in sharks [108]. These metals can also accumulate in the shark's skin and cartilage, depending on the species and the type of metal [108]. The liver is often the primary site of heavy metal accumulation in sharks because it plays a critical role in the detoxification and processing of these metals. The kidneys also play a similar role in removing toxic metals from the shark's body. However, in some cases, the concentration of heavy metals in the muscle tissue can also be high enough to make the consumption of shark meat potentially hazardous to humans. According to

Wosnick et al. [32], *S. canicula* exposure to heavy metals could lead to alterations in hepatic markers with the bioaccumulation of Co, Fe, and Hg in the liver of sharks, in urea and lactate with the bioaccumulation of Fe and Hg in the gills. Moreover, in sharks, the rectal gland plays an important role in shark osmoregulation and associated homeostatic balance. In this context, alteration in phosphorus content with the bioaccumulation of Co, Mn, and Hg in the rectal gland of sharks was also observed.

5. Research Gaps and Opportunities

Scientific progress in the field of environmental pollution studies is of paramount importance as, unfortunately, recent years are witnessing an increase in environmental pollution with certain future consequences on human health as well. Precisely for this reason, we believe that disseminating information and making the community increasingly aware of the risks linked to pollution is of fundamental importance. Indeed, toxicological studies are part of the priorities of those who wish to protect the environment, organisms, and also human health, in accordance with the "One Health" approach.

One point that must be borne in mind is that different contaminants will be present in aquatic ecosystems at the same time and as mixtures, so they may have synergistic, antagonistic, or neutral effects on each other toxicity. Briefly, a synergistic effect entails an increase in the effect of the toxicant on the established endpoints, whereas an antagonistic effect leads to a reduction in the contaminant toxicity with direct results on the biomarkers analyzed. Therefore, toxicological studies of aquatic environment contaminants require knowledge of the impact of individual contaminants, as well as the effects resulting from their interactions.

Moreover, although there is a growing general interest in the scientific community and more data are available in the literature for some of the emerging contaminants reported in this review, according to our research, effects related to personal care products and pharmaceuticals are still under-investigated, and fairly recent data of heavy metals detection on model organisms such as *Mytilus galloprovincialis*, in the Mediterranean Sea, are not still available.

Of critical importance also turns out to be recognizing these effects on a large ecological scale, from the individual organism to the biodiversity-level interaction. Indeed, the health of an ecosystem depends on the interaction of multiple physical, chemical, and biological factors. Particularly, ecosystem processes, such as productivity and nutrient recycling, are directly related to the functional diversity of biotic communities, which in turn are determined by species biodiversity. Nevertheless, species biodiversity can be altered in response to pressures by environmental changes, and this is directly reflected in ecosystem processes' functionality [109].

For these reasons, in accordance with the needs arising from the adverse effects of emerging contaminants, it is of paramount importance for the scientific community to continue in this direction carrying out investigations related especially to aquatic environments, which play the role of one of the greatest pollutant sinks, with a special focus on the effects at different ecological levels, from the individual organism to the influence on biodiversity.

6. Conclusions

The research conducted highlighted how starting from our daily habits, as well as societal and industrial development, it is made possible to negatively interfere at different biological and ecological levels. A crucial point is to make today's society aware of the impact it has on its surroundings, which is reflected both in animal organisms and their biodiversity, a very important index of health in any environment, as well as indirectly on our own health. Therefore, the purpose of this review is to provide information highlighting the toxic effects on suitable model organisms in order to raise awareness of the responsible and conscious use of the major part of the products we mentioned whose use is now extremely common. From the analysis carried out in this review, it is crucial to monitor

contamination levels and implement effective management strategies to prevent further damage to the environment and vulnerable species. It is imperative that action is taken to mitigate the impact of emerging pollutants on the Mediterranean Sea and its inhabitants. Our objective was to describe the type of difference between two different organisms used as models, albeit belonging to different levels of the food web. In this review, it was highlighted how all effects are refined at the level of the physiological system; if we do not pay sufficient attention to how to defend the environment from contaminants, we risk incurring serious diseases and subsequently human extinction.

Author Contributions: Conceptualization, F.I. and C.R.M.; methodology, F.I., C.R.M. and F.F.; resources, F.I., C.R.M. and F.F.; writing—original draft preparation, F.I., C.R.M. and F.F.; writing—review and editing, F.I., C.R.M., P.R., F.F. and C.F.; supervision, F.F., G.P. and C.F.; project administration, F.F., G.P. and C.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zicarelli, G.; Multisanti, C.R.; Falco, F.; Faggio, C. Evaluation of Toxicity of Personal Care Products (PCPs) in Freshwaters: Zebrafish as a Model. *Environ. Toxicol. Pharmacol.* **2022**, *94*, 103923. [CrossRef] [PubMed]
2. Monique, M.; Giuseppe, P.; Francesca, F.; Davide, D.P.; Savoca, S.; Gioele, C.; Teresa, R.; Giovanni, P.; Eleonora, G.; Nunziacarla, S.; et al. Investigating the Effects of Microplastic Ingestion in *Scyliorhinus canicula* from the South of Sicily. *Sci. Total Environ.* **2022**, *850*, 157875. [CrossRef]
3. Spada, L.; Annicchiarico, C.; Cardellicchio, N.; Giandomenico, S.; DI Leo, A. Heavy Metals Monitoring in Mussels *Mytilus galloprovincialis* from the Apulian Coasts (Southern Italy). *Mediterr. Mar. Sci.* **2013**, *14*, 99. [CrossRef]
4. Boldrocchi, G.; Spanu, D.; Polesello, S.; Valsecchi, S.; Garibaldi, F.; Lanteri, L.; Ferrario, C.; Monticelli, D.; Bettinetti, R. Legacy and Emerging Contaminants in the Endangered Filter Feeder Basking Shark *Cetorhinus maximus*. *Mar. Pollut. Bull.* **2022**, *176*, 113466. [CrossRef] [PubMed]
5. Brumovský, M.; Bečanová, J.; Kohoutek, J.; Borghini, M.; Nizzetto, L. Contaminants of Emerging Concern in the Open Sea Waters of the Western Mediterranean. *Environ. Pollut.* **2017**, *229*, 976–983. [CrossRef]
6. López-Serna, R.; Postigo, C.; Blanco, J.; Pérez, S.; Ginebreda, A.; de Alda, M.L.; Petrović, M.; Munné, A.; Barceló, D. Assessing the Effects of Tertiary Treated Wastewater Reuse on the Presence Emerging Contaminants in a Mediterranean River (Llobregat, NE Spain). *Environ. Sci. Pollut. Res.* **2012**, *19*, 1000–1012. [CrossRef]
7. Munschy, C.; Marchand, P.; Venisseau, A.; Veyrand, B.; Zendong, Z. Levels and Trends of the Emerging Contaminants HBCDs (Hexabromocyclododecanes) and PFCs (Perfluorinated Compounds) in Marine Shellfish along French Coasts. *Chemosphere* **2013**, *91*, 233–240. [CrossRef]
8. Dachs, J.; Méjanelle, L. Organic Pollutants in Coastal Waters, Sediments, and Biota: A Relevant Driver for Ecosystems during the Anthropocene? *Estuaries Coasts* **2010**, *33*, 1–14. [CrossRef]
9. Impellitteri, F.; Curpăn, A.-S.; Plăvan, G.; Ciobica, A.; Faggio, C. Hemocytes: A Useful Tool for Assessing the Toxicity of Microplastics, Heavy Metals, and Pesticides on Aquatic Invertebrates. *Int. J. Environ. Res. Public Health* **2022**, *19*, 16830. [CrossRef]
10. Pagano, M.; Fabrello, J.; Multisanti, C.R.; Zicarelli, G.; Ciscato, M.; Boldrin, F.; Giacobbe, S.; Matozzo, V.; Faggio, C. A First Insight into Haemocytes of *Pinctada imbricata radiata*: A Morpho-functional Characterization. *Microsc. Res. Tech.* **2023**, *86*, 368–377. [CrossRef]
11. Pagano, M.; Savoca, S.; Impellitteri, F.; Albano, M.; Capillo, G.; Faggio, C. Toxicological Evaluation of Acetylsalicylic Acid in Non-Target Organisms: Chronic Exposure on *Mytilus galloprovincialis* (Lamarck, 1819). *Front. Physiol.* **2022**, *13*, 1165. [CrossRef]
12. Wehner, F.; Olsen, H.; Tinel, H.; Kinne-Saffran, E.; Kinne, R.K.H. Cell Volume Regulation: Osmolytes, Osmolyte Transport, and Signal Transduction. In *Reviews of Physiology, Biochemistry and Pharmacology*; Springer: Berlin/Heidelberg, Germany, 2003; pp. 1–80.
13. Tresnakova, N.; Famulari, S.; Zicarelli, G.; Impellitteri, F.; Pagano, M.; Presti, G.; Filice, M.; Caferro, A.; Gulotta, E.; Salvatore, G.; et al. Multi-Characteristic Toxicity of Enantioselective Chiral Fungicide Tebuconazole to a Model Organism Mediterranean Mussel *Mytilus galloprovincialis* Lamarck, 1819 (Bivalve: Mytilidae). *Sci. Total Environ.* **2023**, *862*, 160874. [CrossRef]
14. Barathinivas, A.; Ramya, S.; Neethirajan, K.; Jayakumararaj, R.; Pothiraj, C.; Balaji, P.; Faggio, C. Ecotoxicological Effects of Pesticides on Hematological Parameters and Oxidative Enzymes in Freshwater Catfish, *Mystus keletius*. *Sustainability* **2022**, *14*, 9529. [CrossRef]

15. Hodkovicova, N.; Hollerova, A.; Svobodova, Z.; Faldyna, M.; Faggio, C. Effects of Plastic Particles on Aquatic Invertebrates and Fish—A Review. *Environ. Toxicol. Pharmacol.* **2022**, *96*, 104013. [CrossRef]
16. Piazzese, D.; Bonanno, A.; Bongiorno, D.; Falco, F.; Indelicato, S.; Milisenda, G.; Vazzana, I.; Cammarata, M. Co-Inertia Multivariate Approach for the Evaluation of Anthropogenic Impact on Two Commercial Fish along Tyrrhenian Coasts. *Ecotoxicol. Environ. Saf.* **2019**, *182*, 109435. [CrossRef]
17. Ravi, R.; Athisuyambulingam, M.; Kanagaraj, S.; Tresnakova, N.; Impellitteri, F.; Viswambaran, G.; Faggio, C. Impact of Chlorpyrifos on Cytopathological Indices in Mangrove Crab, *Episesarma tetragonum* (Fabricius). *Vet. Sci.* **2023**, *10*, 53. [CrossRef]
18. Moreira, S.M.; Guilhermino, L. The Use of *Mytilus galloprovincialis* Acetylcholinesterase and Glutathione S-Transferases Activities as Biomarkers of Environmental Contamination along the Northwest Portuguese Coast. *Environ. Monit. Assess.* **2005**, *105*, 309–325. [CrossRef]
19. Lam, P.K.S. Use of Biomarkers in Environmental Monitoring. *Ocean Coast. Manag.* **2009**, *52*, 348–354. [CrossRef]
20. González-Fernández, C.; Albentosa, M.; Campillo, J.A.; Viñas, L.; Romero, D.; Franco, A.; Bellas, J. Effect of Nutritive Status on *Mytilus galloprovincialis* Pollution Biomarkers: Implications for Large-Scale Monitoring Programs. *Aquat. Toxicol.* **2015**, *167*, 90–105. [CrossRef]
21. Beyer, J.; Green, N.W.; Brooks, S.; Allan, I.J.; Ruus, A.; Gomes, T.; Bråte, I.L.N.; Schøyen, M. Blue Mussels (*Mytilus edulis* Spp.) as Sentinel Organisms in Coastal Pollution Monitoring: A Review. *Mar. Environ. Res.* **2017**, *130*, 338–365. [CrossRef]
22. Multisanti, C.R.; Merola, C.; Perugini, M.; Aliko, V.; Faggio, C. Sentinel Species Selection for Monitoring Microplastic Pollution: A Review on One Health Approach. *Ecol. Indic.* **2022**, *145*, 109587. [CrossRef]
23. Curpan, A.-S.; Impellitteri, F.; Plavan, G.; Ciobica, A.; Faggio, C. Review: *Mytilus galloprovincialis*: An Essential, Low-Cost Model Organism for the Impact of Xenobiotics on Oxidative Stress and Public Health. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2022**, *256*, 109302. [CrossRef] [PubMed]
24. Álvarez-Ruiz, R.; Picó, Y.; Campo, J. Bioaccumulation of Emerging Contaminants in Mussel (*Mytilus galloprovincialis*): Influence of Microplastics. *Sci. Total Environ.* **2021**, *796*, 149006. [CrossRef] [PubMed]
25. Tiktak, G.P.; Butcher, D.; Lawrence, P.J.; Norrey, J.; Bradley, L.; Shaw, K.; Preziosi, R.; Megson, D. Are Concentrations of Pollutants in Sharks, Rays and Skates (Elasmobranchii) a Cause for Concern? A Systematic Review. *Mar. Pollut. Bull.* **2020**, *160*, 111701. [CrossRef]
26. Dulvy, N.K.; Fowler, S.L.; Musick, J.A.; Cavanagh, R.D.; Kyne, P.M.; Harrison, L.R.; Carlson, J.K.; Davidson, L.N.; Fordham, S.V.; Francis, M.P.; et al. Extinction Risk and Conservation of the World's Sharks and Rays. *Elife* **2014**, *3*, e00590. [CrossRef]
27. Falco, F.; Bono, G.; Cammarata, M.; Cavalca, J.; Vazzana, I.; Dara, M.; Scannella, D.; Guicciardi, S.; Faggio, C.; Ragonese, S. Stress Related Blood Values in *Scyliorhinus canicula* as Live-Indicators of Physiological Status after Bottom Trawling Capture Activity. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **2023**, *263*, 110802. [CrossRef]
28. Rodríguez-Cabello, C.; Sánchez, F.; Fernández, A.; Olaso, I. Is the Lesser Spotted Dogfish (*Scyliorhinus canicula*) Population from the Cantabrian Sea a Unique Stock? *Fish. Res.* **2004**, *69*, 57–71. [CrossRef]
29. Esposito, G.; Prearo, M.; Renzi, M.; Anselmi, S.; Cesarani, A.; Barcelò, D.; Dondo, A.; Pastorino, P. Occurrence of Microplastics in the Gastrointestinal Tract of Benthic by–Catches from an Eastern Mediterranean Deep–Sea Environment. *Mar. Pollut. Bull.* **2022**, *174*, 113231. [CrossRef]
30. Barbieri, M.; Maltagliati, F.; Roldán, M.I.; Castelli, A. Molecular Contribution to Stock Identification in the Small-Spotted Catshark, *Scyliorhinus canicula* (Chondrichthyes, Scyliorhinidae). *Fish. Res.* **2014**, *154*, 11–16. [CrossRef]
31. Kousteni, V.; Karachle, P.K.; Megalofonou, P. Diet of the Small-Spotted Catshark *Scyliorhinus canicula* in the Aegean Sea (Eastern Mediterranean). *Mar. Biol. Res.* **2017**, *13*, 161–173. [CrossRef]
32. Wosnick, N.; Niella, Y.; Hammerschlag, N.; Chaves, A.P.; Hauser-Davis, R.A.; da Rocha, R.C.C.; Jorge, M.B.; de Oliveira, R.W.S.; Nunes, J.L.S. Negative Metal Bioaccumulation Impacts on Systemic Shark Health and Homeostatic Balance. *Mar. Pollut. Bull.* **2021**, *168*, 112398. [CrossRef]
33. Smith, L.E. Plastic Ingestion by *Scyliorhinus canicula* Trawl Captured in the North Sea. *Mar. Pollut. Bull.* **2018**, *130*, 6–7. [CrossRef]
34. Muñoz-Baquero, M.; Marco-Jiménez, F.; García-Domínguez, X.; Ros-Santaella, J.L.; Pintus, E.; Jiménez-Movilla, M.; García-Párraga, D.; García-Vazquez, F.A. Comparative Study of Semen Parameters and Hormone Profile in Small-Spotted Catshark (*Scyliorhinus canicula*): Aquarium-Housed vs. Wild-Captured. *Animals* **2021**, *11*, 2884. [CrossRef]
35. Storelli, M.M.; Barone, G.; Santamaria, N.; Marcotrigiano, G.O. Residue Levels of DDTs and Toxic Evaluation of Polychlorinated Biphenyls (PCBs) in *Scyliorhinus canicula* Liver from the Mediterranean Sea (Italy). *Mar. Pollut. Bull.* **2006**, *52*, 696–700. [CrossRef]
36. Avio, C.G.; Pittura, L.; d'Errico, G.; Abel, S.; Amorello, S.; Marino, G.; Gorbi, S.; Regoli, F. Distribution and Characterization of Microplastic Particles and Textile Microfibers in Adriatic Food Webs: General Insights for Biomonitoring Strategies. *Environ. Pollut.* **2020**, *258*, 113766. [CrossRef]
37. Baldwin, W.S.; Bain, L.J.; Di Giulio, R.; Kullman, S.; Rice, C.D.; Ringwood, A.H.; van den Hurk, P. 20th Pollutant Responses in Marine Organisms (PRIMO 20): Global Issues and Fundamental Mechanisms Caused by Pollutant Stress in Marine and Freshwater Organisms. *Aquat. Toxicol.* **2020**, *227*, 105620. [CrossRef]
38. Lipej, L.; Cumani, F.; Acquavita, A.; Bettoso, N. Plastic Impact on Sharks and Rays. In *Plastic Pollution and Marine Conservation*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 153–185.

39. Aiguo, Z.; Di, S.; Chong, W.; Yuliang, C.; Shaolin, X.; Peiqin, L.; Guohuan, X.; Huijuan, T.; Jixing, Z. Characteristics and Differences of Microplastics Ingestion for Farmed Fish with Different Water Depths, Feeding Habits and Diets. *J. Environ. Chem. Eng.* **2022**, *10*, 107189. [CrossRef]
40. Guerranti, C.; Martellini, T.; Perra, G.; Scopetani, C.; Cincinelli, A. Microplastics in Cosmetics: Environmental Issues and Needs for Global Bans. *Environ. Toxicol. Pharmacol.* **2019**, *68*, 75–79. [CrossRef] [PubMed]
41. Salimi, M.; Esrafili, A.; Gholami, M.; Jonidi Jafari, A.; Rezaei Kalantary, R.; Farzadkia, M.; Kermani, M.; Sobhi, H.R. Contaminants of Emerging Concern: A Review of New Approach in AOP Technologies. *Environ. Monit. Assess.* **2017**, *189*, 414. [CrossRef] [PubMed]
42. Sehonova, P.; Plhalova, L.; Blahova, J.; Doubkova, V.; Marsalek, P.; Prokes, M.; Tichy, F.; Skladana, M.; Fiorino, E.; Mikula, P.; et al. Effects of Selected Tricyclic Antidepressants on Early-Life Stages of Common Carp (*Cyprinus carpio*). *Chemosphere* **2017**, *185*, 1072–1080. [CrossRef] [PubMed]
43. Chromcova, L.; Blahova, J.; Zivna, D.; Plhalova, L.; Casuscelli, F.; Tocco, D.; Divisova, L.; Prokes, M.; Faggio, C.; Tichy, F.; et al. NeemAzal T/S—Toxicity to Early-Life Stages of Common Carp (*Cyprinus carpio* L.). *Vet. Med.* **2015**, *60*, 23–30. [CrossRef]
44. Cappello, T.; De Marco, G.; Oliveri Conti, G.; Giannetto, A.; Ferrante, M.; Mauceri, A.; Maisano, M. Time-Dependent Metabolic Disorders Induced by Short-Term Exposure to Polystyrene Microplastics in the Mediterranean Mussel *Mytilus galloprovincialis*. *Ecotoxicol. Environ. Saf.* **2021**, *209*, 111780. [CrossRef]
45. Capolupo, M.; Franzellitti, S.; Valbonesi, P.; Lanzas, C.S.; Fabbri, E. Uptake and Transcriptional Effects of Polystyrene Microplastics in Larval Stages of the Mediterranean Mussel *Mytilus galloprovincialis*. *Environ. Pollut.* **2018**, *241*, 1038–1047. [CrossRef]
46. Gedik, K.; Eryaşar, A.R. Microplastic Pollution Profile of Mediterranean Mussels (*Mytilus galloprovincialis*) Collected along the Turkish Coasts. *Chemosphere* **2020**, *260*, 127570. [CrossRef]
47. Mancia, A.; Chenet, T.; Bono, G.; Geraci, M.L.; Vaccaro, C.; Munari, C.; Mistri, M.; Cavazzini, A.; Pasti, L. Adverse Effects of Plastic Ingestion on the Mediterranean Small-Spotted Catshark (*Scyliorhinus canicula*). *Mar. Environ. Res.* **2020**, *155*, 104876. [CrossRef]
48. Neves, D.; Sobral, P.; Ferreira, J.L.; Pereira, T. Ingestion of Microplastics by Commercial Fish off the Portuguese Coast. *Mar. Pollut. Bull.* **2015**, *101*, 119–126. [CrossRef]
49. Valente, T.; Sbrana, A.; Scacco, U.; Jacomini, C.; Bianchi, J.; Palazzo, L.; de Lucia, G.A.; Silvestri, C.; Matiddi, M. Exploring Microplastic Ingestion by Three Deep-Water Elasmobranch Species: A Case Study from the Tyrrhenian Sea. *Environ. Pollut.* **2019**, *253*, 342–350. [CrossRef]
50. López-López, L.; Preciado, I.; González-Irusta, J.M.; Arroyo, N.L.; Muñoz, I.; Punzón, A.; Serrano, A. Incidental Ingestion of Meso- and Macro-Plastic Debris by Benthic and Demersal Fish. *Food Webs* **2018**, *14*, 1–4. [CrossRef]
51. Bellas, J.; Martínez-Armental, J.; Martínez-Cámara, A.; Besada, V.; Martínez-Gómez, C. Ingestion of Microplastics by Demersal Fish from the Spanish Atlantic and Mediterranean Coasts. *Mar. Pollut. Bull.* **2016**, *109*, 55–60. [CrossRef]
52. Alomar, C.; Deudero, S.; Compa, M.; Guijarro, B. Exploring the Relation between Plastic Ingestion in Species and Its Presence in Seafloor Bottoms. *Mar. Pollut. Bull.* **2020**, *160*, 111641. [CrossRef]
53. Madgett, A.S.; Yates, K.; Webster, L.; McKenzie, C.; Brownlow, A.; Moffat, C.F. The Concentration and Biomagnification of PCBs and PBDEs across Four Trophic Levels in a Marine Food Web. *Environ. Pollut.* **2022**, *309*, 119752. [CrossRef] [PubMed]
54. Boudjema, K.; Badis, A.; Moulai-Mostefa, N. Study of Heavy Metal Bioaccumulation in *Mytilus galloprovincialis* (Lamark 1819) from Heavy Metal Mixture Using the CCF Design. *Environ. Technol. Innov.* **2022**, *25*, 102202. [CrossRef]
55. Mille, T.; Cresson, P.; Chouvelon, T.; Bustamante, P.; Brach-Papa, C.; Bruzac, S.; Rozuel, E.; Bouchoucha, M. Trace Metal Concentrations in the Muscle of Seven Marine Species: Comparison between the Gulf of Lions (North-West Mediterranean Sea) and the Bay of Biscay (North-East Atlantic Ocean). *Mar. Pollut. Bull.* **2018**, *135*, 9–16. [CrossRef] [PubMed]
56. Chouvelon, T.; Cresson, P.; Bouchoucha, M.; Brach-Papa, C.; Bustamante, P.; Crochet, S.; Marco-Miralles, F.; Thomas, B.; Knoery, J. Oligotrophy as a Major Driver of Mercury Bioaccumulation in Medium-to High-Trophic Level Consumers: A Marine Ecosystem-Comparative Study. *Environ. Pollut.* **2018**, *233*, 844–854. [CrossRef]
57. Marques, A.F.S.; Alves, L.M.F.; Moutinho, A.; Lemos, M.F.L.; Novais, S.C. *Scyliorhinus canicula* (Linnaeus, 1758) Metal Accumulation: A Public Health Concern for Atlantic Fish Consumers? *Mar. Pollut. Bull.* **2021**, *169*, 112477. [CrossRef]
58. Antelo, L.T.; Ordóñez-del Pazo, T.; Lopes, C.; Franco-Uría, A.; Pérez-Martín, R.I.; Alonso, A.A. Pollutant Levels in Discarded Fish Species by Spanish Trawlers Operating in the Great Sole Bank and the Atlantic Coast of the Iberian Peninsula. *Mar. Pollut. Bull.* **2016**, *108*, 303–310. [CrossRef]
59. Bouchoucha, M.; Chekri, R.; Leufroy, A.; Jitaru, P.; Millour, S.; Marchond, N.; Chafey, C.; Testu, C.; Zinck, J.; Cresson, P.; et al. Trace Element Contamination in Fish Impacted by Bauxite Red Mud Disposal in the Cassidaigne Canyon (NW French Mediterranean). *Sci. Total Environ.* **2019**, *690*, 16–26. [CrossRef]
60. Veron, A.; Dell'Anno, A.; Angelidis, M.O.; Aloupi, M.; Danovaro, R.; Radakovitch, O.; Poirier, A.; Heussner, S. Pollutant Pb Burden in Mediterranean Centroscymnus Coelolepis Deep-Sea Sharks. *Mar. Pollut. Bull.* **2022**, *174*, 113245. [CrossRef]
61. Ibor, O.R.; Andem, A.B.; Eni, G.; Arong, G.A.; Adeougn, A.O.; Arukwe, A. Contaminant Levels and Endocrine Disruptive Effects in Clarias Gariepinus Exposed to Simulated Leachate from a Solid Waste Dumpsite in Calabar, Nigeria. *Aquat. Toxicol.* **2020**, *219*, 105375. [CrossRef]
62. Aliko, V.; Multisanti, C.R.; Turani, B.; Faggio, C. Get Rid of Marine Pollution: Bioremediation an Innovative, Attractive, and Successful Cleaning Strategy. *Sustainability* **2022**, *14*, 11784. [CrossRef]

63. Auta, H.S.; Emenike, C.U.; Fauziah, S.H. Distribution and Importance of Microplastics in the Marine Environment: A Review of the Sources, Fate, Effects, and Potential Solutions. *Environ. Int.* **2017**, *102*, 165–176. [CrossRef]
64. Cincinelli, A.; Martellini, T.; Guerranti, C.; Scopetani, C.; Chelazzi, D.; Giarrizzo, T. A Potpourri of Microplastics in the Sea Surface and Water Column of the Mediterranean Sea. *Trends Anal. Chem.* **2019**, *110*, 321–326. [CrossRef]
65. Güven, O.; Gökdağ, K.; Jovanović, B.; Kıdeyş, A.E. Microplastic Litter Composition of the Turkish Territorial Waters of the Mediterranean Sea, and Its Occurrence in the Gastrointestinal Tract of Fish. *Environ. Pollut.* **2017**, *223*, 286–294. [CrossRef]
66. Conkle, J.L.; Báez Del Valle, C.D.; Turner, J.W. Are We Underestimating Microplastic Contamination in Aquatic Environments? *Environ. Manag.* **2018**, *61*, 1–8. [CrossRef]
67. Koelmans, A.A.; Redondo-Hasselerharm, P.E.; Nor, N.H.M.; de Ruijter, V.N.; Mintenig, S.M.; Kooi, M. Risk Assessment of Microplastic Particles. *Nat. Rev. Mater.* **2022**, *7*, 138–152. [CrossRef]
68. Filella, M. Questions of Size and Numbers in Environmental Research on Microplastics: Methodological and Conceptual Aspects. *Environ. Chem.* **2015**, *12*, 527. [CrossRef]
69. Llorca, M.; Álvarez-Muñoz, D.; Ábalos, M.; Rodríguez-Mozaz, S.; Santos, L.H.M.L.M.; León, V.M.; Campillo, J.A.; Martínez-Gómez, C.; Abad, E.; Farré, M. Microplastics in Mediterranean Coastal Area: Toxicity and Impact for the Environment and Human Health. *Trends Environ. Anal. Chem.* **2020**, *27*, e00090. [CrossRef]
70. Ma, J.; Zhao, J.; Zhu, Z.; Li, L.; Yu, F. Effect of Microplastic Size on the Adsorption Behavior and Mechanism of Triclosan on Polyvinyl Chloride. *Environ. Pollut.* **2019**, *254*, 113104. [CrossRef]
71. Kik, K.; Bukowska, B.; Sicińska, P. Polystyrene Nanoparticles: Sources, Occurrence in the Environment, Distribution in Tissues, Accumulation and Toxicity to Various Organisms. *Environ. Pollut.* **2020**, *262*, 114297. [CrossRef]
72. Miller, M.E.; Hamann, M.; Kroon, F.J. Bioaccumulation and Biomagnification of Microplastics in Marine Organisms: A Review and Meta-Analysis of Current Data. *PLoS ONE* **2020**, *15*, e0240792. [CrossRef]
73. Bråte, I.L.N.; Blázquez, M.; Brooks, S.J.; Thomas, K.V. Weathering Impacts the Uptake of Polyethylene Microparticles from Toothpaste in Mediterranean Mussels (*M. galloprovincialis*). *Sci. Total Environ.* **2018**, *626*, 1310–1318. [CrossRef] [PubMed]
74. Trestrail, C.; Walpitagama, M.; Miranda, A.; Nugegoda, D.; Shimeta, J. Microplastics Alter Digestive Enzyme Activities in the Marine Bivalve, *Mytilus galloprovincialis*. *Sci. Total Environ.* **2021**, *779*, 146418. [CrossRef] [PubMed]
75. Picot-Groz, M.; Fenet, H.; Martinez Bueno, M.J.; Rosain, D.; Gomez, E. Diurnal Variations in Personal Care Products in Seawater and Mussels at Three Mediterranean Coastal Sites. *Environ. Sci. Pollut. Res.* **2018**, *25*, 9051–9059. [CrossRef] [PubMed]
76. Montes-Grajales, D.; Fennix-Agudelo, M.; Miranda-Castro, W. Occurrence of Personal Care Products as Emerging Chemicals of Concern in Water Resources: A Review. *Sci. Total Environ.* **2017**, *595*, 601–614. [CrossRef]
77. Sadutto, D.; Andreu, V.; Ilo, T.; Akkanen, J.; Picó, Y. Pharmaceuticals and Personal Care Products in a Mediterranean Coastal Wetland: Impact of Anthropogenic and Spatial Factors and Environmental Risk Assessment. *Environ. Pollut.* **2021**, *271*, 116353. [CrossRef]
78. Pinheiro, M.; Martins, I.; Raimundo, J.; Caetano, M.; Neuparth, T.; Santos, M.M. Stressors of Emerging Concern in Deep-Sea Environments: Microplastics, Pharmaceuticals, Personal Care Products and Deep-Sea Mining. *Sci. Total Environ.* **2023**, *876*, 162557. [CrossRef]
79. Hawash, H.B.; Moneer, A.A.; Galhoum, A.A.; Elgarahy, A.M.; Mohamed, W.A.A.; Samy, M.; El-Seedi, H.R.; Gaballah, M.S.; Mubarak, M.F.; Attia, N.F. Occurrence and Spatial Distribution of Pharmaceuticals and Personal Care Products (PPCPs) in the Aquatic Environment, Their Characteristics, and Adopted Legislations. *J. Water Process Eng.* **2023**, *52*, 103490. [CrossRef]
80. Brausch, J.M.; Rand, G.M. A Review of Personal Care Products in the Aquatic Environment: Environmental Concentrations and Toxicity. *Chemosphere* **2011**, *82*, 1518–1532. [CrossRef]
81. Fisher, M.; MacPherson, S.; Braun, J.M.; Hauser, R.; Walker, M.; Feeley, M.; Mallick, R.; Bérubé, R.; Arbuckle, T.E. Paraben Concentrations in Maternal Urine and Breast Milk and Its Association with Personal Care Product Use. *Environ. Sci. Technol.* **2017**, *51*, 4009–4017. [CrossRef]
82. Freitas, R.; Silvestro, S.; Coppola, F.; Costa, S.; Meucci, V.; Battaglia, F.; Intorre, L.; Soares, A.M.V.M.; Pretti, C.; Faggio, C. Toxic Impacts Induced by Sodium Lauryl Sulfate in *Mytilus galloprovincialis*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2020**, *242*, 110656. [CrossRef]
83. Yeltekin, A.Ç.; Oğuz, A.R. Toxic Effects of Sodium Lauryl Sulfate on Antioxidant Defense System and DNA Damage in Fish Primary Hepatocyte Cultures. *Maced. Vet Rev.* **2022**, *45*, 169–175. [CrossRef]
84. Walker, C.H.; Sibly, R.M.; Sibly, R.M.; Peakall, D.B. *Principles of Ecotoxicology*; CRC Press: Boca Raton, FL, USA, 2005; ISBN 9780429109515.
85. Martins, M.F.; Costa, P.G.; Bianchini, A. Maternal Transfer of Pharmaceuticals and Personal Care Products in the Brazilian Guitarfish *Pseudobatos horkelii*. *Environ. Adv.* **2022**, *8*, 100228. [CrossRef]
86. Martins, M.F.; Costa, P.G.; Bianchini, A. Maternal Transfer of Polycyclic Aromatic Hydrocarbons in an Endangered Elasmobranch, the Brazilian Guitarfish. *Chemosphere* **2021**, *263*, 128275. [CrossRef]
87. Martins, M.F.; Costa, P.G.; da Guerreiro, A.S.; Bianchini, A. Consequences of Prenatal Exposure to Contaminants in Elasmobranchs: Biochemical Outcomes during the Embryonic Development of *Pseudobatos horkelii*. *Environ. Pollut.* **2023**, *323*, 121276. [CrossRef]
88. Desbiolles, F.; Malleret, L.; Tiliacos, C.; Wong-Wah-Chung, P.; Laffont-Schwob, I. Occurrence and Ecotoxicological Assessment of Pharmaceuticals: Is There a Risk for the Mediterranean Aquatic Environment? *Sci. Total Environ.* **2018**, *639*, 1334–1348. [CrossRef]

89. Feo, M.L.; Bagnati, R.; Passoni, A.; Riva, F.; Salvagio Manta, D.; Sprovieri, M.; Traina, A.; Zuccato, E.; Castiglioni, S. Pharmaceuticals and Other Contaminants in Waters and Sediments from Augusta Bay (Southern Italy). *Sci. Total Environ.* **2020**, *739*, 139827. [CrossRef]
90. Yuan, M.; Faggio, C.; Perugini, M.; Aliko, V.; Wang, Y. Editorial: Pharmaceuticals, Personal Care Products and Endocrine Disrupting Chemicals: The Physiological Consequences of Exposure to Pollutants in Aquatic Animals. *Front. Physiol.* **2023**, *14*, 122. [CrossRef]
91. Gelsleichter, J.; Szabo, N.J. Uptake of Human Pharmaceuticals in Bull Sharks (*Carcharhinus leucas*) Inhabiting a Wastewater-Impacted River. *Sci. Total Environ.* **2013**, *456–457*, 196–201. [CrossRef]
92. Corsolini, S.; Guerranti, C.; Perra, G.; Focardi, S. Polybrominated Diphenyl Ethers, Perfluorinated Compounds and Chlorinated Pesticides in Swordfish (*Xiphias gladius*) from the Mediterranean Sea. *Environ. Sci. Technol.* **2008**, *42*, 4344–4349. [CrossRef]
93. Triassi, M.; Nardone, A.; Giovinetti, M.C.; De Rosa, E.; Canzanella, S.; Sarnacchiaro, P.; Montuori, P. Ecological Risk and Estimates of Organophosphate Pesticides Loads into the Central Mediterranean Sea from Volturno River, the River of the "Land of Fires" Area, Southern Italy. *Sci. Total Environ.* **2019**, *678*, 741–754. [CrossRef]
94. Comoretto, L.; Chiron, S. Comparing Pharmaceutical and Pesticide Loads into a Small Mediterranean River. *Sci. Total Environ.* **2005**, *349*, 201–210. [CrossRef] [PubMed]
95. Stara, A.; Kubec, J.; Zuskova, E.; Buric, M.; Faggio, C.; Kouba, A.; Velisek, J. Effects of S-Metolachlor and Its Degradation Product Metolachlor OA on Marbled Crayfish (*Procambarus virginalis*). *Chemosphere* **2019**, *224*, 616–625. [CrossRef] [PubMed]
96. Sánchez-Bayo, F. Insecticides Mode of Action in Relation to Their Toxicity to Non-Target Organisms. *J. Environ. Anal. Toxicol.* **2012**, *s4-002*. [CrossRef]
97. Bado-Nilles, A.; Gagnaire, B.; Thomas-Guyon, H.; Le Floch, S.; Renault, T. Effects of 16 Pure Hydrocarbons and Two Oils on Haemocyte and Haemolymphatic Parameters in the Pacific Oyster, *Crassostrea gigas* (Thunberg). *Toxicol. Vitr.* **2008**, *22*, 1610–1617. [CrossRef]
98. Matozzo, V.; Zampieri, C.; Munari, M.; Marin, M.G. Glyphosate Affects Haemocyte Parameters in the Clam *Ruditapes philippinarum*. *Mar. Environ. Res.* **2019**, *146*, 66–70. [CrossRef]
99. Del Giudice, G.; Prisco, M.; Agnese, M.; Verderame, M.; Rosati, L.; Limatola, E.; Andreuccetti, P. Effects of Nonylphenol on Vitellogenin Synthesis in Adult Males of the Spotted Ray Torpedo Marmorata. *J. Fish. Biol.* **2012**, *80*, 2112–2121. [CrossRef]
100. Marsili, L.; Coppola, D.; Giannetti, M.; Casini, S.; Fossi, M.C.; Van Wyk, J.H.; Sperone, E.; Tripepi, S.; Micarelli, P.; Rizzuto, S. Skin Biopsies as a Sensitive Non-Lethal Technique for the Ecotoxicological Studies of Great White Shark (*Carcharodon carcharias*) Sampled in South Africa. *Expert Opin. Environ. Biol.* **2016**, *4*, 2. [CrossRef]
101. Storelli, M.M.; Marcotrigiano, G.O. Bioindicator Organisms: Heavy Metal Pollution Evaluation in the Ionian Sea (Mediterranean Sea—Italy). *Environ. Monit. Assess.* **2005**, *102*, 159–166. [CrossRef]
102. Salvo, A.; Potortì, A.G.; Cicero, N.; Bruno, M.; Lo Turco, V.; Di Bella, G.; Dugo, G. Statistical Characterisation of Heavy Metal Contents in *Paracentrotus lividus* from Mediterranean Sea. *Nat. Prod. Res.* **2014**, *28*, 718–726. [CrossRef]
103. Kemp, M.; Wepener, V.; de Kock, K.N.; Wolmarans, C.T. Metallothionein Induction as Indicator of Low Level Metal Exposure to Aquatic Macroinvertebrates from a Relatively Unimpacted River System in South Africa. *Bull. Environ. Contam. Toxicol.* **2017**, *99*, 662–667. [CrossRef]
104. Riani, E.; Cordova, M.R.; Arifin, Z. Heavy Metal Pollution and Its Relation to the Malformation of Green Mussels Cultured in Muara Kamal Waters, Jakarta Bay, Indonesia. *Mar. Pollut. Bull.* **2018**, *133*, 664–670. [CrossRef]
105. Kwon, Y.-K.; Jung, Y.-S.; Park, J.-C.; Seo, J.; Choi, M.-S.; Hwang, G.-S. Characterizing the Effect of Heavy Metal Contamination on Marine Mussels Using Metabolomics. *Mar. Pollut. Bull.* **2012**, *64*, 1874–1879. [CrossRef]
106. Vlahogianni, T.H.; Valavanidis, A. Heavy-Metal Effects on Lipid Peroxidation and Antioxidant Defence Enzymes in Mussels *Mytilus galloprovincialis*. *Chem. Ecol.* **2007**, *23*, 361–371. [CrossRef]
107. Bonsignore, M.; Salvagio Manta, D.; Oliveri, E.; Sprovieri, M.; Basilone, G.; Bonanno, A.; Falco, F.; Traina, A.; Mazzola, S. Mercury in Fishes from Augusta Bay (Southern Italy): Risk Assessment and Health Implication. *Food Chem. Toxicol.* **2013**, *56*, 184–194. [CrossRef]
108. Reinero, F.R.; Milazzo, C.; Minervino, M.; Marchio, C.; Filice, M.; Bevacqua, L.; Giglio, G.; Leonetti, F.L.; Micarelli, P.; Tripepi, S.; et al. Parasitic Load, Hematological Parameters, and Trace Elements Accumulation in the Lesser Spotted Dogfish *Scyliorhinus canicula* from the Central Tyrrhenian Sea. *Biology* **2022**, *11*, 663. [CrossRef]
109. Humbert, J.-F.; Dorigo, U. Biodiversity and Aquatic Ecosystem Functioning: A Mini-Review. *Aquat. Ecosyst. Health Manag.* **2005**, *8*, 367–374. [CrossRef]

CHAPTER II

Postharvest Technologies and Enhancing the Quality of Seafood

As mentioned in the introduction to this thesis, this second chapter focuses on enhancing the quality of seafood, understood as a tool to guarantee consumers increasingly healthy, safe and traceable fishery and aquaculture products. At the same time, fishermen are provided with new tools for innovating onboard storage and packaging. All of this is in line with a holistic approach to the use of fishery resources (sensu lato): by combining the three pillars of sustainability (environmental, social and economic), it is possible to meet the needs of the present without compromising the ability of future generations of fishermen to meet their own needs.

The following data and publications were produced during my three-year doctoral research studies.

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 27 July 2022
DOI 10.3389/fnut.2022.955216

OPEN ACCESS

EDITED BY
James Owusu-Kwarteng,
University of Energy and Natural Resources, Ghana

REVIEWED BY
Eduardo Esteves,
University of Algarve, Portugal
Shengjun Chen,
South China Sea Fisheries Research Institute (CAFS), China

*CORRESPONDENCE
Gioacchino Bono
gioacchino.bono@cnr.it

SPECIALTY SECTION
This article was submitted to Nutrition and Sustainable Diets, a section of the journal Frontiers in Nutrition

RECEIVED 28 May 2022
ACCEPTED 04 July 2022
PUBLISHED 27 July 2022

CITATION
Rusanova P, Bono G, Dara M, Falco F, Gancitano V, Lo Brutto S, Okpala COR, Nirmal NP, Quattrocchi F, Sardo G and Hassoun A (2022) Effect of different packaging methods on the free amino acid profiles of the deep-water rose shrimp (*Parapenaeus longirostris*) during frozen storage. *Front. Nutr.* 9:955216. doi: 10.3389/fnut.2022.955216

Effect of different packaging methods on the free amino acid profiles of the deep-water rose shrimp (*Parapenaeus longirostris*) during frozen storage

Polina Rusanova[1,2], Gioacchino Bono[1]*, Manuela Dara[3], Francesca Falco[1], Vita Gancitano[1], Sabrina Lo Brutto[4], Charles Odilichukwu R. Okpala[5], Nilesh Prakash Nirmal[6], Federico Quattrocchi[1], Giacomo Sardo[1] and Abdo Hassoun[7,8]

[1]Institute for Biological Resources and Marine Biotechnologies, National Research Council (IRBIM-CNR), Mazara del Vallo, Italy, [2]Department of Biological, Geological and Environmental Sciences (BiGeA)–Marine Biology and Fisheries Laboratory of Fano (PU), University of Bologna, Bologna, Italy, [3]Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Rome, Italy, [4]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy, [5]Department of Functional Food Products Development, Faculty of Biotechnology and Food Science, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland, [6]Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand, [7]Sustainable AgriFoodtech Innovation & Research (SAFIR), Arras, France, [8]Syrian Academic Expertise (SAE), Gaziantep, Turkey

The composition of free amino acids (FAAs) in seafood products contributes to characterizing their flavor, as well as freshness and quality during storage. Deep-water rose shrimps (*Parapenaues longirostris*, Lucas, 1846) (DWRS) are being increasingly harvested in the Mediterranean Sea, and the captured specimens are quickly frozen onboard fishing trawlers to preserve freshness and post-harvest quality. Here, we quantified the FAA profiles of DWRS packaged using five methods: (1) 100% N_2; (2) vacuum; (3) 50% N_2 + 50% CO_2; (4) commercial anhydrous sodium sulfite; and (5) air (control). All samples were quickly frozen at −35°C and stored for 12 months at −18°C. Arginine (661 mg/100 g), proline (538 mg/100 g), and glycine (424 mg/100 g) were the most abundant FAAs, whereas the least abundant were tyrosine (67 mg/100 g), histidine (58 mg/100 g), and aspartic acid (34 mg/100 g). FAAs in all samples gradually (and significantly) increased in the first 6 to 8 months of storage, and then significantly decreased. The sodium sulfite treatment (Method 4) kept the initial FAA contents lower than the other treatments, due to the strong antioxidant action of sulfite agents. Interestingly, similar results were obtained for vacuum packaging (Method 2). Thus, combining frozen storage with vacuum packaging represents an alternative approach to chemical additives in shrimp/prawn processing to meet the increasing demand for high-quality seafood products with long shelf-life.

KEYWORDS

shrimp, free amino acids, modified atmosphere packaging, vacuum, sulfites, freezing shelf-life, seafood quality

Introduction

Free amino acids (FAAs) are among the most essential fractions of non-protein nitrogen compounds found in the tissues and muscles of several seafood products (1–3). Some FAAs, such as alanine, glycine, lysine, and taurine occur at relatively high concentrations in fishery products and are the basis for a balanced healthy diet (4). Many important fractions of non-protein nitrogenous compounds could be used as indicators of food product spoilage, as they are the precursors of biogenic amines (5). FAAs also determine the sweetness, sourness, bitterness, and umami taste of fish products (3, 6). For instance, glycine, alanine, and glutamic acid are associated with the typical "umami" taste in crustacean products (1). In particular, FAAs can interact with reducing sugars, which is demonstrated *via* the Maillard reaction or non-enzymatic browning in seafood products, altering aroma, color, and taste (3). The composition of FAAs in seafood products inevitably changes during storage as a function of food packaging and processing technologies (3).

In the Mediterranean region, after harvesting, shrimp products are preserved directly onboard fishing trawlers using air blast freezing techniques (at −35°C), followed by storage at −18°C in a static freezer (7). Although the freezing process prevents bacterial activity, biochemical processes continue to take place at a very slow rate during storage, irreversibly altering the freshness and flavor (8). In addition, the preservative success of freezing is affected by the size and muscle structure of fishery products (7, 9). Bono et al. (7, 10, 11) showed that the ability of MAPs to prevent fishery products from deteriorating increases when they are combined with other preservation methods, such as freezing. For instance, Bono et al. (10) showed that lipid oxidation and volatile amines were significantly reduced in DWRS when MAP was combined with frozen storage. Yet, the freshness of a fish product varies noticeably depending on packaging method (N_2, CO_2, or vacuum package), storage temperature, and species. Therefore, it is important to quantify FAAs from a production perspective of biogenic amines, due to the capacity of MAP/vacuum package to preserve the FAA profile of seafood products (7), as well as documented changes in FAA concentrations during processing and storage (12).

In countries bordering the Mediterranean Sea (i.e., Italy, France, Spain, Algeria, Tunisia, Greece, and Turkey), DWRS are one of the most highly harvested crustaceans (13–15). DWRS are usually caught by bottom trawling vessels, and are quickly frozen onboard to preserve the high quality of the fresh harvest. However, dark discoloration that arises post mortem makes the shrimp unacceptable for purchase by consumers (16). Previous studies demonstrated that post mortem discoloration could be prevented by pretreating DWRS with various chemical approaches, such as those using chemical antioxidants (e.g., resorcinol, sulfites), melanosis-inhibiting formulations, phenolic extract derived from olive vegetation water, or applying non-thermal processes, such as MAP combined with frozen storage (10, 17–19). Quantifying FAAs in fishery products could advance our current understanding of various autolytic processes, as well as muscle degradation, which decisively influences FAA evolution (20, 21). Investigating how FAA levels in DWRS change under frozen storage combined with MAP/vacuum could help promote the use of this approach by the fishermen and associated stakeholders in the Mediterranean basin. It might also provide a baseline for assessing general risks to health from consuming seafood products packaged under different methods and conditions.

Thus, here, we aimed to determine FAA content in DWRS after harvesting and to assess the impact of five different packaging methods, followed by frozen storage at −18°C for 12 months on FAA profiles.

Materials and methods

Overview of the experimental program

An overview of the experimental program is presented in Figure 1. This schematic representation depicts the major stages of this work, including harvesting DWRS samples by bottom trawl vessels, onboard collection, handling, packaging protocols, and analytical determination of FAAs. FAA analysis was initiated on day 6 after the catch and onboard packaging of DWRS, due to the time required for landing and transfer of samples to the laboratory. Thus, "time zero" captured this delay used in the following sections and graphs. Before laboratory analyses, DWRS samples were thawed, beheaded, and peeled. All chemicals and reagents employed were of analytical grade.

Onboard handling, packaging, and frozen storage of shrimp

Handling and packaging

DWRS samples were processed directly onboard a shrimp trawler, according to the method described by Bono et al. (7, 10) with minor modifications. A bottom trawler equipped with a semi-automatic MAP system (Mondini, Brescia, Italy) was used to collect 50 kg of shrimp during a single haul (to remove any bias caused by sampling time and/or fishing operation). Shrimp had an average carapace length of 22 ± 5 mm. Shrimp were washed in flowing seawater and pre-chilled (1 ± 0.5°C) within 1 h of capture, by dipping them in a 1:1 mixture of seawater and ice. After approximately 15 min, when the core temperature of the shrimp was equal to the temperature of the ice-seawater mixture, the ice water was drained from the shrimp, and the shrimp were randomly separated into five lots for the five treatment/packaging protocols (Figure 1):

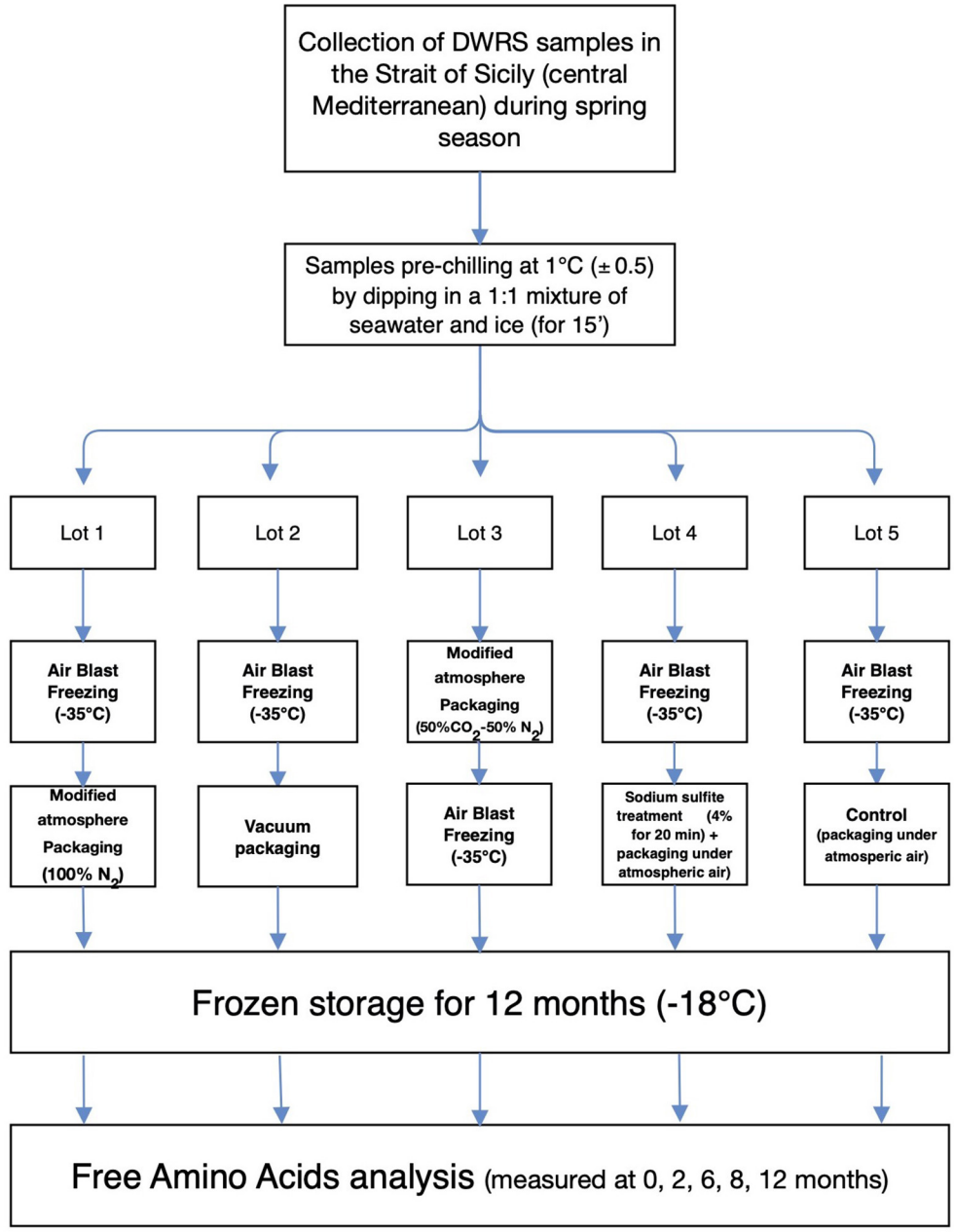

FIGURE 1
Schematic representation showing an overview of the experimental program, starting from the harvesting of DWRS samples by bottom trawl vessels to the analytical determination of FAAs.

- Lot 1 was quickly frozen in an air blast freezer room at −35°C (air speed of 4–5 m/s). When the water at the thermal center of the DWRS samples became ice, they were packaged under 100% N_2 gas using the semi-automatic MAP system;
- Lot 2 was frozen in air in the blast freezer room at −35°C (similar to Lot 1). It was then vacuum-packaged using the semi-automatic MAP system;
- Lot 3 was packaged under 50% N_2 + 50% CO_2 gas mixture to allow the full dissolution of CO_2 in the shrimp tissue (22), and was then frozen in the air blast freezer room at −35°C;
- Lot 4 was dipped in seawater solution (4% w/v) of commercial anhydrous sodium sulfite (shrimp-to-dipping solution ratio of 1:4), according to the preservation techniques and materials usually employed

by Mediterranean fleet crews. After 20 min dipping, shrimp were dried, frozen in the air blast freezer room at −35°C, and finally packed under atmospheric air using the semi-automatic MAP system;

- Lot 5 was frozen in the air blast freezer room at −35°C with no treatment, packed under atmospheric air using the semi-automatic MAP system and served as the control for the current study.

Packaging materials

DWRS samples were packaged using the method previously described by Bono et al. (7). Transparent A.PET/EVOH/PE barrier bags (bag size: 290 × 200 mm; bag volume: 1.8 L; laminate density: 1.39 g/cm^3; thickness: 500 μm) (Arcoplastica Srl, Andezeno, Italy) were used for packaging, and were manufactured with oxygen (O_2) and water vapor permeabilities of 1.8 cm^3 m^{-2} day^{-1} atm^{-1} and 4 g m^{-2} day^{-1}, respectively. Packaging bags were heat-sealed by a semi-automatic packaging machine (Mondini S.p.A., Brescia, Italy), which employed a multiflex OPP/EVOH/PE (Cryovac, Sealed Air Corp., Italy) film (weight: 72 g m^2; thickness: 75 μm) that operated O_2, CO_2, and water vapor permeabilities of 3 cm^3 m^{-2} day^{-1} atm^{-1}, 10 cm^3 m^{-2} day^{-1} atm^{-1} and 3 g m^{-2} day^{-1}, respectively. For MAP samples, a gas-to-product ratio (v/w) of 2.25:1 was applied. An additional anti-pinhole lamina (weight: 340 g/m^{-2}; thickness: 250 μm) was inserted in the headspace, which helped to minimize damage to the multiflex film by shrimp horns.

Frozen storage

When all packaging operations onboard were complete, all packaged DWRS samples were subjected to frozen storage (−18°C) for 12 months. According to Blond and le Meste (23), this is the optimum storage temperature when considering both the financial costs of freezing and shelf-life of frozen foods. The frozen storage of DWRS samples adhered consistently to widely recognized storage regulations in Europe and other countries globally, particularly for the temperatures of frozen foods (7).

Determination of FAA profiles

The FAAs were analyzed, from extraction to chromatography, according to a previously described method (7, 24), with minor modifications. Ten grams of shrimp and 40 mL extracting solvent (75% methanol in distilled deionized water) were homogenated, transferred to a 100 mL volumetric flask, and stored for 60 min at 4°C. The contents of the flask were transferred to a 50 mL centrifuge tube and centrifuged at 15,000 rpm for 40 min at 4°C. The supernatant was filtered on a PTFE 0.2 μm filter membrane (Gelman Sciences), before derivatization. By way of o-phthaldialdehyde (OPA) pre-column derivatization, the OPA Thiol Reagent (OPT) was prepared 24 h before use by dissolving 27 mg of o-phthaldialdehyde in 500 μL absolute alcohol. Then, 5 mL of 0.1 M sodium tetraborate ($Na_2B_4O_7$,10H_2O) (pH 9.5) were added, followed by 50 μL mercaptoethanol, which were mixed and stored in the dark. The amino acid standard stock solution was prepared by dissolving the equivalent of 2,500 nmol of each amino acid in 0.05 M NaH_2PO_4 buffer (pH 5.5), which was then diluted for the calibration curve. Then, 400 μL of OPT was added to 100 μL of the amino acid standard or 189 diluted sample supernatant under High Pressure Liquid Chromatography (HPLC), and the sample was manually injected onto the column of the HPLC system. Mobile phase A was made up of 0.05 M sodium phosphate buffer (pH 5.5), methanol, and tetrahydrofuram (THF) (80: 19:1). Mobile phase B was made up of 80% methanol and 20% of the 0.05 M NaH_2PO_4 buffer. The pH of the phosphate buffer was adjusted to 5.5. The mobile phases were filtered through 0.2 μm filter membranes (Gelman Sciences, Ann Arbor, MI) and were degassed by vacuum for 5 min. The HPLC column was an Ultrasphere ODS with 5 μm particle size, 4.6 mm × 25 cm (Beckman Instruments, Inc., Fullerton, CA). The elution gradient was generated using an Elite LaChrom equipped with a L-7100 pump (LaChrom, Hitachi), oven L-7350 (LaChrom, Merck), programmable fluorescence detector, which had an excitation monochromator setting of 330 nm and emission cut-off filter of 418 nm. Chromatographic data were processed using software EZChrom Elite (Agilent Tech., Santa Clara, CA 95051, USA). The FAA results are presented as the mean of four replicates ± standard deviation and expressed in mg/100 g.

Statistical analyses

For each FAA, we tested the differences between treatments and the existence of linear and simple polynomial trend as function of the storage time. Ordinary least square models were used introducing the treatment as factor (fixed with 5 levels) and time as continuous variable. Models were fitted in which the highest order polynomial term can be linear, quadratic, or cubic function of time. This analysis, also called trend analysis, is basically an extended ANCOVA which allows us to model quantitative predictors with higher-order polynomials. Fitting a polynomial allowed us to express the impact of the continuous predictor (time in this case) on the response to separately evaluate the contributions of linear and non-linear components of the polynomial. The best formulation was chosen using the small-sample equivalent Akaike Information Criterion (AICc). A Tukey test was used to perform pairwise comparisons between levels of the factor treatment when it was significant in the regression analysis.

We used heat maps to visualize, graphically and simultaneously, clusters of FAA concentrations across

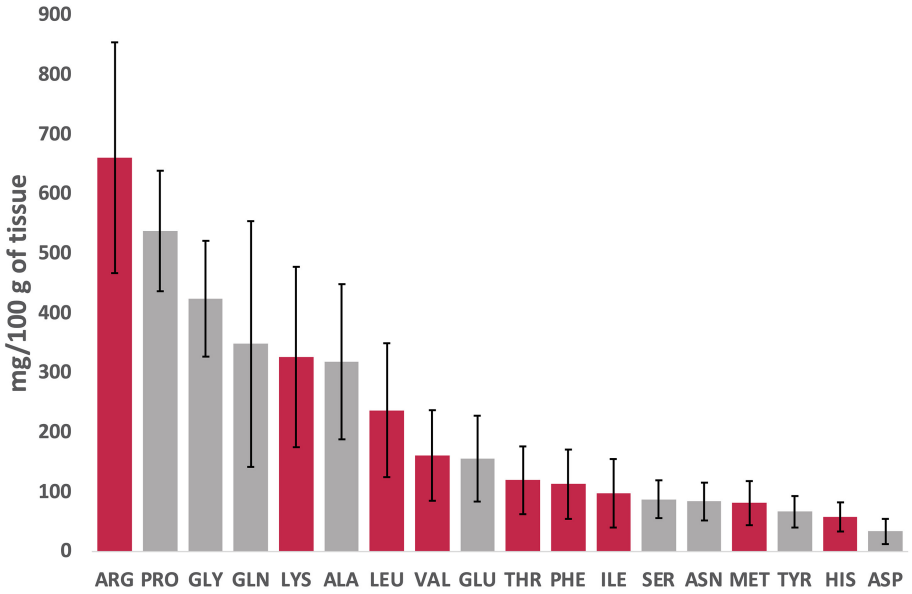

FIGURE 2
Overview of free amino acids (FAAs) concentrations (essential, red; non-essential, gray) in DWRS arranged in decreasing order (left to right), regardless of the effect of storage time (12 months at −18°C) or packaging method.

treatments and storage time. Hierarchical clustering on the Euclidean dissimilarity matrix was first performed on both the treatments and time storage, using the complete linkage method in which the distances between two clusters are defined as the maximum value of all pairwise distances between elements in of different clusters. Then, it was visualized by re-ordering the observations based on their similarity according to the hierarchical clustering results. The heat maps were constructed by using the "heatmap" functions in the "stats" package which is part of R statistical software (37).

Results and discussion

Overall snapshot of FAAs

Figure 2 presents a snapshot of each FAA (essential and non-essential AAs are presented in red and gray, respectively) in DWRS caught in the Strait of Sicily during spring season. The data are arranged in decreasing order (from left to right), regardless of the effects of storage time (12 months at −18°C) and the five packaging methods.

Three FAAs constituted more than 40% of total FAAs (Figure 2). Arginine was the most prominent (661 mg/100 g; 17% of total), followed by proline (538 mg/100 g equal to 14%) and glycine (424 mg/100 g equal to 11%). Aspartic acid, histidine, and tyrosine had the least concentrations. The high levels of glycine and arginine detected corroborated that reported for the Norway lobster *Nephrops norvegicus* (Linnaeus 1758) in the same region (11), and prawns (25). The ratio of essential amino acids (EAAs) to non-essential amino acids (NEAAs) was 0.9 (data not shown), which was higher than that reported by Iwasaki and Harada (26) for many other seafood products (0.7). According to Gómez-Limia et al. (3), the amount of EAAs is an important factor that affects the nutritional value of proteins.

High levels of typical (sweet) FAAs such as arginine, proline, and glycine (27) detected in this study might be associated with the high acceptability and palatability, particularly for shrimp and lobsters. In addition, high quantities of FAAs detected in DWRS (such as glutamic acid, glutamine, lysine and arginine) could exhibit anxiolytic-like and/or antidepressant-like activity, and might reduce corticosterone and cortisol levels in stressed animals (28). There was also a noticeable variation for given FAAs (which was more marked in some), which might be associated with storage time (one year) and/or packaging method.

According to Chen and Zhang (27) and Dai et al. (29), FAAs can be grouped into two main categories: pleasant FAAs (PFAAs), including threonine, glutamic acid, aspartic acid, alanine, glycine, serine, proline and arginine, and unpleasant FAAs (UPFAAs), including leucine, phenylalanine, isoleucine, lysine, valine, methionine, tyrosine, histidine and cysteine. Based on this classification, Figure 3 shows the trends in total FAAs, total PFAAs, and total UPFAAs in DWRP after 12 months of storage at −18°C, regardless of the effects of packaging. The total FAA content of all five packaging methods increased over the treatment period (start: 2,781 mg/100 g; after 6 months storage:

10.3389/fnut.2022.955216

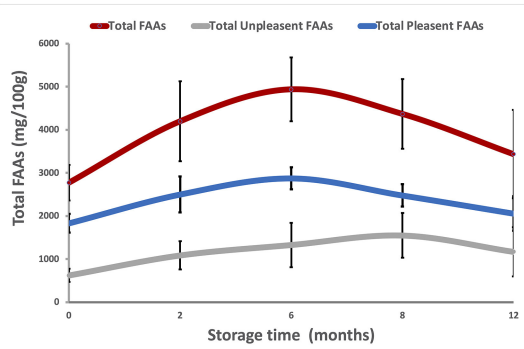

FIGURE 3
Trends in total FAAs (red), pleasant FAAs (PFAAs, blue), and unpleasant FAAs (UPFAAs, gray) in DWRP taking frozen storage time into account (12 months at −18°C), regardless of the effects of packaging methods.

4,937 mg/100 g). Then, the total FAAs content decreased slightly until the end of storage (12 months; mean 3,535 mg/100 g). The increase of total FAAs during the first/eight months of storage might be associated with the degradation of proteins *via* endogenous protease activity (30). Over the 12-month period, mean PFAA content ranged between 1,832 and 2,058 mg/100 g in the five groups. Similarly, over the 12 month period, total UPFAA concentrations ranged between 624 and 1,171 mg/100 g. Comparing our results with those in existing literature, the zero timepoint FAA observation in our study was about 30% higher than that previously recorded for the same species (31). This difference might be explained by the 6 days of time-lapse between the shrimps catch and the FAAs analysis or attributed to geographical factors (i.e., different fishing areas; Central Mediterranean vs. East Atlantic Ocean) or biological factors (i.e., trophic conditions, catch period, size and developmental stage of specimens, quantity and distribution of lipids in muscle, especially in lean specimens).

Changes to FAAs during frozen storage time

Trends in the concentrations of each essential and non-essential FAA (pleasant, unpleasant and flat/tasteless) in DWRS samples per packaging/treatment method and frozen storage time were evaluated (Figures 4, 5, respectively). The concentrations of each FAA differed with packaging method, several of which depicted diverse non- and bow-like shapes. On day 6 after catch (time 0 on the graph), the concentrations of some FAAs already exhibited significant differences among the five packaging methods, especially for both sulfited and vacuum lots when compared to N_2, N_2/CO_2, and control. Thus, FAAs appear to change immediately after the catch, with preservative treatment having a strong effect.

During the first 6–8 months of frozen storage time, all FAAs (essential/non-essential, pleasant/unpleasant), except aspartic acid, showed a gradual significant increase, and then declined at 8–12 months ($p < 0,05$). Pleasant FAAs that constituted over 40% of total FAAs (i.e., arginine (Figure 4C), proline (Figure 5H), and glycine (Figure 5E) exhibited a similar pattern, rising until the sixth month, and declining until the 12th month of frozen storage, with ultimate values being slightly lower than those detected at zero time. The similarity among arginine, proline, glycine, and glutamine was also confirmed by the heat-map coupled with cluster analysis depicted in Figure 6 in which the hierarchical clustering was applied only on the FAAs. Proline appeared to be strongly correlated with glycine, while glutamine (one of flat/tasteless FAAs) was correlated with arginine (red cluster). On the other hand, Figure 7 showed the hierarchical clustering applied on both the treatments and FAAs.

When considering the effect of treatments on each FAA, the cluster analysis also highlighted three other groupings. Threonine, phenylalanine, isoleucine, valine, and glutamic acid were clustered together to form a second group (yellow lines). Serine, asparagine, methionine, tyrosine, histidine, and aspartic acid (green lines) were clustered together to form a third group. Lysine, alanine, and leucine (violet lines) were clustered together to form a fourth group.

Hong et al. (32) found that the loss of quality is generally accompanied by an increase in glycine, whereas higher glycine levels denote high quality and acceptability of fish products. Certain FAAs (such as arginine, proline, and glycine) have important roles in the freshness and palatability of fish and shellfish muscle. Our results showed that DWRS samples remained in good condition throughout the 12 months of storage. The best condition for DWRS samples was reached at the sixth month, when arginine, proline, and glycine approached maximum values (>500 mg/100 g) (27, 33).

Protein degradation by endogenous protease should also be evaluated when exploring shelf-life during frozen storage, as it caused FAAs to increase, particularly at 6–8 months of storage. Furthermore, protein degradation is impacted by the rate of freezing, effect of ice crystals, and packaging method. The general reduction in FAAs observed in the last 3 months, reflected that also recorded for Norway lobster by Bono et al. (11). This phenomenon might be attributed to decarboxylation by decarboxylase enzymes, and the consequent formation of breakdown products such as biogenic amines (34, 35) that can cause relevant modifications in the sensory properties, nutritional and safety quality of seafood products with negative effects on consumers health. Indeed, consuming seafood products with high levels of histamine, tyramine and cadaverine (formed by decarboxylation of histidine, tyrosine and lysine, respectively) can produce some acute forms of urticaria, vomiting and diarrhea, as well as could be involved in more

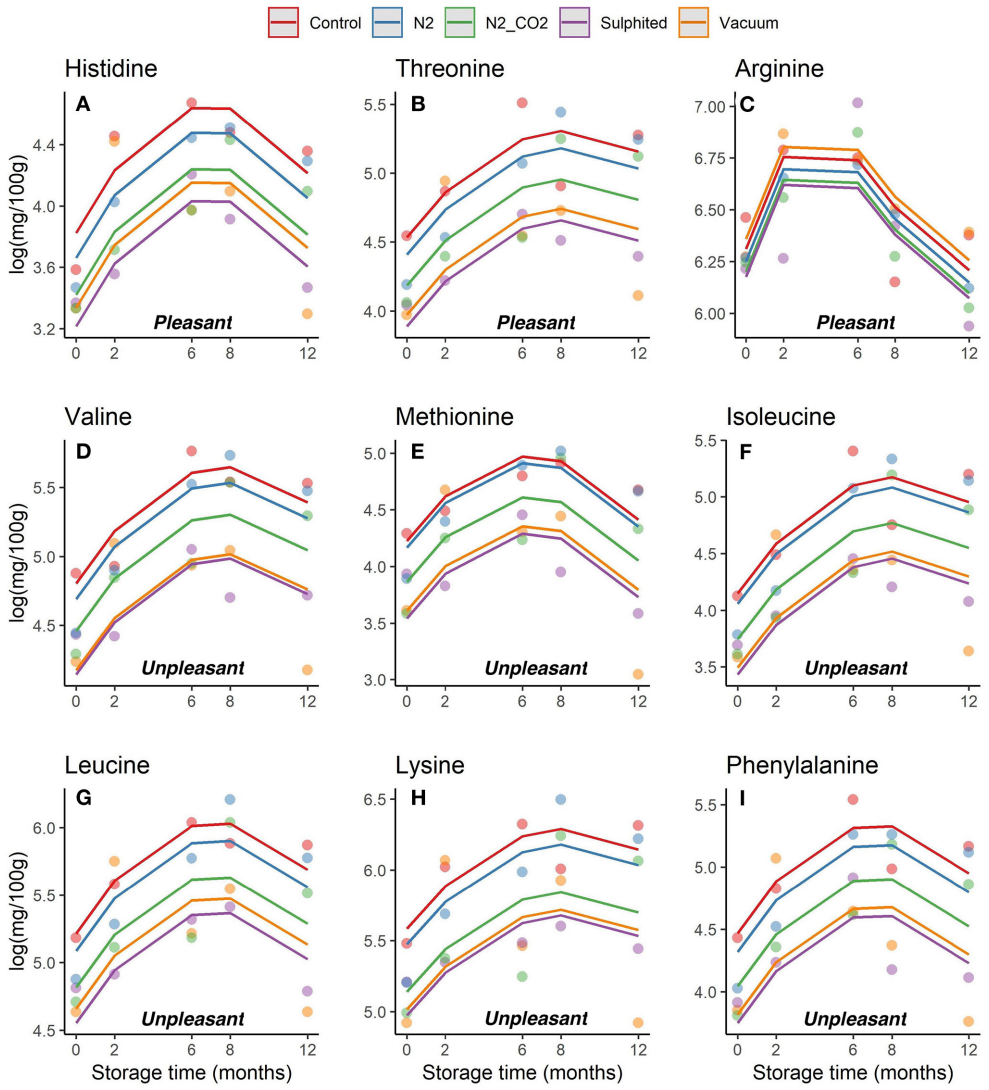

FIGURE 4
(A–I) Trends obtained for each essential FAA in DWRS samples in relation to the packaging method over 12 months of frozen storage. FAAs were grouped as pleasant and unpleasant.

serious complications affecting the nervous and vascular system, or become potential precursors of carcinogens (3).

Changes to FAAs under the five packaging methods

For all five tested packaging methods, the levels of arginine, proline, and glycine (first, second, and third most abundance FAAs, respectively), as well as glutamic acid, glutamine, alanine, and tyrosine were similar (Figures 4, 5; Supplementary table 1). A similar result was obtained by Bono et al. (7) for Giant red shrimp *Aristeomorpha foliacea* (Risso, 1827) caught in the same fishing area. On the other hand, Supplementary table 2 shows the result of the Tukey test used to perform a paired comparison between levels of the factor treatment when it was significant in the regression analysis.

The sulfiting treatment maintained the initial state of FAAs lower than the four other preservation methods. This result was obtained for most detected FAAs, and can be attributed to the strong antioxidant action of sulfite additives (36). Vacuum packed samples had the most similar results to sulfited samples for FAAs. Heat map analysis also confirmed the stability of FAAs with sulfiting agents during storage (Figure 6). Furthermore, heat maps confirmed our second unexpected finding on the stability of vacuum FAAs, especially at 8–12 months storage (Figure 6, two blue rectangles for sulfited and vacuum methods). In contrast, the heatmap showed that nitrogen preservation

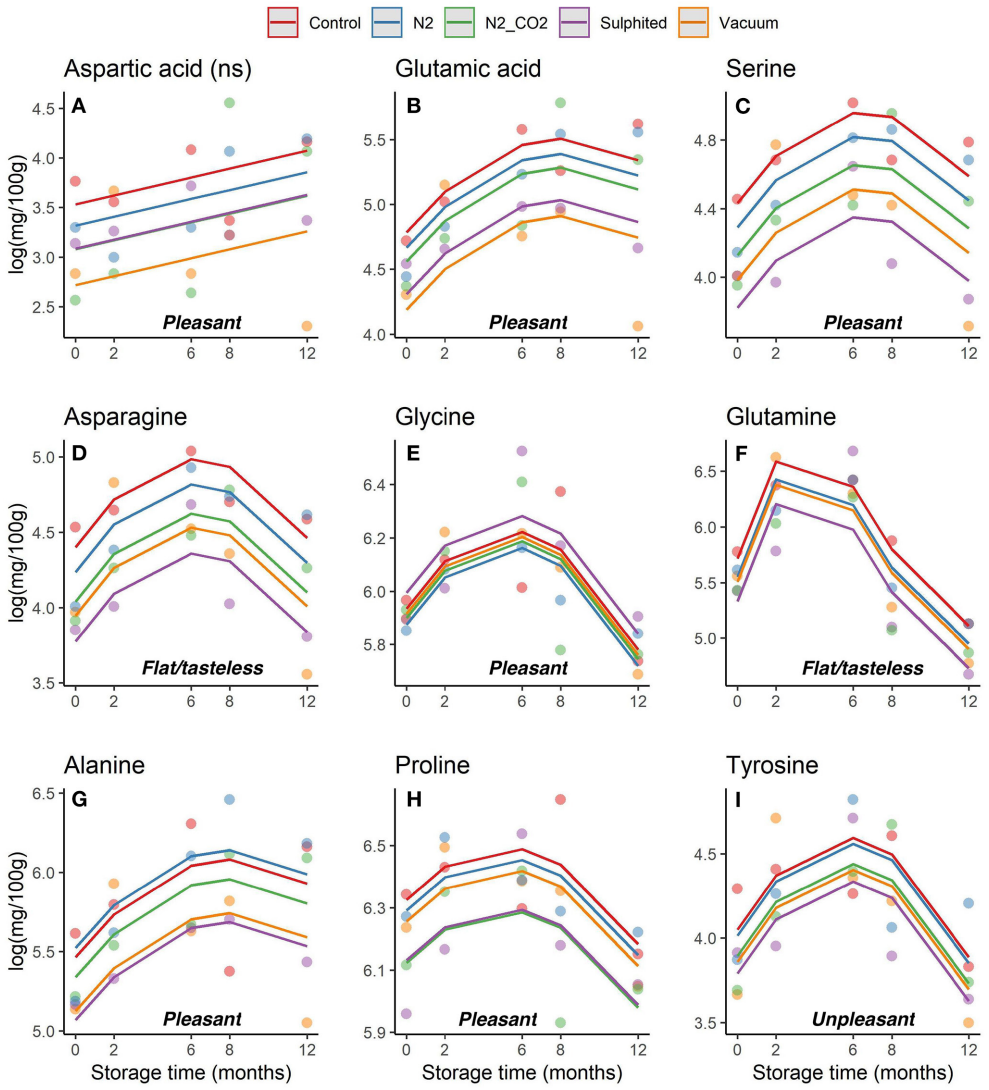

FIGURE 5
(A–I) Trends obtained for each non-essential FAA in DWRS samples in relation to the packaging method over 12 months of frozen storage. FAAs were grouped as pleasant, unpleasant, and flat/tasteless.

caused FAAs to change in a similar way to the control sample, especially in the last 6 months (intense coloring; two black rectangles Figure 6).

The good performance of FAAs in samples packed under vacuum to resemble those of sulfite-treated samples required further considerations. This result was novel, and was not obtained in the two previous analogous studies on Giant red shrimp (7) and Norway lobster (11) caught in the same geographical region. If the high performance of vacuum samples was due to the almost total absence of oxygen in the headspace (which is directly involved in the oxidative denaturation/deamination of proteins and FAAs), we should have obtained similar results for samples preserved in N_2 (100%), as they were also packaged in anoxic atmosphere. Indeed, in our similar study on FAAs in giant red shrimp (7), 100% N_2-treated group appeared with improved individual FAAs compared with other treatments.

As for FAAs in shrimp samples preserved in N_2/CO_2 (whose content was slightly higher compared to sulfited/vacuum, but lower compared to N_2 and control samples), this result might be due to the positive acidification effect of CO_2 on muscle tissue.

However, the FAA content of our samples was higher for N_2 (100%) compared to N_2/CO_2 (50%-50%), and was similar to the control sample. As equivalent studies are currently not available in the published literature, more research on this phenomenon is required to draw conclusions on the evolution of FAAs in frozen seafood products.

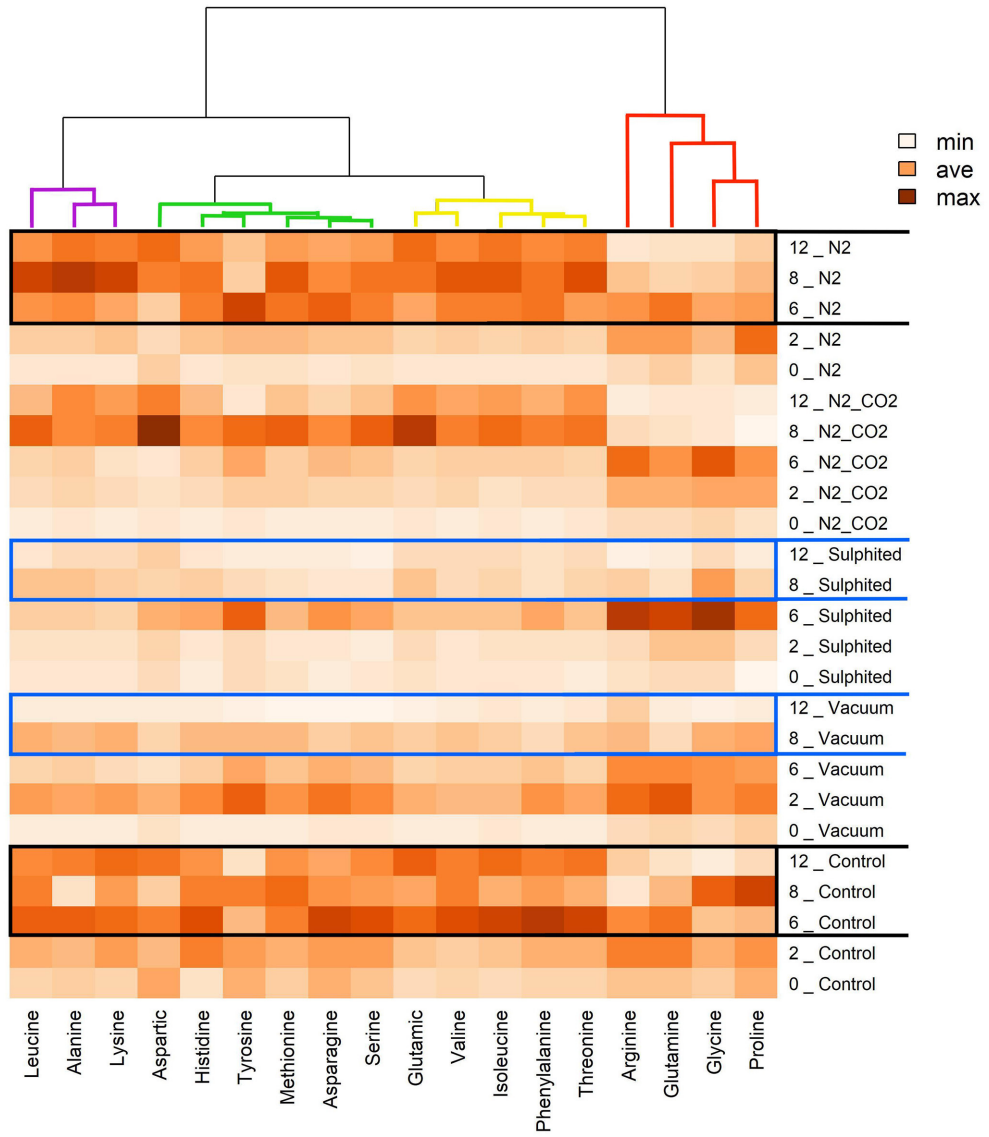

FIGURE 6
Heat map visualization and clustering results of FAAs of deep water rose shrimps during frozen storage. The heat map was generated using hierarchical clustering analysis to identify the color intensity for each FAA (minimum intensity, no color; average intensity, yellow; maximum intensity, brown). Values adjacent to packaging methods represent frozen storage time in months.

Conclusions

In this current study, changes in FAA profiles of post-harvest DWRS subjected to five different MAP methods, quickly frozen at −35°C, and thereafter kept for 12 months in frozen storage (−18°C) were investigated. Although the concentrations of FAAs differed with packaging and treatment methods, they appeared very high compared to the control. Furthermore, the ratio between essential vs. non-essential amino acids, as well as pleasant vs. unpleasant FFAs, was, interestingly, unbalanced in favor of the first ones (i.e., essential and pleasant amino acids). Additionally, regardless of the effects of treatment/packaging methods, the typical (sweet) FAAs arginine, proline and glycine constituted more than 40% of total FAAs. This might be associated with the high acceptability and palatability that characterize this valuable DWRS product. More so, the sulfiting treatment maintained the initial state of FAAs lower than the four other preservation methods. Overall, the frozen storage combined with MAP continues to be a promising alternative to chemical additives in shrimp/prawn processing, which not only could help sustain the FAAs as this current work has demonstrated, but more so, would help cater for

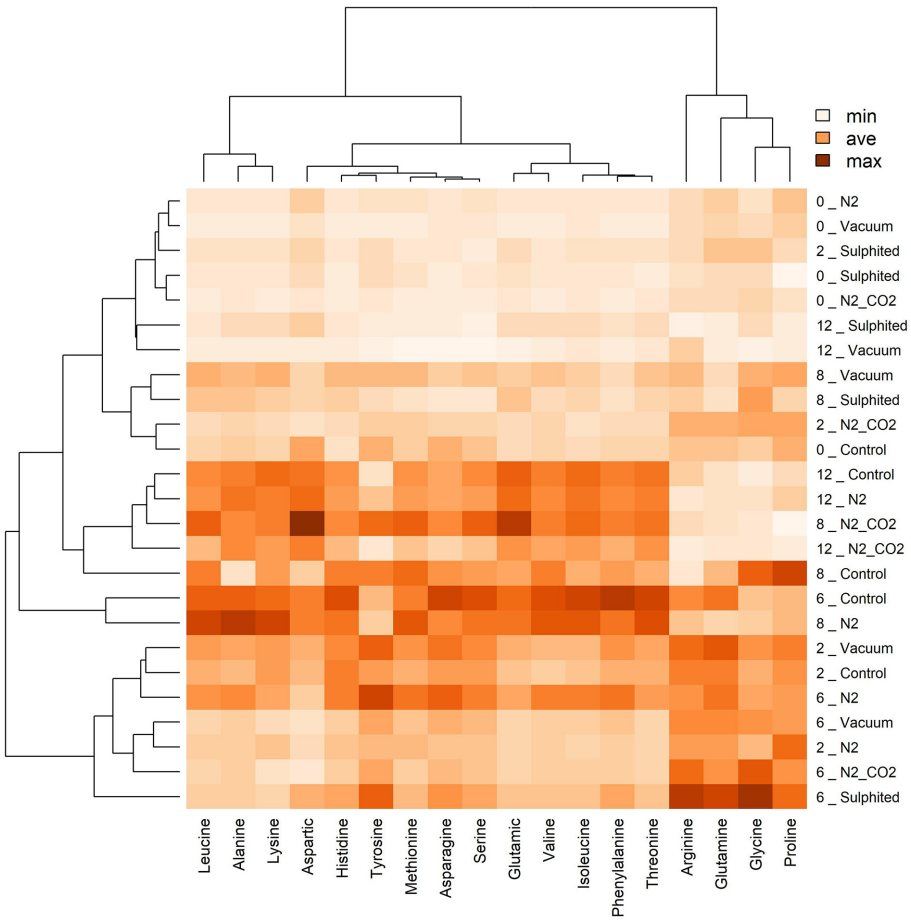

FIGURE 7
Heat map visualization and clustering results of FAAs in deep water rose shrimps during frozen storage, regardless of the effects packaging method (y-axis). Similar to Figure 6, the heat map was generated using hierarchical clustering analysis to identify the color intensity of each FAA (minimum intensity, no color; average intensity, yellow; maximum intensity, brown). Values adjacent to packaging methods represent frozen storage time in months.

increasing global demand for high-quality/lengthened shelf seafood products. Given the findings of this current work, the direction of future studies should be to subject the DWRS under the same treatment conditions to further shelf-life studies, especially biogenic amines and sensorial analysis.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

GB and CO provided conceptualization and study design. GB, PR, FQ, MD, FF, VG, and GS conducted experiments and performed data analysis. GB, PR, and MD drafted the initial manuscript. NN, SL, and AH critically reviewed and revised the manuscript. All authors approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those

of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.955216/full#supplementary-material

References

1. Gunlu A, Gunlu N. Taste activity value, free amino acid content and proximate composition of Mountain trout (Salmo trutta macrostigma Dumeril, 1858) muscles. *Iranian J Fish Sci.* (2014) 13:58–72.

2. Tejero J, Biswas A, Wang ZQ, Page RC, Haque MM, Hemann C, et al. Stabilization and characterization of a heme-oxy reaction intermediate in inducible nitric-oxide synthase. *J Biol Chem.* (2008) 283:33498–507. doi: 10.1074/jbc.M806122200

3. Gómez-Limia L, Cutillas R, Carballo J, Franco I, Martínez S. Free amino acids and biogenic amines in canned european eels: influence of processing step, filling medium and storage time. *Foods*. (2020) 9:1377. doi: 10.3390/foods9101377

4. Calanche J, Tomas A, Martinez S, Jover M, Alonso V, Roncalés P, et al. Relation of quality and sensory perception with changes in free amino acids of thawed seabream (Sparus aurata). *Food Res Int*. (2019) 119:126–34. doi: 10.1016/j.foodres.2019.01.050

5. Saito M, Kunisaki N. Proximate composition, fatty acid composition, free amino acid contents, mineral contents, and hardness of muscle from wild and cultured puffer fish takifugu rubripes. *Nippon Suisan Gakkaishi.* (1998) 64:116–20. doi: 10.2331/suisan.64.116

6. Bian C, Cheng H, Yu H, Mei J, Xie J. Effect of multi-frequency ultrasound assisted thawing on the quality of large yellow croaker (Larimichthys crocea). *Ultrason Sonochem*. (2022) 82:105907. doi: 10.1016/j.ultsonch.2021.105907

7. Bono G, Okpala COR, Alberio GRA, Messina CM, Santulli A, Giacalone G, et al. Toward shrimp consumption without chemicals: Combined effects of freezing and modified atmosphere packaging (MAP) on some quality characteristics of giant red shrimp (Aristaeomorpha foliacea) during storage. *Food Chemist.* (2016) 197:581–8. doi: 10.1016/j.foodchem.2015.10.146

8. Johnston WA, Nicholson FJ, Roger A, Stroud GD. *Freezing and Refrigerated Storage in Fisheries*. Rome (1994).

9. Okpala COR, Bono G. Investigating the biometric and physicochemical characteristics of freshly harvested Pacific white shrimp (Litopenaeus vannamei): a comparative approach. *J Sci Food Agric*. (2016) 96:1231–40. doi: 10.1002/jsfa.7211

10. Bono G, Badalucco CV, Cusumano S, Palmegiano GB. Toward shrimp without chemical additives: a combined freezing-MAP approach. *LWT Food Sci Technol.* (2012) 46:274–9. doi: 10.1016/j.lwt.2011.09.020

11. Bono G, Okpala COR, Badalucco CV, Milisenda G, Vitale S. Influence of freezing and oxygen-free packaging methods on lipid oxidation and other flesh quality parameters of Norway lobster (Nephrops norvegicus). *Eur J Lipid Sci Technol.* (2017) 119:1600035. doi: 10.1002/ejlt.201600035

12. Özden Ö. Changes in amino acid and fatty acid composition during shelf-life of marinated fish. *J Sci Food Agric.* (2005) 85:2015–20. doi: 10.1002/jsfa.2207

13. Holthuis LB. *Shrimps and Prawns of the World. An Annotated Catalogue of Species of Interest to Fisheries*. FAO Fisheries Synopsis, editor. Vol. 1. FAO (1980).

14. Lo Brutto S, Maggio T, Arculeo M. Isolation By Distance (IBD) signals in the deep-water rose shrimp Parapenaeus longirostris (Lucas, 1846) (Decapoda, Panaeidae) in the mediterranean sea. *Marine Environ Res.* (2013) 190:1–8. doi: 10.1016/j.marenvres.2013.05.006

15. Bono G, Falsone F, Falco F, di Maio F, Gabriele M, Gancitano V, et al. Microplastics and alien black particles as contaminants of deepwater rose shrimp (Parapenaeus longistroris Lucas, 1846) in the central mediterranean sea. *J Adv Biotechnol Bioeng.* (2020) 8:23–8. doi: 10.12970/2311-1755.2020.08.04

16. Bono G, Badalucco C, Corrao A, Cusumano S, Mammina L, Palmegiano GB, et al. Effect of temporal variation, gender and size on cuticle polyphenol oxidase activity in deep-water rose shrimp (Parapenaeus longirostris). *Food Chemistry*. (2010) 123:489–93. doi: 10.1016/j.foodchem.2010.04.055

17. Martínez-Alvarez O, Elvira López-Caballero M, Montero P, del Carmen Gómez-Guillén M. The effect of different melanosis-inhibiting blends on the quality of frozen deep-water rose shrimp (Parapenaeus longirostris). *Food Control*. (2020) 109:106889. doi: 10.1016/j.foodcont.2019.106889

18. Miraglia D, Castrica M, Menchetti L, Esposto S, Branciari R, Ranucci D, et al. Effect of an olive vegetation water phenolic extract on the physico-chemical, microbiological and sensory traits of shrimp (parapenaeus longirostris) during the shelf-life. *Foods*. (2020) 9:1647. doi: 10.3390/foods9111647

19. Okpala COR, Bono G, Pipitone V, Vitale S, Cannizzaro L. Toward crustacean without chemicals: a descriptive analysis of consumer response using price comparisons. *Food Nutr Res.* (2016) 60:30955. doi: 10.3402/fnr.v60.30955

20. Ruiz-Capillas C, Moral A. Changes in free amino acids during chilled storage of hake (Merluccius merluccius L.) in controlled atmospheres and their use as a quality control index. *Eur Food Res Technol.* (2001) 212:302–7. doi: 10.1007/s002170000232

21. Ruiz-Capillas C, Moral A. Free amino acids and biogenic amines in red and white muscle of tuna stored in controlled atmospheres. *Amino Acids.* (2004) 26:125–32. doi: 10.1007/s00726-003-0054-4

22. Devlieghere F, Debevere J, van Impe J. Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. *Int J Food Microbiol.* (1998) 43:105–13. doi: 10.1016/S0168-1605(98)00101-9

23. Blond G, le Meste M. Principles of frozen storage. In: Hui YH, editor. *Handbook of Frozen Foods.* New York, NY: Marcel Dekker (2004). p. 25–54.

24. Antoine F, Wei C, Littell R, Marshall M. HPLC method for analysis of free amino acids in fish using o-phthaldialdehyde precolumn derivatization. *J Agric Food Chem.* (1999) 47:5100–7. doi: 10.1021/jf990032+

25. Kato H, Rhue MR, Nishimura T. Role of free amino acids and peptides in food taste. In: Teranishi R, Buttery RG, Shahidi F, editors. *Flavor Chemistry*, Vol. 388. Washington, DC: American Chemical Society (1989). p. 158–74. doi: 10.1021/bk-1989-0388.ch013

26. Iwasaki M, Harada R. Proximate and amino acid composition of the roe and muscle of selected marine species. *J Food Sci.* (1985) 50:1585–7. doi: 10.1111/j.1365-2621.1985.tb10539.x

27. Chen DW, Zhang M. Non-volatile taste active compounds in the meat of Chinese mitten crab (Eriocheir sinensis). *Food Chemist.* (2007) 104:1200–5. doi: 10.1016/j.foodchem.2007.01.042

28. Martínez-Alvarez O. *Hormone-like Peptides Obtained by Marine-protein Hydrolysis and Their Bioactivities. In: Marine Proteins and Peptides*. Chichester, UK: John Wiley and Sons, Ltd (2013). p. 351–67. doi: 10.1002/9781118375082.ch16

29. Dai W, Gu S, Xu M, Wang W, Yao H, Zhou X, et al. The effect of tea polyphenols on biogenic amines and free amino acids in bighead carp (Aristichthys nobilis) fillets during frozen storage. *LWT*. (2021) 150:111933. doi: 10.1016/j.lwt.2021.111933

30. Lv Y, Xie J. Effects of freeze–thaw cycles on water migration, microstructure and protein oxidation in cuttlefish. *Foods*. (2021) 10:2576. doi: 10.3390/foods10112576

31. Rosa N, Nunes M. Seasonal patterns of nucleic acid concentrations and amino acid profiles of Parapenaeus longirostris (Crustacea, Decapoda): relation to growth and nutritional condition. *Hydrobiologia.* (2005) 537:207–16. doi: 10.1007/s10750-004-2841-7

32. Hong H, Regenstein JM, Luo Y. The importance of ATP-related compounds for the freshness and flavor of post-mortem fish and shellfish muscle: a review. *Crit Rev Food Sci Nutr.* (2017) 57:1787–98. doi: 10.1080/10408398.2014.1001489

33. Konosu S, Yamaguchi K. The flavor components in fish and shellfish. In: Martin RE, editor. *Chemistry and Biochemistry of Marine Food Products*. A. V. I. Publishing. Westport (1982). p. 367–404.

34. Sikorski ZE, Kołakowska A. Changes in proteins in frozen stored fish. In: *Seafood Proteins*. New York, NY: Springer US. (1994). p. 99–112. doi: 10.1007/978-1-4615-7828-4_8

35. Dabadé DS, Jacxsens L, Miclotte L, Abatih E, Devlieghere F, de Meulenaer B, et al. Survey of multiple biogenic amines and correlation to microbiological quality and free amino acids in foods. *Food Control*. (2021) 120:107497. doi: 10.1016/j.foodcont.2020.107497

36. Vally H, Misso NL. Adverse reactions to the sulphite additives. *Gastroenterol Hepatol Bed Bench*. (2012) 5:16–23.

37. R Core Team. *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing*, Vienna, Austria (2021). Available online at: https://www.R-project.org/

Internship experience

University of Thessaly

Using microbiome analyses to characterize the high-value Norway lobster (*Nephrops norvegicus*)

"The study described below has been completed and a manuscript is currently being prepared for possible scientific publication"

Introduction

Nephrops norvegicus, commonly known as the Norway lobster, is found in the deep-sea regions of Norway, south of Libya, west of the Canary Islands and east of the Aegean Sea. These diverse habitats are characterized by cold temperatures, high pressures and different substrate compositions (Sealifebase).

The Norway lobster can be considered an ecological indicator of the deep-sea ecosystem. As a benthic organism, it influences the structure and dynamics of marine communities through its feeding habits, burrowing behavior and interactions with other species (Phillips, 2006).

From a commercial standpoint, *Nephrops norvegicus* is an economically valuable species (Brčić et al., 2018, European Commission).

For this reason, understanding the factors influencing the abundance, distribution and sustainability of *Nephrops norvegicus* populations is important to the international commercial market and can help optimize fishing practices, ensuring the longevity of this resource.

Microbiota serve as valuable geographic indicators, reflecting environmental conditions across diverse habitats (Yatsunenko et al., 2012). Geographic conditions, diet, age and physical activity influence the composition and diversity of microbiota (Буйваленко & Покровская, 2022). Even within the distinct taxonomic profiles of populations inhabiting different regions, studies have revealed a positive correlation between Firmicutes abundance and latitude, alongside a negative correlation between Bacteroidetes abundance and latitude (Suzuki & Worobey, 2014). Shared microbial signatures across closely related species occupying similar geographic areas underscore the influence of common environmental factors. In some cases, environmental conditions can drive microbiota variability, further highlighting the significant influence of geography on microbial communities (Lu et al., 2021).

Gut microbiota plays a crucial role in the health and functioning of marine organisms, influencing digestion, nutrient absorption and immune responses (Cai et al., 2024).

Environmental conditions (such as temperature, salinity and available food sources) are the most important factors in the variability of communities within gut microbiota (Nikouli et al., 2021).

The Norway lobster's gut microbiota can also influence its metabolism and immunity (S. Wu et al., 2024; Y. Wu et al., 2022). Comparative analyses of the Norway lobster's gut microbiota across commercially important geographical locations offer the opportunity to explore how these interactions may vary in response to distinct environmental factors.

Scientific studies on the Norway lobster have primarily addressed aspects impacting its commercial viability, focusing on issues such as quality degradation and its adverse effects on the animal's physical conditions (Albalat et al., 2012; Stentiford et al., 1999, 2000; Stentiford, Neil, & Atkinson, 2001; Stentiford et al., 2015) and the possible decline in populations (Beevers et al., 2012).

Studies have notably honed in on microbiological spoilage-induced degradation and the prevalence of detrimental factors like Hematodinium, Dinoflagellate, parasitic infections (Briggs & McAliskey, 2002; Field et al., 1992; Field & Appleton, 1995, 1996) and seasonal infections (Stentiford, Neil, & Coombs, 2001).

These works highlight the paramount importance of delving into the intricate interplay between the Norway lobster and factors that can compromise both its market value and physical conditions.

Considering the commercial importance of the Norway lobster and the global risks related to fraud in the seafood chain (Donlan & Luque, 2019; Horreo et al., 2017; Silva et al., 2020), the first research objective was to use microbiome analyses (Liu et al., 2020; Singh et al., 2022) to characterize the geographical origin of Norway lobster (*Nephrops norvegicus*) caught in three different fishing areas in the North and the Mediterranean Sea. Results can be used for ecological purposes such as understanding ecological interactions. The variability of GUT microbiota provides important information about its adaptive strategies, ecological resilience and possible response to environmental changes, which can be used to devise conservation strategies and to optimize commercial aspects.

Materials & methods

Specimen collection

Nephrops norvegicus specimens were collected in three distinct geographical regions (fig.1): the Mediterranean Sea (Strait of Sicily, Italy), the Aegean Sea (Kavala, Greece) and the North Sea (Kattegat-Skagerrak, Sweden).

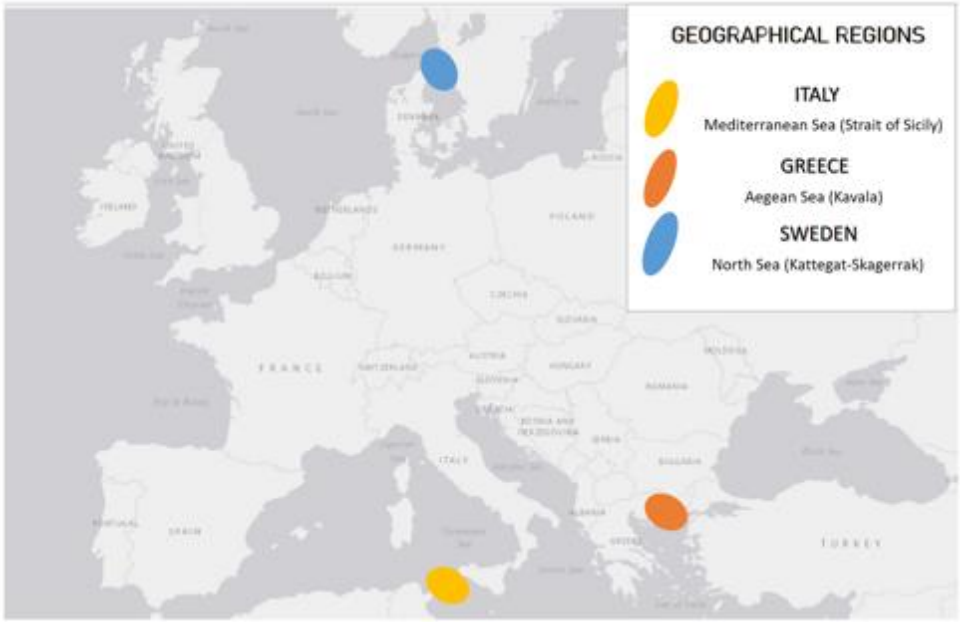

Figure 1 –Geographical location of sampling regions

Samples were collected from the end of August (Italy) to the end of October 2023 (Greece and Sweden). After collection, the *N. norvegicus* specimens from the Strait of Sicily (Italy) were immediately transferred on ice to the laboratory, whereas extractions from Aegean and North Sea specimens were completed on board. Biometric data, including sex, weight and carapace length, was also collected during extraction (Fig.2). A total of 63 samples were collected, the details of which are reported in Table1.

Figure 2 – Collecting biometric data

Table 1 - Sampling metadata

Sites	***Sampling Month***	***№ of specimens/gender***	***Total average weight ±S.D.***	***Total average length ±S.D.***
Aegean Sea (Kavala, Greece)	October	11F/12M	78.6 ±46.8	45.2 ± 9
Mediterranean Sea (Strait of Sicily, Italy),	September	10F/10M	34.8 ±8.8	35.9 ±3.4
North Sea (Kattegat-Skagerrak, Sweden)	August	10F/10M	65.4 ±2.3	45 ±0

*presence of eggs in samples (collected separately)

Extraction process (tissue dissection):

The GUT (i.e. whole intestine without stomach) was subsequently removed from specimens: the gut of Mediterranean Sea (Italy) specimens was aseptically removed within three hours of capture, and then stored in ice, whereas gut extraction in Aegean Sea (Greece) and North Sea (Sweden) specimens took place on board directly after capture. Each GUT extraction took about 1-2 minutes. The first step of extraction involved cutting the muscle with a sterile scalpel along the perpendicular of the posterior border of the carapace and then cutting with a sterile scalpel (or scissors) both sides (right and left) of the last segment of the tail, near the telson (Fig.3). The second step of GUT extraction involved removing the GUT from tissue by pulling the telson: in this case the intestine (red arrow) also came out easily (Fig.4).

Figure 3 – First step of GUT extraction

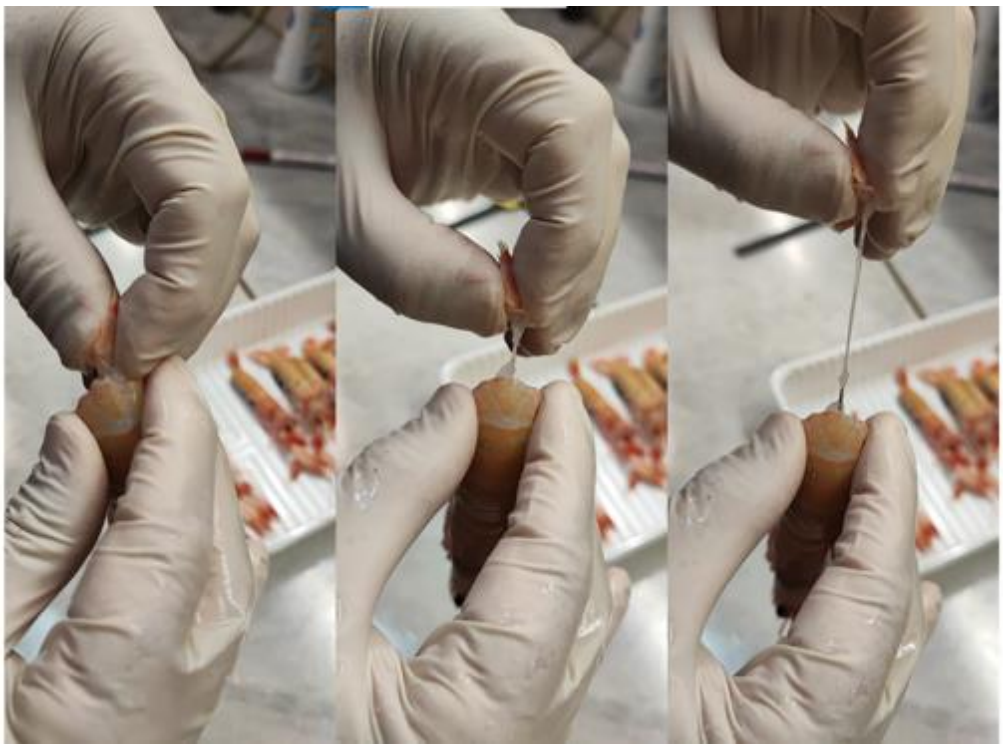

Figure 4 – Second step of GUT extraction

The GUT was immediately stored in 2 ml Eppendorf tubes, adding about 1 ml of DNA/RNA Shield (Zymo Research) (Fig.5). Samples could then be stored for one month at room temperature, or preferably in the refrigerator at +2/4°C.

Figure 5 – GUT sample in a solution of DNA/RNA Shield (Zymo Research)

DNA isolation was performed on 42 gut tissues from all sampling months using the QIAamp DNA Mini Kit (Qiagen Inc.) following the manufacturer's standard protocol (7 female and male/country + 5 eggs).

Preliminary results

Statistically significant differences were found between: (a) males-females from the Greek and Swedish populations, (b) the Greek eggs and Greek adults (both males and females), and (c) between the total of the three populations (males+females). The dominant bacterial operational taxonomic units (OTUs) were from the Firmicutes, Fusobacteriota, Bacteroidota, Desulfobacterota, Actinobacteriota (prevailed almost exclusively in eggs) and Spirocahetota phyla (Figs.1, 2); several unclassified bacterial taxa were also found, especially in the Greek females. The core microbiota of the three populations comprised 253 OTUs of the 2362 total OTUs found in the adults of the three populations. The eggs and the GRE adults shared 142 OTUs (9.5% of the total OTUs in eggs and GRE adults).

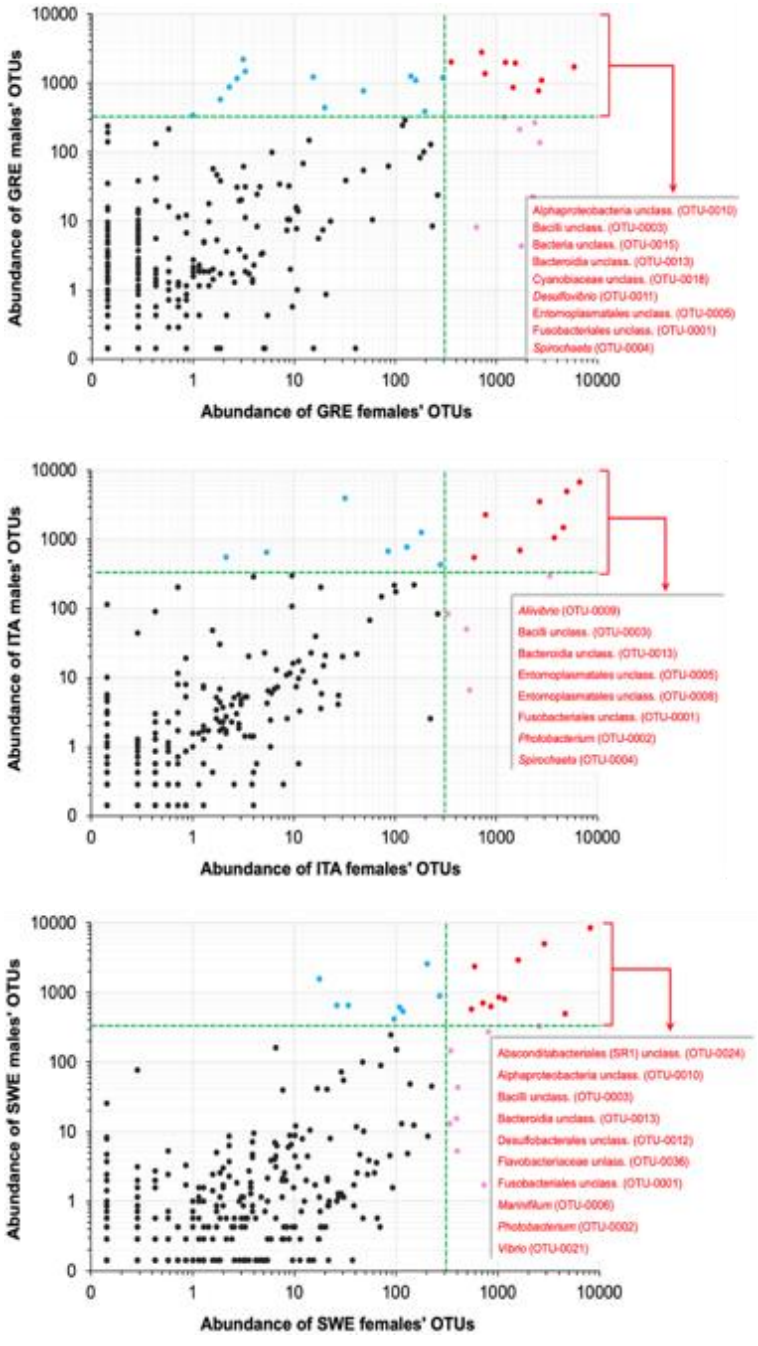

Figure 1 - OTU abundance in males and females across zones (Greece, Italy, Sweden)

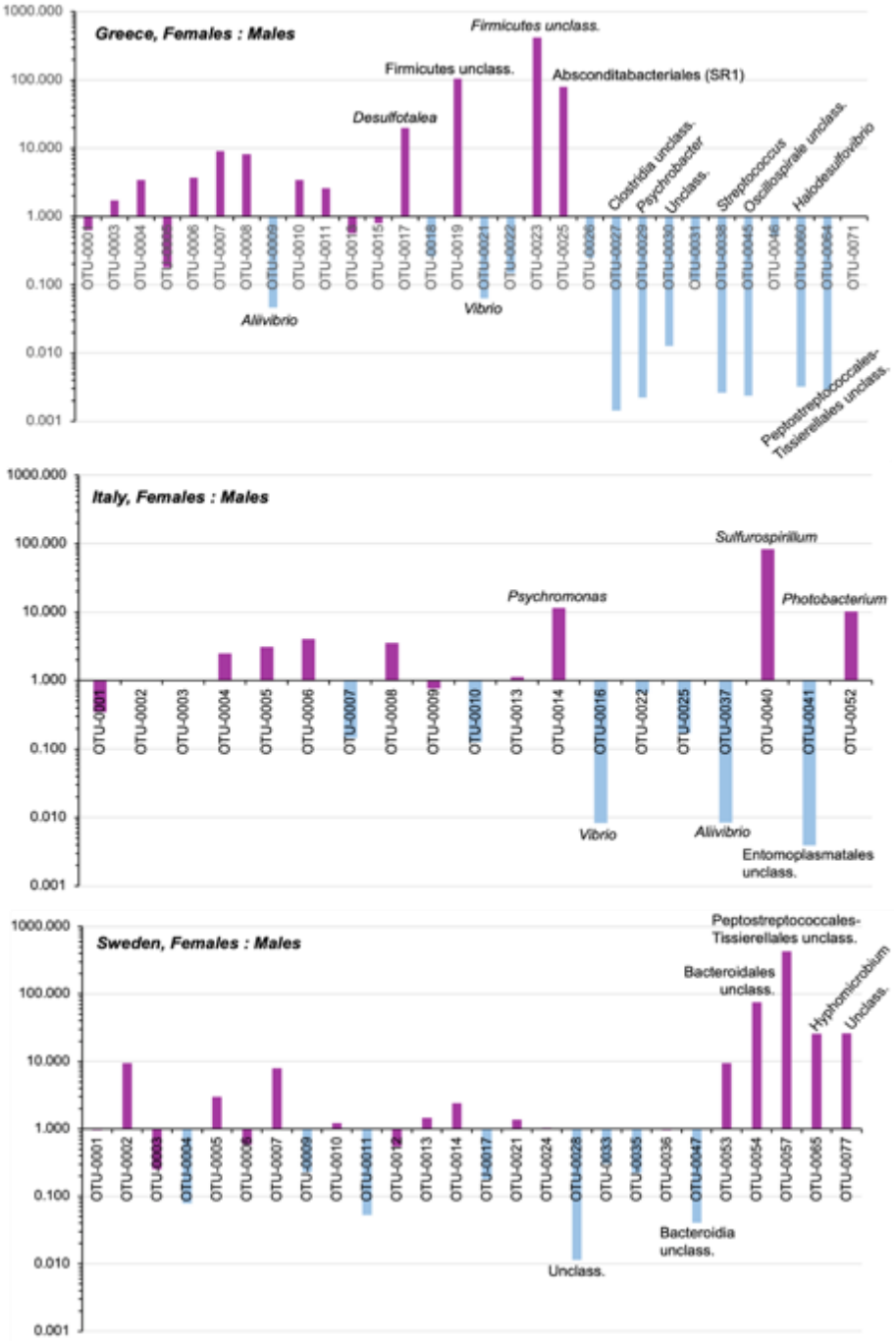

Figure 2 - Differential OTU abundance ratios in females and males across zones (Greece, Italy, Sweden)

The data set in Table 1 presents statistical differences in bacterial taxa in eggs, females and males across geographical regions (Greece, Italy and Sweden). Significant differences ($p < 0.05$) were noted between eggs and females ($p = 0.027$) and between eggs and males ($p = 0.046$) in Greece, as well as between males from Italy and Sweden ($p = 0.021$). These results suggest regional and sex-specific variations in bacterial communities.

Table 1 - Statistical Analysis of Bacterial Taxa Variation by Sex and Region

	Eggs-Females	Eggs-Males	Female-Males		Females	Males
GRE	p=0.027* F=3.162	p=0.046* F=2.190	p=0.897 F=1.565	GRE-ITA	p=0.032* F=2.916	p=0.580 F=1.579
ITA	X	X	p=1.000 F=0.616	ITA-SWE	p=0.027* F=2.981	p=0.021* F=2.668
SWE	X	X	p=0.960 F=1.668	GRE-SWE	p=0.008*** F=3.611	p=0.015* F=3.438

Despite the genetic homogeneity of Norway lobster populations, this study found differences in the gut microbiota of geographically distant populations, suggesting that environmental and/or sex factors are important.

Preliminary research results were presented at the Hologenomics Conference 2024 in Denmark.

References

Albalat, A., Gornik, S. G., Beevers, N., Atkinson, R. J. A., Miskin, D., & Neil, D. M. (2012). Hematodinium sp. infection in Norway lobster Nephrops norvegicus and its effects on meat quality. *Diseases of Aquatic Organisms*, *100*(2). https://doi.org/10.3354/dao02500

Beevers, N. D., Kilbride, E., Atkinson, R. J. A., & Neil, D. M. (2012). Hematodinium infection seasonality in the Firth of Clyde (Scotland) Nephrops norvegicus population: A re-evaluation. *Diseases of Aquatic Organisms*, *100*(2). https://doi.org/10.3354/dao02473

Brčić, J., Herrmann, B., Mašanović, M., Baranović, M., Šifner, S. K., & Škeljo, F. (2018). Size selection of Nephrops norvegicus (L.) in commercial creel fishery in the Mediterranean Sea. *Fisheries Research*, *200*. https://doi.org/10.1016/j.fishres.2017.12.006

Briggs, R. P., & McAliskey, M. (2002). The prevalence of Hematodinium in Nephrops norvegicus from the western Irish Sea. *Journal of the Marine Biological Association of the United Kingdom*, *82*(3). https://doi.org/10.1017/S0025315402005684

Cai, J., Yin, B., Wang, Y., Pan, K., Xiao, Y., & Wang, X. (2024). Gut microbiome play a crucial role in geographical and interspecies variations in mercury accumulation by fish. *Science of the Total Environment*, *912*. https://doi.org/10.1016/j.scitotenv.2023.169381

Donlan, C. J., & Luque, G. M. (2019). Exploring the causes of seafood fraud: A meta-analysis on mislabeling and price. *Marine Policy*, *100*. https://doi.org/10.1016/j.marpol.2018.11.022

Field, R. H., & Appleton, P. L. (1995). A Hematodinium-like dinoflagellate infection of the Norway lobster Nephrops norvegicus: Observations on pathology and progression of infection. *Diseases of Aquatic Organisms*, *22*(2). https://doi.org/10.3354/dao022115

Field, R. H., & Appleton, P. L. (1996). An indirect fluorescent antibody technique for the diagnosis of Hematodinium sp. infection of the Norway lobster Nephrops norvegicus. *Diseases of Aquatic Organisms*, *24*(3). https://doi.org/10.3354/dao024199

Field, R. H., Chapman, C. J., Taylor, A. C., Neil, D. M., & Vickerman, K. (1992). Infection of the Norway lobster Nephrops norvegicus by a Hematodinium-like species of dinoflagellate

on the west coast of Scotland. *Diseases of Aquatic Organisms*, *13*(1). https://doi.org/10.3354/dao013001

Horreo, J. L., Machado-Schiaffino, G., & García-Vázquez, E. (2017). Forensic assignment to geographic origin, a useful tool in seafood fraud control. *Forensic Science International*, *272*. https://doi.org/10.1016/j.forsciint.2017.01.003

Liu, X., Teixeira, J. S., Ner, S., Ma, K. V., Petronella, N., Banerjee, S., & Ronholm, J. (2020). Exploring the Potential of the Microbiome as a Marker of the Geographic Origin of Fresh Seafood. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00696

Lu, J., Zhang, L., Zhai, Q., Zhao, J., Zhang, H., Lee, Y. K., Lu, W., Li, M., & Chen, W. (2021). Chinese gut microbiota and its associations with staple food type, ethnicity, and urbanization. *Npj Biofilms and Microbiomes*, *7*(1). https://doi.org/10.1038/s41522-021-00245-0

Nikouli, E., Meziti, A., Smeti, E., Antonopoulou, E., Mente, E., & Kormas, K. A. (2021). Gut Microbiota of Five Sympatrically Farmed Marine Fish Species in the Aegean Sea. *Microbial Ecology*, *81*(2). https://doi.org/10.1007/s00248-020-01580-z

Phillips, B. F. (2006). Lobsters: Biology, Management, Aquaculture and Fisheries. In *Lobsters: Biology, Management, Aquaculture and Fisheries*. https://doi.org/10.1002/9780470995969

Silva, A. J., Hellberg, R. S., & Hanner, R. H. (2020). Seafood fraud. In *Food Fraud: A Global Threat with Public Health and Economic Consequences*. https://doi.org/10.1016/B978-0-12-817242-1.00008-7

Singh, P., Williams, D., Velez, F. J., & Nagpal, R. (2022). Comparison of the Gill Microbiome of Retail Oysters from Two Geographical Locations Exhibited Distinct Microbial Signatures: A Pilot Study for Potential Future Applications for Monitoring Authenticity of Their Origins. *Applied Microbiology*, *3*(1). https://doi.org/10.3390/applmicrobiol3010001

Stentiford, G. D., Neil, D. M., Albalat, A., Milligan, R. J., & Bailey, N. (2015). The effect of parasitic infection by hematodinium sp. On escape swimming and subsequent recovery in the Norway lobster, nephrops norvegicus (L.). *Journal of Crustacean Biology*, *35*(1). https://doi.org/10.1163/1937240X-00002296

Stentiford, G. D., Neil, D. M., & Atkinson, R. J. A. (2001). The relationship of Hematodinium infection prevalence in a Scottish Nephrops norvegicus population to season, moulting and sex. *ICES Journal of Marine Science*, *58*(4). https://doi.org/10.1006/jmsc.2001.1072

Stentiford, G. D., Neil, D. M., & Coombs, G. H. (1999). Changes in the plasma free amino acid profile of the Norway lobster Nephrops norvegicus at different stages of infection by a parasitic dinoflagellate (genus Hematodinium). *Diseases of Aquatic Organisms*, *38*(2). https://doi.org/10.3354/dao038151

Stentiford, G. D., Neil, D. M., & Coombs, G. H. (2000). Alterations in the biochemistry and ultrastructure of the deep abdominal flexor muscle of the Norway lobster Nephrops norvegicus during infection by a parasitic dinoflagellate of the genus Hematodinium. *Diseases of Aquatic Organisms*, *42*(2). https://doi.org/10.3354/dao042133

Stentiford, G. D., Neil, D. M., & Coombs, G. H. (2001). Development and application of an immunoassay diagnostic technique for studying Hematodinium infections in Nephrops norvegicus populations. *Diseases of Aquatic Organisms*, *46*(3). https://doi.org/10.3354/dao046223

Suzuki, T. A., & Worobey, M. (2014). Geographical variation of human gut microbial composition. *Biology Letters*, *10*(2). https://doi.org/10.1098/rsbl.2013.1037

Wu, S., Pan, M., Zan, Z., Jakovlić, I., Zhao, W., Zou, H., Ringø, E., & Wang, G. (2024). Regulation of lipid metabolism by gut microbiota in aquatic animals. In *Reviews in Aquaculture* (Vol. 16, Issue 1). https://doi.org/10.1111/raq.12819

Wu, Y., Jha, R., Li, A., Liu, H., Zhang, Z., Zhang, C., Zhai, Q., & Zhang, J. (2022). Probiotics (Lactobacillus plantarum HNU082) Supplementation Relieves Ulcerative Colitis by Affecting Intestinal Barrier Functions, Immunity-Related Gene Expression, Gut Microbiota, and Metabolic Pathways in Mice. *Microbiology Spectrum*, *10*(6). https://doi.org/10.1128/spectrum.01651-22

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. In *Nature* (Vol. 486, Issue 7402). https://doi.org/10.1038/nature11053

CIRCLES

Controlling mIcRobiomes CircuLations for bEtter food Systems

D5.2 Report

Subtask 5.5.3 Improving food quality

1. Introduction

The aim of subtask 5.5.3 was to compare three batch of farmed gilthead sea bream, one fed on a conventional diet (Control) and two fed on two new experimental probiotic diets (ED1 and ED2). Analyses were carried out in collaboration with IRBIM CNR of Messina and Bolton (Italy). Each seabream batch was mainly assessed for nutritional quality, in terms of optimization of the presence and functional effects of the proximate composition (as an indicator of foodstuff quality), fatty acid contents (in particular PUFA contents) and amino acid composition (both total and free) of flesh samples.

2. Materials and methods

2.1 Fish processing and analysis of sea bream

Prior to starting the feeding trial with the two probiotic diets (October 2022), 18 gilthead sea breams with an average weight of approximately 89 grams underwent all essential analyses. This was done to facilitate comparison between the initial and final sample analyses at the conclusion of the experimental trial.

At the end of May 2023 (7 months after the start of the feeding trial and when specimens reached an average weight of 256 g ± 36) the three lots of gilthead sea bream were ready to be processed and analysed in order to collect information about their nutritional composition and others physical-chemical properties.

A total of 15 specimens were randomly collected from each lot of fish fed with either of the two new experimental diets, ED1 and ED2, and a control group that was fed with a conventional pellet diet (CD) (Table 2.1). After following the IRBIM CNR protocol for sacrificing specimens, biometric and biological parameters (total length, total weight, etc.) were recorded in the laboratory (Fig.1). The fish were then hand filleted, and both fillets were packed under vacuum in PE Barrier bags, quick-frozen and stored at -80°C until analysis (Fig.2).

Table 2.1 - Composition of three diets used in the rearing experiment

	Composition
Control Diet (CD) Linea Blu 4 – Veronesi	[Pellet size (mm): 4.6-5.6; Protein (%): 43.00; Fat (%): 21; Fibre (%):2,5; Ash (%) 5,6; Carbohydrates (%): 18.90; Phosphorus (%): 0.9; Vitamin C (mg/Kg): 160; Vitamin E (mg/Kg): 160; Digestible energy (mj/Kg): 19.76]

Experimental Diet 1 (ED1)	control diet + yeast Saccharomyces cerevisiae, caprylic acid and vitamin B complex (1% of the total)
Experimental Diet 2 (ED2)	control diet + Short Chain FaSy Acids – SCFA (1% of the total)

Figure 1 – Preparation of gilthead sea bream

Figure 2 – Fillets (left), fish waste (center) and fish skin (right)

2.2 Fatty acid (FA) profile

The Fatty Acid Profile was determined by Gas Chromatography analysis of fatty acid methyl esters present in flesh. Fatty acid methyl esters (FAMEs) were prepared by direct trans-esterification with a mixture of sulfuric acid:methanol (1:9, v/v). These FAMEs were then analyzed using a split inlet gas chromatograph, flame ionization detector (FID) and a fused silica column 30m x 0.25 mm I.D, 0.25 µm film thickness. To identify the fatty acids, the retention times of each FAME peak were compared with those of standards.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818290.

2.3 Free Amino acids (FAAs)

Free amino acids were extracted using diluted hydrochloric acid. Co-extracted nitrogenous macromolecules were precipitated with sulfosalicylic acid and removed by filtration. The filtered solution was then adjusted to pH = 2.20. The amino acids were separated by gas chromatography.

2.4 Total Amino acids (TAAs)

Total amino acids were determined by GC-FID analysis. Before hydrolysis, cysteine and methionine were oxidized to cysteic acid and methionine sulfone, respectively. Tyrosine, however, was not oxidized and was determined in non-oxidized samples. All other amino acids listed in the paragraph were determined in both oxidized and non-oxidized samples. The oxidation process was carried out with a performic acid/phenol mixture at 0 °C, and the excess of the oxidation reagent was decomposed using sodium metabisulfite. The oxidized and non-oxidized samples were hydrolyzed with hydrochloric acid (concentration of 6 mol/L) for 23 hours. The hydrolyzate was then adjusted to a pH of 2.20, and the amino acids were finally separated through gas chromatography.

2.5 Total lipids

Total lipids were measured using the Bligh-Dyer method. This technique enables the extraction of lipids from food samples with high moisture content. It involves mixing a portion of the sample with a chloroform-methanol mixture, followed by the addition of diluted sulfuric acid.

2.6 Proteins

Protein content was determined using the Kjeldahl method to measure nitrogenous substances and expressed as g/100 g of edible portion.

2.7 Minerals (Na, K, Ca, Mg, P, Fe, Zn, Mn)

The method used to determine minerals and metals in foods was UNI EN 13805:2014 + UNI EN ISO 17294-2:2016. This method involves inductively coupled plasma mass spectrometry. The sample solution was mineralized by digestion under pressure. It was then nebulized and introduced into a high-frequency inductively coupled plasma where the aerosol was dried, atomized, and ionized. A series of cones (sampler and skimmer) extracted the ions from the plasma, which were then transferred to the mass spectrometer. The ions were separated based on their charge-mass ratio and detected by a dual-mode secondary ion multiplier (SEM).

2.8 Color

The color of the inner surface of the fillets was assessed by recording the L* (lightness), a* (redness) and b* (yellowness) values using a colorimeter (Konica Minolta, Osaka, Japan).

2.9 Other parameters: Cholesterol, Shear force

Cholesterol was measured according to AOAC 994.10, shear force according to ASPA 1996.

3. Results and discussions

3.1 Biometric parameters

Table 3.1 shows the values of selected biometric parameters collected in both aggregate form (ALL) and for individual samples (control, ED1 and ED2). Overall, there are no significant differences between the three batches. It suggests that the new ingredients added to the diets listed in tab 2.1 were not potent enough to modify the growth performance of ED1 and ED2 specimens with respect to the control specimen. It may be that fattening times exceeding the seven months considered are required to assess the potential effects of the new diets.

Table 3.1 Mean biometric parameters and main condition factors for the three gilthead sea bream batches fed on different diets

	ALL		**Control**		**ED-1**		**ED-2**	
	(mean)	SD	(mean)	SD	(mean)	SD	(mean)	SD
Total weight (g)	256.2	36.0	264.4	37.7	252.3	33.5	252.1	36.4
Total length (cm)	24.5	1.3	24.7	0.6	24.3	1.3	24.4	1.7
Fork length (cm)	27.0	31.2	22.5	0.7	22.2	0.9	22.5	1.2
Standard length (cm)	20.7	0.8	20.7	0.5	20.6	0.8	21.0	1.1
Liver weight (g)	3.6	0.7	3.8	0.6	3.6	0.9	3.4	0.6
Visceral weight (g)	15.4	4.8	16.5	4.7	14.8	4.3	14.8	5.4
Fat weight (g)	5.3	3.2	6.2	2.7	4.6	2.8	5.0	4.0
Right fillet weight (g)	65.1	9.8	68.1	10.2	63.2	8.7	64.0	9.8
Left fillet weight (g)	67.3	10.1	68.9	10.7	66.1	9.1	66.9	10.5
Waste weight (g)	96.8	12.5	99.1	12.9	94.3	13.9	96.9	10.6
Condition factors								
T. weight/T. length	10.5	1.2	10.7	1.4	10.4	1.2	10.3	1.1
T. weight/F. length	11.2	2.0	11.1	3.1	11.4	1.3	11.2	1.1
Liver weight/T. weight	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
Fat weight/T. weight	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818290.

3.2 Nutritional composition of gilthead sea bream at the beginning of the feeding trial

3.2.1 Chemical composition

The chemical composition of gilthead seabream fillets is presented in Table 3.2. The average moisture content was around 72% of wet weight,. crude proteins 20.5 g/100g,. lipids 6.4 g/100g.

Table 3.2 – Chemical composition (g/100 of dry matter) of gilthead sea bream flesh fillets (n = 6) at the beginning of the feeding experiment

	pH	Moisture (g/100g)	Proteins (g/100g)	Lipids (g/100g)	Ash (g/100g)
Mean	6.20	71.76	20.48	6.41	1.38
SD	0.04	0.18	0.16	0.32	0.05

The flesh fatty acid (FA) profile is summarized in Table 3.3,. where it is expressed in g 100 g−1 total FA. The most abundant FAs were oleic acid (C18:1n9 = 33.6 g 100 g^{-1} FA),. palmitic acid (C16:0 =18.4 g 100 g−1 FA), and linoleic acid (C18:2n6 = 13.8 g 100 g−1 FA). The combined data for SFA, MUFA and PUFA are consistent with published data on the same sector (D. S. Lenas et al. 2011).

Table 3.3 - Fatty acid (FA) composition (g/100 g of total FA) of gilthead sea bream flesh fillets (n = 6) at the beginning of the feeding experiment.

	mean (n=6)	SD
C14:0	2.47	0.15
C16:0	18.44	0.44
C16:1	4.52	0.39
C18:0	4.57	0.35
C18:1n9	33.59	1.12
C18:2n6	13.79	0.15
C18:3n6	0.25	0.09
C18:3n3	2.47	0.08
C18:4n3	0.74	0.08
C20:1n9	2.37	0.27
C20:2	0.49	0.08
C20:4n6	0.62	0.14
C20:4n3	0.66	0.07
C20:5n3 (EPA)	4.16	0.56

C22:1n9	1.33	0.34
C22:5n3	1.51	0.19
C22:6n3 (DHA)	8.02	1.40
SFA	25.48	0.76
MUFA	41.82	1.78
PUFA	32.71	2.15
Ratio n-3/n-6	0.91	
Ratio EPA/DHA	0.51	

Table 3.4 – Mineral composition (Na, K, Ca, Mg, P Fe, Zn, Mn) (mg/kg) of gilthead sea bream flesh fillets (n = 6) at the beginning of the feeding experiment.

	mg/kg	SD
Na (mg/kg)	796.95	73.91
K (mg/kg)	5074.49	393.34
Ca (mg/kg)	244.87	62.39
Mg (mg/kg)	381.82	35.90
P (mg/kg)	2917.42	333.71
Fe (mg/kg)	5.35	2.58
Zn (mg/kg)	4.10	0.78
Mn (mg/kg)	0.33	0.37

3.3 Nutritional composition of gilthead sea bream after the feeding experimental trial

3.3.1 Chemical composition

Figure 3.1 shows the chemical composition of the sea bream fillets. The moisture content for all three batches was approximately 69 grams per 100 grams wet weight. The crude protein content was 20 grams per 100 grams, while the fat content was 9 grams per 100 grams. No significant differences were found between the three analysed batches (control, ED1 and ED2) and the initial batch of juvenile sea bream.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818290.

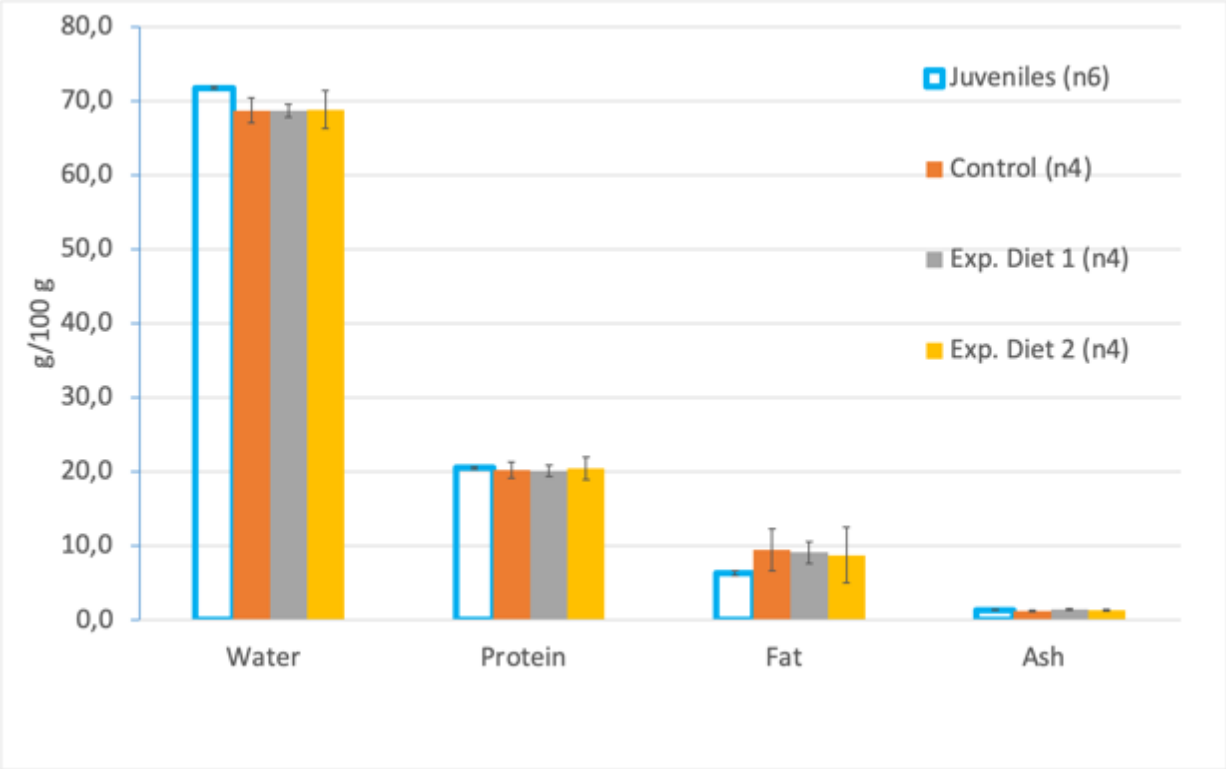

Fig. 3.1. Comparison between the chemical composition (water, crude protein, crude fat and ash) of gilthead sea bream flesh fillets at the start (juveniles) and after feeding for 7-months on three different diets.

Figure 3.2 summarizes the fatty acid (FA) profiles of three batches of gilthead sea bream after feeding for 7 months on three different diets. These profiles were then compared with the starting batch of juveniles.

The following fatty acids (FAs) were present in the highest proportion among the samples:

- Oleic acid (C18:1n9) accounted for around 33% in all three batches (Control, ED1 and ED2) after the 7-month feeding trial. No significant differences were detected between the three final batches, and values remained consistent with the starting batch (juveniles).
- Linoleic acid (C18:2n6) accounted for around 19% and showed no significant differences between the three batches after the feeding trial. However, a significant growth of approximately 5 percentage points was detected with respect to the starting batch (juveniles). This increase could be attributed to the change in diet between the juvenile phase and the specific fattening phase.
- Palmitic acid (C16:0) accounted for 16-17% with no differences between the three post-trial samples. There was a slight decrease compared to the initial value (19%) of the starting batch (juveniles).

- Docosahexaenoic acid (DHA - C22:6n3) in ED1 and ED2 decreased slightly with respect to the control. It decreased by a good 40% (from 8% to 5%) after the feeding trial.
- Overall, when analyzing the combined data for saturated, monounsaturated and polyunsaturated fatty acids (SFA, MUFA and PUFA) (Fig.3.3), no significant differences were found between the three groups. Results are consistent with published data on the same sector (D. Lenas et al. 2010; D. S. Lenas et al. 2011).

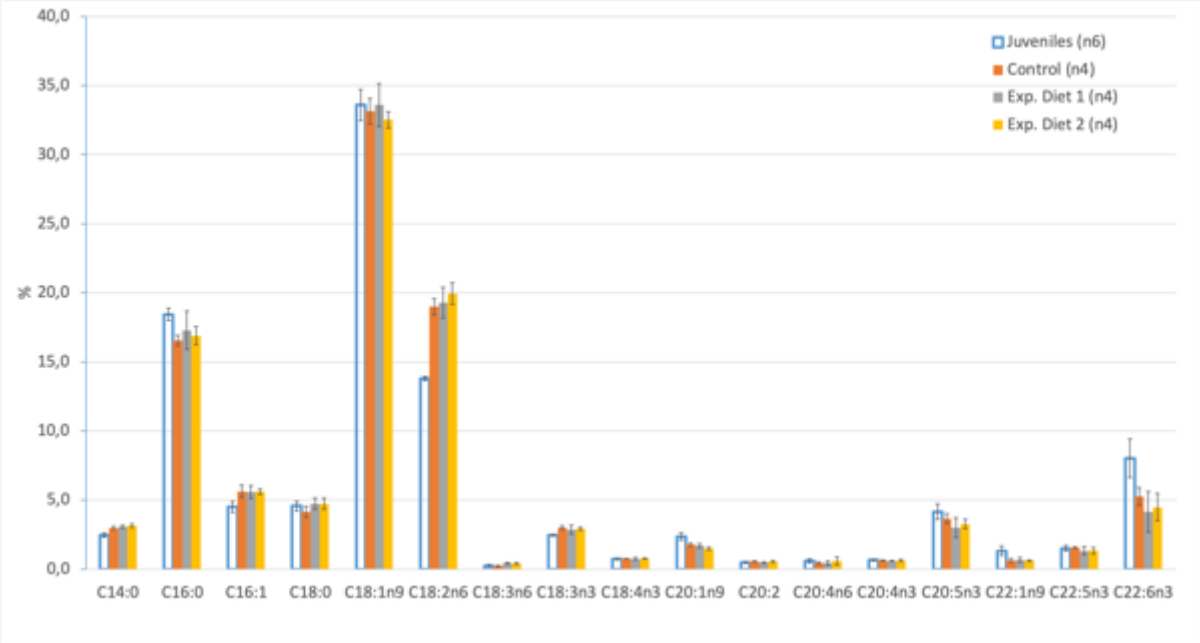

Fig. 3.2. Fatty acid (FA) composition of gilthead sea bream: comparison between the initial population (juveniles) and after feeding for 7-months on three different diets (Control, ED1 and ED2).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818290.

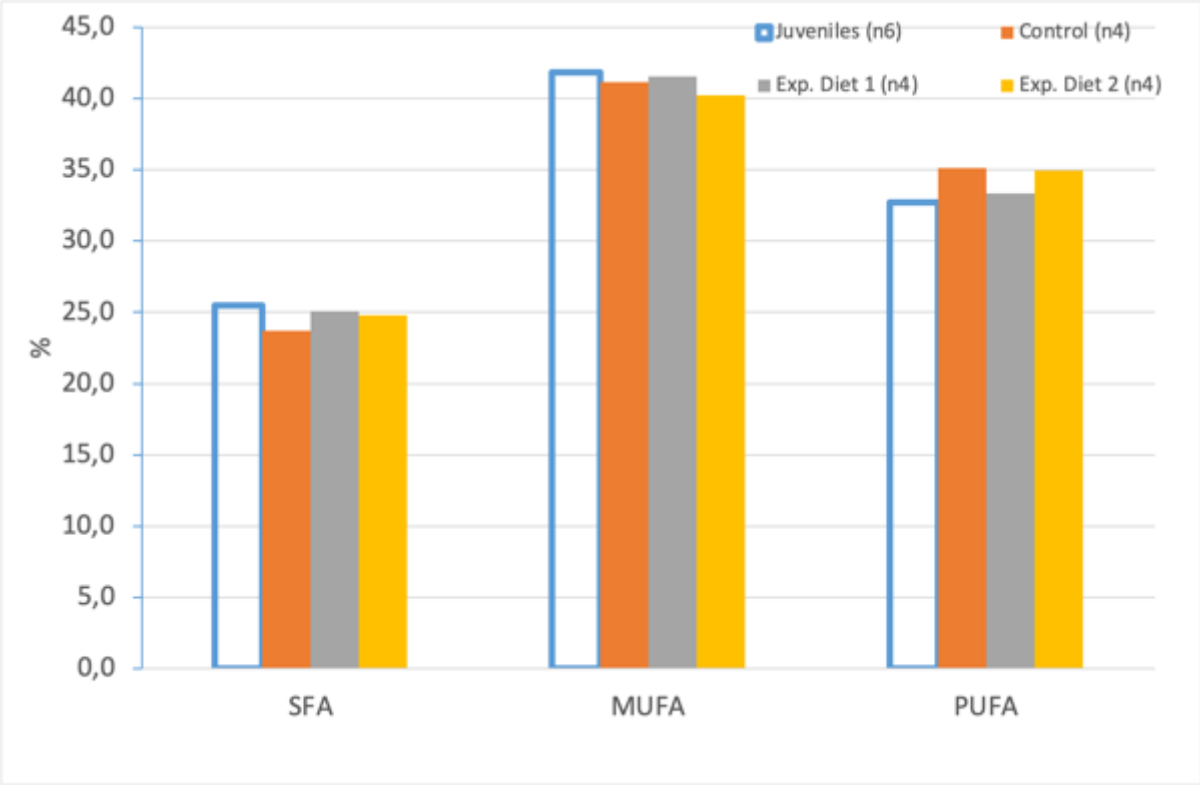

Fig. 3.3. SFA, MUFA and PUFA profiles for the initial gilthead sea bream population (juveniles) and after feeding for 7 months on three different diets (Control, ED1 and ED2).

Among the unsaponifiable fraction of lipids, no significant difference in the average cholesterol content was detected among the three samples after the feeding trial. It ranged between 67 and 70mg/100g (Fig. 3.4), in line with data collected on the same farmed species by Orban et al. (2003).

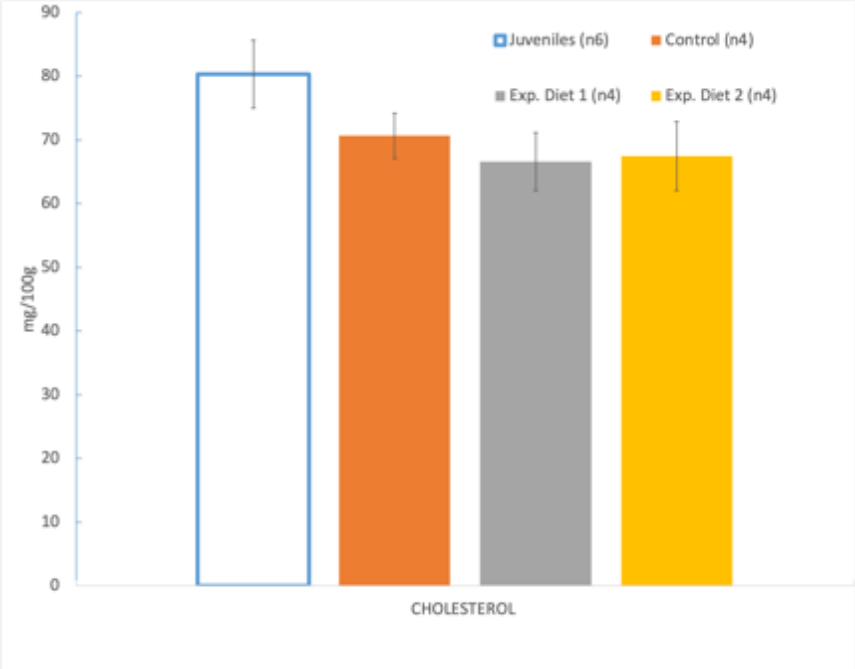

Fig. 3.4. Cholesterol content in the initial gilthead sea bream population (juveniles) after feeding for 7-months on three different diets (control, ED1, and ED2).

The nutritional value of gilthead seabream, as well as the atherogenic index (AI) and the thrombogenic index (TI) were determined in specimens from all feeding trials. The lower these indices, the lower the probability of cardiovascular disease. Both AI indices and TI indices were calculated as follows:

$$AI = (C12:0 + 4 \times C14:0 + C16:0)/[\Sigma MUFA + \Sigma(n-6) + \Sigma(n-3)]$$

$$TI = (C14:0 + C16:0 + C18:0)/[0.5 \times \Sigma MUFA + 0.5 \times \Sigma(n-6) + 3 \times \Sigma(n-3) + \Sigma(n-3)/\Sigma(n-6)]$$

The values of the atherogenic (AI) and thrombogenic (TI) indices did not vary significantly among the three examined batches of sea bream (control, ED1 and ED2). Values were in line with those reported (Álvarez et al. 2020) for the same species fed on an oil diet in which 75% of the fish oil was replaced by a vegetable oil.

The total amino acid composition of fish fillets is shown in Figure 3.5. Glutamine was the most abundant amino acid (2.4 to 2.8 g/100g), followed by lysine (2.0 to 2.25 g/100g), asparagine (1.9 to 2.1 g/100g) and leucine/isoleucine (1.8 to 2.3 g/100g). There were no significant differences observed among specimens fed on the three diets (control, ED1 and ED2). Overall, the amino acid profile of the fillets was similar to that reported by Kaya Öztürk et al. (2020) for the same species.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818290.

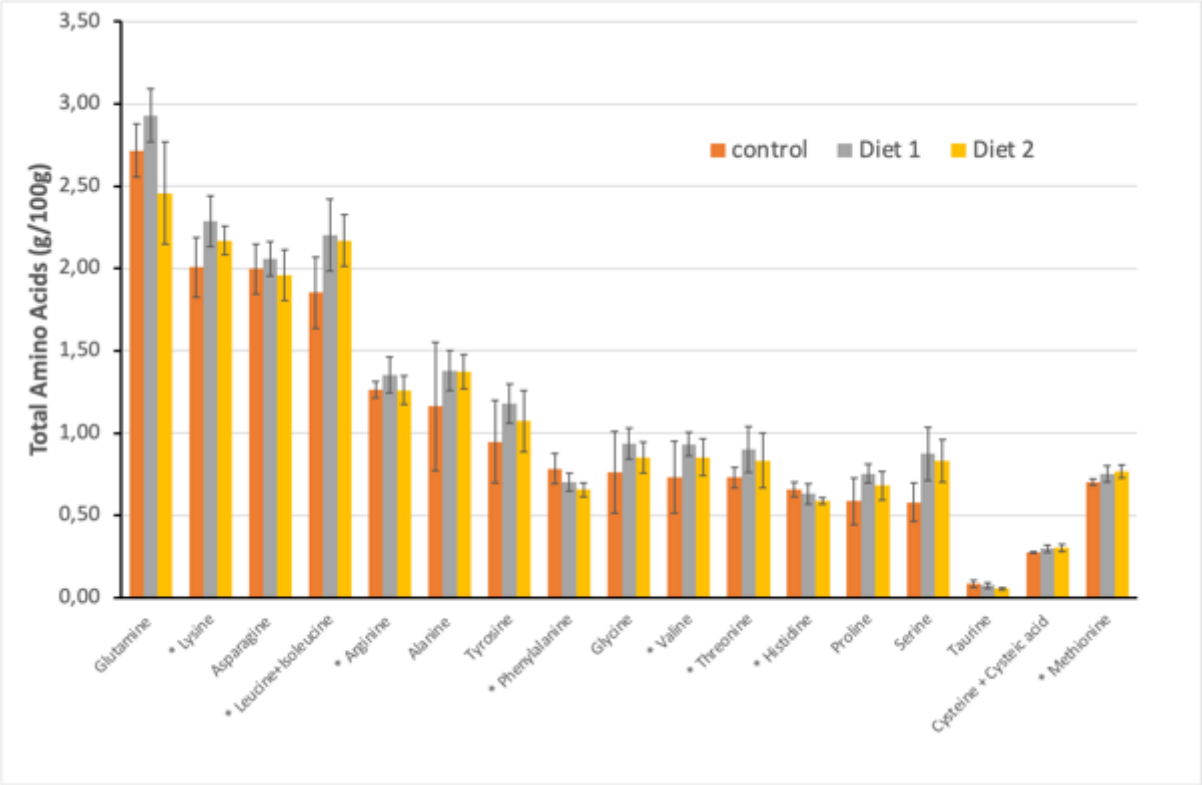

Fig. 3.5. Overview of free amino acid (FAA) concentrations (essential marked with *) in gilthead sea bream after the feeding trial

Figure 3.6 presents an overview of the Free Amino Acid (FAA) profile. Leucine/isoleucine accounted for over 30% of the total FAAs (0.5/100g), while Glycine is the second most prominent FAA (0.2g/100g). Proline, glutamine, histidine, threonine, valine, alanine, Arginine and serine were all below 0.1g/100g. Phenylalanine, Tyrosine, Methionine, Asparagine and Cysteine were all below 0.05 g/100g. The study revealed no significant differences among the three tested diets.

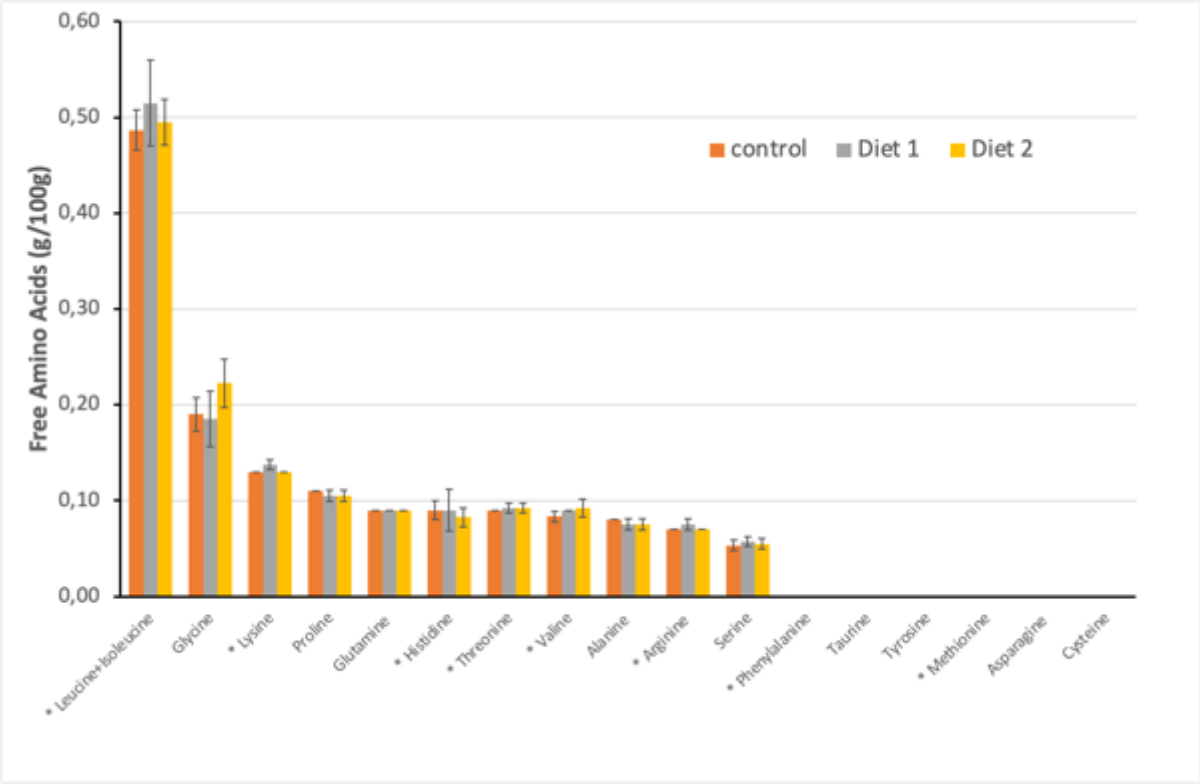

Fig. 3.6. Overview of free amino acid (FAAs) concentrations (amino acids are marked with an asterisk) in gilthead sea bream after the feeding trial (phenyalanine, tyrosine, methionine, asparigine and cysteine values were <0.05 g/100g).

The major mineral content is shown in Tables 3.7 and 3.8. As expected, all samples showed a high level of potassium and phosphorus (5 and 3 g/kg respectively) without significant differences between the three batches. The only element that stood out among the three diets was iron. which in the ED2 sample reached 5 mg/kg compared to 3 mg/kg in the control and ED1. These results are consistent with those of (Öztürk et al. 2019) on muscles of the same species raised in Turkish waters and processed in the same season.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818290.

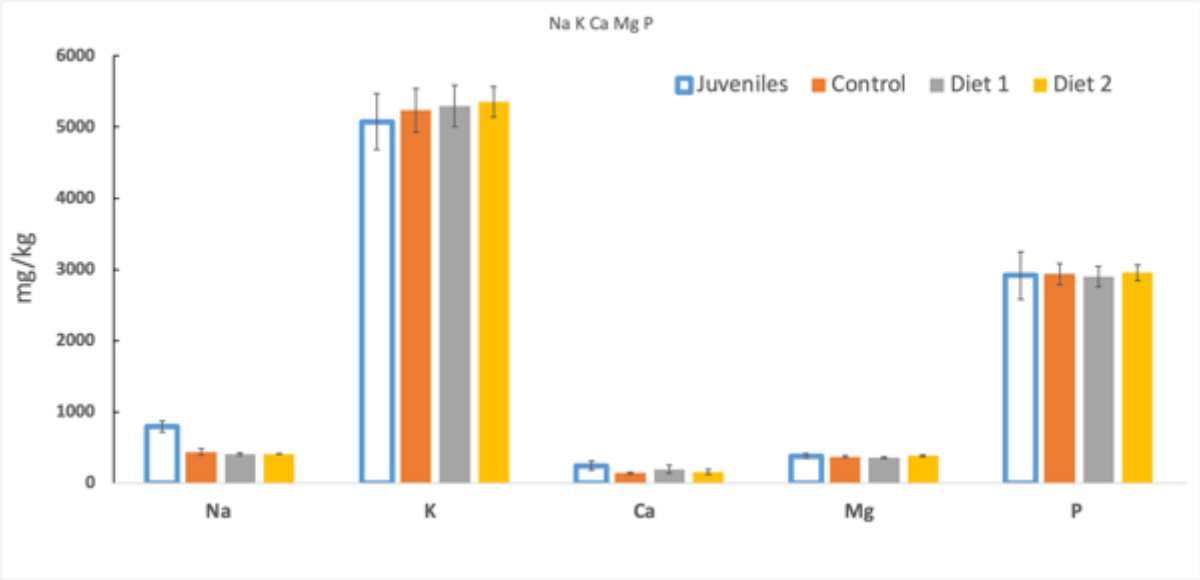

Fig.3.7. Mineral content (Na, K, Ca, Mg, P) in the initial gilthead sea bream population (juveniles) and after feeding for 7-months on three different diets (control, ED1 and ED2).

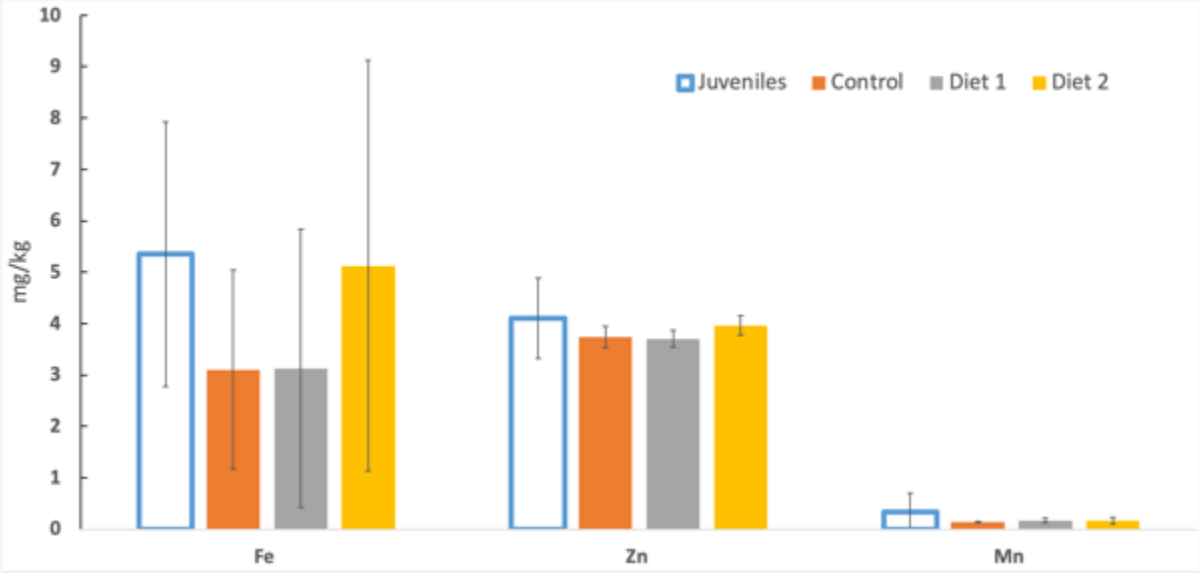

Fig. 3.8. Mineral content (Fe, Zn, Mn) in the initial gilthead sea bream population (juveniles) and after feeding for 7-months on three different diets (control, ED1 and ED2).

Table 3.5 shows the results of color analysis. The change in the color of GSB was evaluated based on L* (brightness), b* (yellowness) and a* (redness) values. The mean values of L* and a* were similar in the three batches (control, ED1 and ED2). However, compared to the ED1 sample, the control and ED2 samples showed an increase in the yellow value (b*), which

according to Khayat and Schwall (1983) could be interpreted as an increase in muscle oxidation.

Table 3.5. Average L*, a* and b* color values for gilthead seabream.

	L* (lightness)	SD	a* (+red/-green)	SD	b* (+yellow/-blue)	SD
Control	45.02	1.13	-2.79	0.18	3.81	0.75
ED1	45.06	0.95	-2.41	0.47	2.28	0.57
ED2	43.80	2.19	-2.41	0.63	3.67	1.20

Table 3.6 summarizes the results of the Shear Force (SF) test carried out on the fillets of the three sea bream batches after feeding for 7-months on three different diets. The SF of the control batch and that of the ED2 batch are very similar (around 0.12 N/mm2), whereas that of the ED2 batch is almost twice as high.

Table 3.6. Changes in the shear force of sea bream (control, ED1 and ED2) after feeding for 7-months on three different diets (n=4).

Sample	**Shear force (N/mm^2)**	DS
Control	0.12	0.06
ED 1	0.21	0.13
ED 2	0.12	0.03

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818290.

4. References

Álvarez, A., R. Fontanillas, A. Hernández-Contreras, and M.D. Hernández. 2020. "Partial Replacement of Fish Oil with Vegetal Oils in Commercial Diets: The Effect on the Quality of Gilthead Seabream (Sparus Aurata)." *Animal Feed Science and Technology* 265 (July):114504. https://doi.org/10.1016/j.anifeedsci.2020.114504.

Kaya Öztürk, Dilara, Recep Öztürk, Birol Baki, İsmihan Karayücel, and Sedat Karayücel. 2020. "Monthly Variation of Growth, Biochemical Composition, Fatty and Amino Acids Patterns of Gilthead Sea Bream (*Sparus Aurata*) in Offshore Cage Systems under Brackish Water Conditions in the Black Sea." *Aquaculture Research* 51 (12): 4961–83. https://doi.org/10.1111/are.14833.

Khayat, A., and D. Schwall. 1983. "Lipid Oxidation in Seafood." *Food Technology* 37:130–40.

Lenas, Dimitrios, Panaghiotis Loghothetis, Grigorios Kanlis, and Cosmas Nathanailides. 2010. "Comparison of Fatty Acids in the Brains of Wild and Reared Sea Bass Dicentrarchus Labrax L. and Sea Bream Sparus Aurata L., and Living in the Same Natural Environment." *International Aquatic Research* 2 (3): 135–41.

Lenas, Dimitrios S., Dimitrios J. Triantafillou, Soumela Chatziantoniou, and Cosmas Nathanailides. 2011. "Fatty Acid Profile of Wild and Farmed Gilthead Sea Bream (Sparus Aurata)." *Journal Für Verbraucherschutz Und Lebensmittelsicherheit* 6 (4): 435–40. https://doi.org/10.1007/s00003-011-0695-2.

Orban, E., T. Nevigato, G. Di Lena, I. Casini, and A. Marzetti. 2003. "Differentiation in the Lipid Quality of Wild and Farmed Seabass (*Dicentrarchus Labrax*) and Gilthead Sea Bream (*Sparus Aurata*)." *Journal of Food Science* 68 (1): 128–32. https://doi.org/10.1111/j.1365-2621.2003.tb14127.x.

Öztürk, Dilara Kaya, Birol Baki, İsmihan Karayücel, Recep Öztürk, Gülşen Uzun Gören, and Sedat Karayücel. 2019. "Determination of Seasonal Vitamin and Mineral Contents of Sea Bream (Sparus Aurata L., 1758) Cultured in Net Cages in Central Black Sea Region." *Biological Trace Element Research* 187 (2): 517–25. https://doi.org/10.1007/s12011-018-1382-2.

Postharvest Technologies and Quality Control of Shrimp

Edited by

Nilesh Prakash Nirmal
Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand

Soottawat Benjakul
The International Center of Excellence in Seafood Science and Innovation, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Gioacchino Bono
Institute of Marine Biological Resources and Biotechnologies, National Research Council (IRBIM-CNR), Mazara del Vallo, Italy; Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy

ACADEMIC PRESS
An imprint of Elsevier

Chapter 2

The impact of the handling process and different storage conditions on shrimp quality

Gioacchino Bono[1,2], **P. Rusanova**[1,3], **C.O.R. Okpala**[4,5] **and Nilesh Prakash Nirmal**[6]

[1]*Institute of Marine Biological Resources and Biotechnologies, National Research Council (IRBIM-CNR), Mazara del Vallo, Italy;* [2]*Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy;* [3]*Department of Biological, Geological and Environmental Sciences (BiGeA)—Marine Biology and Fisheries Laboratory of Fano (PU), University of Bologna, Bologna, Italy;* [4]*Department of Functional Food Products Development, Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences, Wrocław, Poland;* [5]*UGA Cooperative Extension, University of Georgia, Athens, GA, United States;* [6]*Institute of Nutrition, Mahidol University, Salaya, Nakhon Pathom, Thailand*

1. Introduction

1.1 A brief taxonomic overview of commercial shrimps and prawns

To this day, Holthuis (1980) and Chan (1998) are the reference works for those interested in, or who wish to deepen their knowledge of, the systematics of shrimps and prawns (scientifically better known as "Macrura Natantia") of interest to fisheries and aquaculture. There are more than 300 commercial species, of which 100 are highly appreciated by gourmets from all over the world for the quality of their meat (Bono, Gai, et al., 2012; Chan, 1998; Heu et al., 2003; Rusanova et al., 2022).

Most of the commercial shrimps and prawns belong to the superfamily Penaeoidea, which includes most of the highly valued commercial marine shrimps, especially several species included in the Penaeidae family, followed by about eight species in the Aristeidae family.

Other species of particular commercial importance are the giant river prawn (*Macrobrachium rosenbergii)* and the oriental river prawn (*Macrobrachium nipponense*), both members of the Palaemonidae family (both

Postharvest Technologies and Quality Control of Shrimp. https://doi.org/10.1016/B978-0-443-16124-7.00002-7

farmed and fished), followed by *Pandalus borealis* (Pandalidae family) and the Akiami paste shrimp *Acetes japonicus* (Sergestidae family).

Most of the abovementioned Penaeidae species occur in shallow waters along the continental shelf (from the infralittoral zone to a depth of 200 m), whereas Aristeidae species occur along the slope (from a depth of 201 m to about 1000 m). Many of the peneids that live in shallow waters can be either wild or farmed, whereas species from the Aristeidae family are only wild. The fishing of wild species mostly involves the use of bottom trawlers, traps and other minor artisanal gear.

1.2 Shrimp and prawn fisheries and aquaculture production

The following tables show the most fished (Table 2.1) and the most farmed (Table 2.2) shrimp and prawn species in 2020 (FAO, 2022). In the wild captured group (3.3 million tonnes in 2020), excluding the nonspecific category of Natantia, the most captured shrimp species were fleshy prawn *Penaeus chinensis* (7% of total captured wild crustaceans), followed by the giant tiger prawn *Penaeus monodon*, the northern prawn *Pandalus borealis* and the Akiami paste shrimp *Acetes japonicus*. Among the aquaculture species, the whiteleg shrimp *Penaeus vannamei* was the most farmed marine species (5.8 million tonnes, Tab. 1b) (FAO, 2022).

In the 2017–20 4-year period, global shrimp catches fluctuated between 3.4 and 3.1 million tonnes. This variability could be due to several factors: (a) the crisis in marine captures that has invested China in recent years, although it remains the world's largest producer of marine shrimps, followed by Ecuador, India, Indonesia and Thailand; (b) the strong decline in the

TABLE 2.1 Main wild commercial shrimps and prawns caught in 2020.

Common name	Scientific name	Family	Million tonnes	Percentage of total crustaceans (%)
Natantian decapods nei, Natantia			0.82	15
Fleshy prawn	*Penaeus chinensis*	Penaeidae	0.37	7
Giant tiger prawn	*Penaeus monodon*	Penaeidae	0.3	5
Northern prawn	*Pandalus borealis*	Pandalidae	0.26	5
Akiami paste shrimp	*Acetes japonicus*	Sergestidae	0.25	4

TABLE 2.2 Main shrimps and prawns farmed in 2020.

Common name	Scientific name	Family	Million tonnes	Percentage of total crustaceans
Whiteleg shrimp	*Penaeus vannamei*	Penaeidae	5.8	51.7
Giant tiger prawn	*Penaeus monodon*	Penaeidae	0.7	6.4
Giant river prawn	*Macrobrachium rosenbergii*	Palaemonidae	0.29	2.6
Oriental river prawn	*Macrobrachium nipponense*	Palaemonidae	0.23	2

production of Akami past shrimp, which almost halved in just 4 years (from 0.45 to 0.25 million tonnes); (c) the increase in other important species such as the northern prawn, which has remained constant (about 0.23 million tonnes), the giant tiger prawn, which has increased significantly (from 0.24 to 0.3 million tonnes) and the fleshy prawn, which even doubled (from 1.8 to 3.7 million tonnes) (FAO, 2022). In this scenario, the role of inland shrimp catches appears negligible.

As for the role of shrimp and prawns in aquaculture, their production has increased steadily over the last decade: in 2020 they abundantly exceeded the captured production, especially along the coasts of some developing countries in Asia and Latin America, where the many brackish mangrove ponds lend themselves well to the breeding of these species (FAO, 2022; Monsalve & Quiroga, 2022; Hassoun, Prieto, et al., 2022).

In the central Mediterranean, especially after the Second World War, wild shrimps (those from aquaculture did not exist yet) were considered a by-catch and were therefore only consumed by fishermen or, in any case, by coastal communities (perhaps following a barter) (personal data or last MS on the GRS history). This was linked to the high perishability and well-known blackening of these products, which made them unattractive and therefore unmarketable already a few hours from capture.

Starting from the 1960–70s (during the economic boom), shrimps began to be treated with synthetic antioxidants, especially sodium sulfites (Na_2SO_3). Especially in the central Mediterranean shrimp fishing sector, sulfites were a turning point in the production and consumption of shrimp in Europe: from an almost exclusively local use, chemical antioxidants coupled with onboard cooling systems (first only to refrigerate, then to freeze the products) made it so that in less than a decade three of the most valuable wild shrimps

(deep-water rose shrimp *Parapenaeus longirostris*, giant red shrimp *Aristaemorpha foliacea* and red shrimp *Aristeus antennatus*) became the main target species for bottom trawlers and a delicacy food.

To date, the deep-water rose shrimp is the most captured shrimp species in the 200−400 m depth range, followed by giant red shrimp and violet shrimp, which have been intensively fished since 1980 in the 400−800 m depth range, especially in the central Mediterranean Sea (FAO GSAs 12, 15, 16, 21). From 2000 onwards, red shrimp fleets have shifted toward the eastern sector of the Mediterranean Sea, toward Crete in particular (GSA 22 and 23), thanks to the Sicilian fishermen who were the first to exploit these depths in search of the valuable crustaceans. Moreover, in the last 10−15 years, giant red shrimp capture by local fishermen has been observed off the Egyptian coasts (GSA 26 and 27) (Fiorentino et al., 2024).

Further back in time, Carbonell et al. (1999) and Cartes et al. (2011) noted that the first landings of valuable red shrimps (*A. antennatus and A. foliacea*) in the western Mediterranean sector (especially in the Balearic Islands) were recorded in around 1940. Similar observations can also be made for GSA 9, where interesting yields were recorded starting in 1930 (Fiorentino et al., 2024).

1.3 Shrimp and prawn consumption and consumer attitudes by geographical area

In the second half of the last century, due to their high price, the consumption of shrimps and prawns was mainly concentrated in high-income countries (North America, Europe and Japan), where the consumption of aquatic food products sometimes reached 80 kg per capita per year. This of course clearly contrasts with low-income countries such as Afghanistan, Tajikistan and Ethiopia, where total seafood consumption, let alone shrimp consumption, did not exceed 1 kg per capita per year (FAO, 2022).

Thanks to the intensive farming of these precious crustaceans, their increased availability in recent years has led to lower prices, promoting an increase in per capita consumption (from 0.4 to 2.2 kg) between 1961 and 2019 (FAO, 2022).

The increasing popularity among consumers of shrimps and related products, which half a century ago were consumed only in the areas of production, has attracted the attention of gourmets worldwide. Accordingly, more research is required to extend shelf-life and quality through new tailored packaging techniques.

As for consumer attitudes and acceptance, there aren't many studies in this sector. To the best of our knowledge, one of the first studies dates back to 1979 (Edmunds & Lillard, 1979); Mills (2001) completed a more recent study, but it is only in 2006 that researchers (Erickson et al., 2007) conducted a more complete study to determine the acceptability of shrimp samples based on their

appearance, texture, flavor and aroma. In 2016, a couple of studies carried out in Italy (Okpala, Bono, Pipitone, et al., 2016; Okpala, Bono, & Gancitano, 2015) provided further insight into how Italian consumers perceive wild shrimps that are either chemical-treated or free of chemicals. Results found that consumers favor chemical-free shrimp products and are willing to pay an additional 15% for these.

2. Handling process

2.1 Handling of wild shrimp from fisheries

The most widely used gear to gather wild shrimp is the bottom otter trawl net (Fig. 2.1) towed by a fishing vessel: depending on the country's fishing ground of interest and its relative distance from the coastline, these vessels can range between 15 and 80 m of Length Overall (LOA) (Fig. 2.2). In some cases, a single fishing vessel can simultaneously tow up to four trawl nets.

Over 90% of the wild shrimps and prawns arriving on consumer tables are caught with bottom trawls like those shown in Fig. 2.2B. This fishing activity is not sustainable, above all because the bottom otter trawl gear is not selective enough: in addition to the target species (e.g., the above-mentioned pink and red shrimps), it catches many other unwanted marine species (e.g., bony fish,

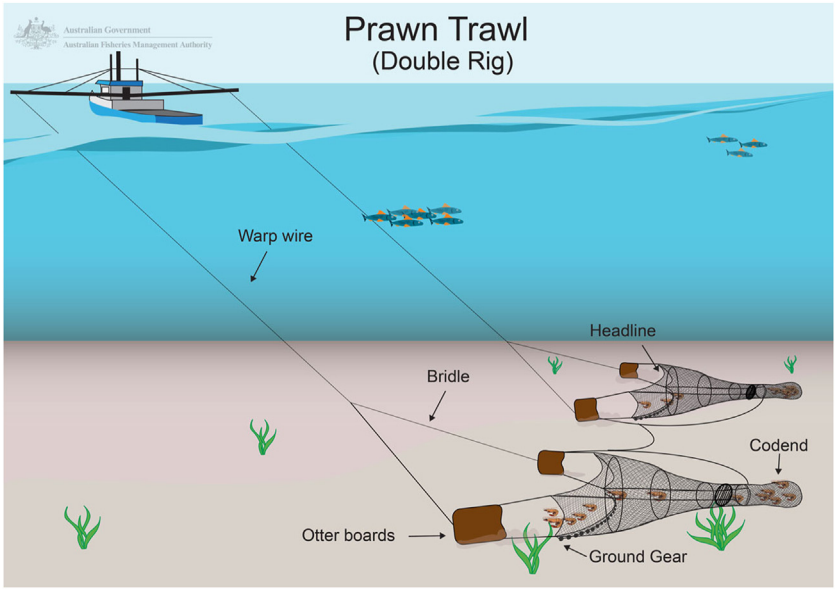

FIGURE 2.1 Schematic overview of bottom otter trawl. Shrimp trawls can be towed in single or in multiples of two, three or four nets. *Source: Australian Fisheries Management Authority - https://www.afma.gov.au/methods-and-gear/trawling.*

(A)

(B)

FIGURE 2.2 (A) a small Spain fishing trawler (LOA 16−18 m; gross tonnage: 15−20); (B) the ALTARIRE, a large fishing trawler of the UK fleet (LOA 80 m; gross tonnage: 3863). *From Alamy Stock Photo.*

elasmobranchs, cephalopods and other low-value crustaceans), the total weight of which can reach or exceed that of the target species and which are generally thrown out into the sea https://www.linkedin.com/in/gioacchino-bono-bb348844/recent-activity/all/.

In contrast to the industrial shrimp fishery in Fig. 2.2B, characterized by a high environmental impact, also in terms of energy consumption and CO_2 emissions, a very ancient and little-known shrimp fishing tradition is still practiced on horseback along the northwest Belgian coasts (Fig. 2.3). Fishing is carried out by pulling a small trawl net (a funnel-shaped net held open by

FIGURE 2.3 Traditional shrimp fishing on horseback. *From Alamy Stock Photo.*

two wooden boards) parallel to the coastline. A chain dragged over the sand creates vibrations, causing the shrimp to jump into the net. Fishermen place the catch in two typical baskets made from plant materials hanging at the horses' sides. This traditional and sustainable fishing activity plays a central role in social and cultural events in the Belgian area, attracting over 10,000 visitors each year.

Other minor shrimp and prawn fisheries make use of special traps (or pots) that are part of the local fishing tradition (Fig. 2.4) or, in a more industrial and mechanized context, are attached to medium-to large-sized fishing vessels (even over 30 m of LOA) capable of handling hundreds of small traps (Fig. 2.5). The gear consists of a mainline and hundreds of secondary short lines (snoods) to which the traps are attached at regular intervals and which are

FIGURE 2.4 An example of traditional shrimp fishing using typical traps adapted to catch some species of the genus Plesionika.

FIGURE 2.5 A Mediterranean fishing vessel (about 30 m of L.O.A.) with a fleet of shrimp traps on the upper deck ready to be set on the main shrimp fishing grounds.

set very close to the bottom. Although the first ancient fishery on *Plesionika narval* can be considered a low-impact activity (due to the natural materials and the low number of single traps used), according to Stevens (2021) the mechanized trap fishing gear shown in Fig. 2.5A can cause significant damage to benthic habitats (i.e., coral, sponges and other benthonic flora and fauna) when the gear is set and then retrieved.

2.1.1 Onboard processing of wild shrimps

Right after the catch, shrimps are traditionally sorted by species and then by specimen size (Fig. 2.6). Both these processes can be carried out manually (Fig. 2.7) in small to medium fishing vessels (about 15−30 m of LOA) or automatically (on board fishing vessels larger than 30 m of LOA) using a catch separator coupled with a shrimp grading machine such as those shown in Fig. 2.8.

Especially in the Mediterranean area, the subsequent stages of processing include washing shrimp with abundant marine water and then manually applying an antioxidant (i.e., sodium sulfite, 4-Hexylresorcinol) in order to inhibit blackspot discoloration (Bono et al., 2010) (Fig. 2.9). The treatment involves dipping shrimp in a seawater solution (4% w/v) of commercial anhydrous sodium sulfite (shrimp-to-dipping solution ratio of 1:4) (Rusanova et al., 2022). When the seawater or air temperature is above 20°C, it is preferable to replace part of the marine water used to prepare the abovementioned

FIGURE 2.6 A traditional Mediterranean selection of giant red shrimps (GRS) (Aristeomorpha foliacea) and Blue and red shrimps (BRS) (Aristeus antennatus). From the largest (I) to the smallest (IV) commercial category. Based on the fishing and processing areas, these four categories can be also labeled as "extra-extra large" (XXL), "extra large" (XL), large (L) and small (S).

FIGURE 2.7 Manual sorting and grading of giant red shrimp onboard the Italian trawler twenty-two (April 2016; North of Creta Island, Mediterranean Sea).

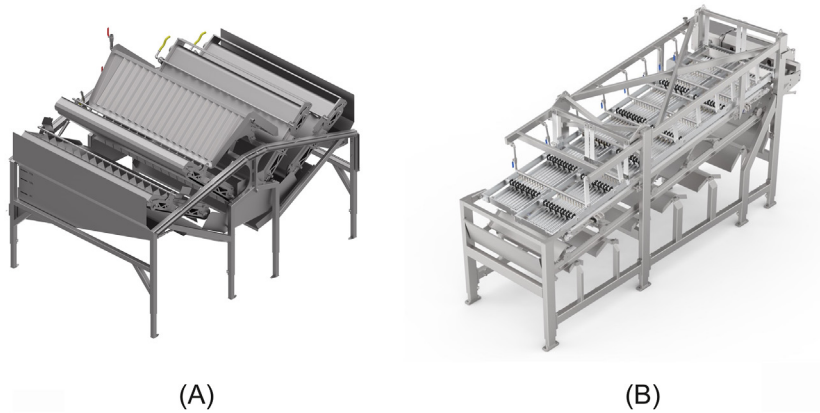

FIGURE 2.8 Example of catch separator (A) and a compact shrimp grader (B) developed by CARSOE for use both onboard medium fishing vessels and during onshore processing. *Source: https://carsoe.com/onboard-seafood-processing/shrimp/*

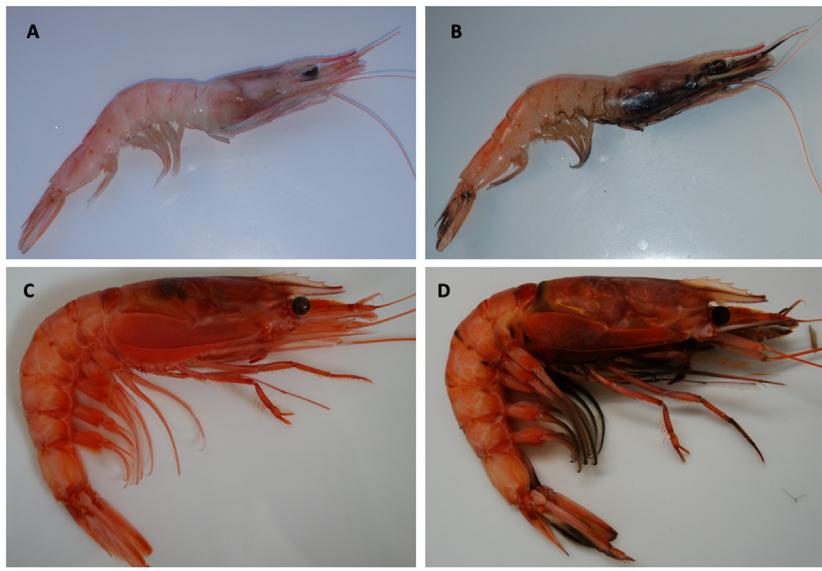

FIGURE 2.9 **Shrimps blackspot.** (A) specimens of deep-water rose shrimp immediately after the catch; (B) the same specimens after 5 h at 2°C without any chemical treatment. (C) Specimens of giant red shrimp immediately after the catch; (D) the same specimens after 5 h at 2°C without any chemical treatment.

antioxidant solution with a fair amount of ice (flake ice or, even better, slurry ice): this quickly reduces the core temperature of shrimps to about 0°C and therefore slows down post-mortem degradation processes immediately after capture.

Other processing techniques that can already be implemented on board medium-large fishing vessels include rapid cooking.

After antioxidant treatment, shrimp are landed as refrigerated or frozen products. This depends on the vessel characteristics (size of vessel and on-board shrimp preservation technologies), the duration of the fishing trip, and the fishing area's distance from the landing port. Fishing trawlers less than 20 m of LOA tend to carry out 1-to 4-day fishing trips: in this case the shrimp can be landed under ice or refrigeration. Industrial shrimp fisheries operating at great distances from the landing port (even hundreds of nautical miles), with trips lasting even more than 3 months, require freezing as a preservation method. More details about refrigeration and freezing methods are provided in Section 3.

In addition to the traditional techniques involving the treatment of shrimp with chemical antioxidants and subsequent refrigeration or freezing, in the last 10 years, thanks to scientific research in the sector (Bono, Badalucco, et al., 2012; Bono et al., 2016), it has been possible to preserve shrimp in a modified atmosphere and/or skins directly on board fishing vessels capable of housing the technologies shown in figures Figs. 2.10 and 2.11. This new packaging method, carried out directly on board within no more than 2 h from capture,

FIGURE 2.10 Onboard packaging of deep-water rose shrimps using a semi-automatic machine for modified atmosphere packaging (100% nitrogen) (Mondini, Brescia, Italy). Shrimps are packed in semi-rigid A.PET/EVOH/PE barrier trays (volume: 1800 cc; laminate density: 1.39 g/cm^3; thickness: 500 μm) with an oxygen permeability of 1.8 cm^3/m^2/day/atm and water vapor permeability of 4 g/m^2/day (Arcoplastica srl, Andenzeno, Italy). The trays are heat-sealed with a multiflex OPP/EVOH/PE film (weight: 72 g/m^2; thickness: 75 μm) with an O_2 permeability of 3 cm^3/m^2/day/atm, a CO_2 permeability of 10 cm^3/m^2/day/atm and a water vapor permeability of 3 g/m^2/day.

FIGURE 2.11 Automatic onboard packaging of giant red shrimp under skin-vacuum technology. *www.facebook.com/gioacchino.bono.980/videos/120396639300418*

can be used to preserve shrimp without the need for chemical additives (sulfites, r4-Hexylresorcinol, etc.) and therefore to distribute a genuine product with the taste and smell of the freshly caught one.

2.2 Handling of shrimp and prawns from aquaculture activities

2.2.1 Preliminary processing after the harvest

As with wild shrimps and prawns, most of the handling protocols for farmed products immediately after the harvest must be carried out quickly and at temperatures close to 0°C.

In light of this, during the operations following shrimp and prawn harvesting, cooling is generally carried out by immersing shrimps and prawns in tubs containing water mixed with ice. Where possible, inside the product processing structures, cooling of the entire processing room is preferred.

According to the rich literature in the field (Sipahutar et al., 2020; Okpala & Bono, 2016; Bono, Badalucco, et al., 2012; Okpala, Bono, Cannizzaro, & Jereb, 2016; Gökoğlu, 2021) the application of low temperatures as early as possible (i.e., cooling of products by dipping them in a 1:1 mixture of seawater and ice until the core temperature of shrimps reaches 0°C) is the most effective and common method to maintain the freshness of shrimp and other seafood products.

Some shrimp species require immediate cooling after harvesting, especially in cases where the product (i.e., *Penaeus vannamei*, *Penaeus monodon*, *Penaeus japonicus*, *Macrobrachium* spp) must be kept alive until it reaches the market and/or is consumed (fish markets, restaurants, etc.). Lowering the temperature of the product has an anesthetic effect and reduces metabolic activity, thereby reducing stress and mortality during transport.

However, aquaculture makes extensive use of chemical anesthetics such as 2-phenoxyethanol, quinaldine, benzocaine, Aqui-STM and methomidate, which can be toxic to both humans and the environment (Li et al., 2018). Among other things, the treatment of marine organisms with these anesthetics requires a recovery period of at least 21 days before the product can be consumed. The same authors propose natural anesthetics such as eugenol, extracted from cloves, which has proven to be relatively safe and effective on crustaceans.

The best methods for keeping shrimp alive during transport involve keeping them in tubs of water, preferably equipped with a closed water recirculation system able to maintain a constant temperature and oxygenation. In the case of short trips, it is possible to wrap the prawns in specially moistened and chilled sawdust (Gökoğlu, 2021).

Once they arrive at their destination, the prawns are usually brought to room temperature and the effects of anesthesia are reversed.

2.2.2 Grading

As in the case of onboard wild shrimp processing, farmed shrimps can be graded manually, especially in small medium companies, or using a mechanical grader such as that shown in Fig. 2.8B. In the latter case, the shrimp are distributed evenly on two oscillating screens with adjustable rails that separate them according to a set size.

3. Different storage conditions

3.1 Prompt postharvest cooling of shrimp and prawns to slow metabolic degradation and prolong shelf life

Compared to many meat products of terrestrial origin, fishery and aquaculture products, including shrimps and prawns, have a high perishability index, partly due to their high water content, the presence of enzymes belonging to the family of polyphenol oxidases (PPOs) and their pH: these factors compromise their texture (firmness, elasticity and chewiness), color (both due to melanosis and dehydration of the exoskeleton), total microbiological load (TPC) and consequently the values of trimethylamine (TMA-N) and total volatile basic nitrogen (TVB-N), as well as the primary (Peroxide Value) and secondary (TBARS) oxidation of the lipid component (Srinivasan et al., 2020; Okpala &

Bono, 2016; Okpala, Bono, Cannizzaro, & Jereb, 2016; Gökoğlu, 2021; Bono et al., 2010).

For this reason, immediately after catching (wild shrimps) or harvesting (farmed shrimps), the products should be immediately cooled and then kept at temperatures not exceeding 4°C, irrespective of whether they are intended to be marketed as fresh products or are waiting to be frozen to considerably lengthen their shelf-life. In particular, the product should be rapidly cooled after capture, especially when fishing operations or harvesting are carried out during the summer season and, in any case, when the air temperature exceeds 20°C or when the products are directly exposed to the sun and wind (Bono, Badalucco, et al., 2012; Gökoğlu, 2021).

Pre-cooling of shrimps and prawns is therefore essential to preserve the high quality of the product and prolong its shelf-life until it is consumed.

The most effective pre-cooling method in the fish sector is hydrocooling, which is performed by immersing the product in mechanically refrigerated water (Fig. 2.12) or by manually adding a suitable quantity of ice to keep the liquid temperature at around zero. Air pre-cooling is not recommended as it is much slower than hydrocooling.

If the preservation process involves treatment with antioxidants (sulfites, 4-Hexylresorcinol, etc.), product pre-cooling should be carried out before antioxidant treatment to avoid antioxidant leaching in the outermost tissues (mainly the exoskeleton) of the shrimp. It is possible to combine the two processes by adding the right dose of antioxidants directly to the container with water and ice used for the pre-hydrocooling of shrimps.

3.2 Traditional storage of shrimp with flake or crushed ice

In the case of wild shrimp and prawns, preservation under ice is mainly practiced by small coastal fishing vessels which land the product daily or at most every two-three days. This is to allow sufficient time for the product to be

FIGURE 2.12 (A) shrimps hydrocooling machine implemented onboard a Mediterranean bottom trawler; (B) conventional hydrocooling method adopted by small-medium shrimp trawlers. *Courtesy of ALFACOLD srl, Italy.*

first sold and then consumed within the canonical 7–8 days of maximum shelf-life extension based on the acceptable limits of TCP, TMA-N, TVB-N and PV (Okpala, 2014). Technically, different types of boxes in synthetic plastic, polystyrene (preferably made with recycled materials) or the newest bioplastic materials with reduced environmental impact can be used for storage under ice. In order to cool the product in the best possible way and to ensure maximum shelf-life, it is preferable to place layers of flake or crushed ice in the box with layers of shrimp in almost equal proportions (shrimp/ice ratio: 1:1 (w/w)) (Fig. 2.13).

The ice can be produced on land and then stored on board in a cold room, for example the same one in which the products will then be stored, or it can be produced directly on board. This is possible thanks to the vast range of ice generators that can be mounted on board medium and large fishing vessels (Fig. 2.14).

In order to reduce unwanted bacterial contamination of the product due to poor quality ice (e.g., ice produced from seawater or microbiologically impure water), the latter could be pre-treated with ozone, which is known to have powerful bactericidal and virucidal effects. This kind of treatment has, among other things, significantly prolonged the shelf life of various fishery products (Bono & Badalucco, 2012; Okpala, Bono, Abdulkadir, & Madumelu, 2015; Bono et al., 2017).

The product must subsequently be kept in the refrigerator at a temperature of 0–4°C. The treatment of farmed prawns is very similar, especially in geographic or market contexts in which the supply chain favors the commerce of fresh products for short-term consumption.

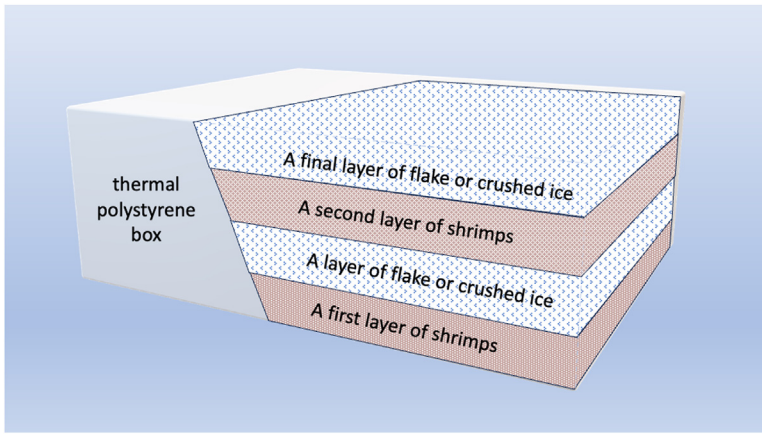

FIGURE 2.13 An example of thermal polystyrene box with shrimps stored under two layers of flake/crushed ice. The shrimp/ice ratio may be modified according to the ambient or refrigerator temperature.

FIGURE 2.14 Flake ice machine implemented onboard a Mediterranean shrimp trawler (LOA: 25–35 m). *Courtesy of Frigotecnica soc. coop. (Mazara del Vallo, Italy).*

One of the weaknesses of using flaked and/or crushed ice is linked to the potential mechanical damage caused by the weight of hard pieces of ice, especially to small shrimp (with a relatively soft exoskeleton) or decapitated or yet to be peeled shrimps.

3.3 Slurry ice as a gentle solution for fresh shrimp storage

Since its industrial introduction around the 2000s, slurry ice has been shown to extend the shelf-life of many fishery and aquaculture products. Composed of a mixture of small ice particles and a carrier fluid, generally freshwater or a solution capable of lowering the freezing point (containing sodium chloride, ethanol, ethylene glycol or propylene glycol), slurry ice provides a higher heat transfer coefficient than flake or crushed ice (Gökoğlu, 2021) (Fig. 2.15).

FIGURE 2.15 Box of deep water rose shrimp (*Parapenaeus longirostris*) under slurry-ice.

Zhang et al. (2015) tested the effects of slurry ice on Pacific white shrimp (*Litopenaeus vannamei*) and compared the results with a sample preserved in flake ice. This study revealed a significant reduction in TVB-N and TBARS, two of the most important parameters used to monitor the quality and shelf-life of fish products in general. The resulting microbial growth, springiness and chewiness variables were comparable to those of flake ice-treated samples. Furthermore, research on the possible application of slurry ice to other fish products has also provided good results which could therefore also apply to shrimps and prawns. In this regard, Zhang et al. (2022) recently confirmed that slurry ice combined with chitosan yielded better results than traditional suspensions. Improved performance has also been obtained by combining liquid ice with ozone to reduce total bacterial growth (Bono et al., 2017; Chen et al., 2016; Zhao et al., 2022), and by combining slurry ice with slightly acidic electrolyzed water (Liu et al., 2021).

3.4 Superchilling

The current international guidelines for the transport and storage of refrigerated fishery products refer to temperatures slightly higher than that of melting ice, more precisely 0−4°C (Duarte et al., 2020; Banerjee & Maheswarappa, 2019).

In this regard, the European Food Safety Authority (EFSA) confirmed that pre-packed fresh fishery products stored at refrigeration temperatures above 0°C (e.g., 3−5°C) comply with the current international rules (European Food and Safety Authority, 2015).

However, from the late 1980s to the present, over 56 studies [data from SCOPUS database searching for: (TITLE-ABS-KEY (superchilling) AND TITLE-ABS-KEY (shrimp) OR TITLE-ABS-KEY (fish) OR TITLE-ABS -KEY (seafood)) have shown that maintaining the temperature of the products between −3 and 0°C (i.e., between the freezing point and the temperature of traditional refrigeration) significantly extends the shelf-life of fish and shellfish and keeps both biochemical and microbiological spoilage within acceptable limits.

Zeng et al. (2005) showed that super chilling (−1.5°C) extends the shelf-life of coldwater shrimp (Pandalus borealis) with respect to storage in flake ice and liquid ice at 1.5°C.

A recent scientific opinion produced for EFSA (Koutsoumanis et al., 2021) extensively analyzed the use of the super chilling technique and compared superchilled fresh fishery products with products preserved in boxes with ice (the currently authorized practice) in terms of survival and growth of biological hazards.

3.5 Freezing

The rapid freezing of shrimp and prawns at a temperature lower than −40°C, especially if done shortly after catch or harvest, and storage at a constant temperature is a suitable way of preserving these fine seafood products for a long period of time.

In agreement with Jiang and Lee (2005), freezing inhibits the growth of poisonous bacteria and greatly reduces the biochemical reactions that normally occur in chilled seafood products. Technically, the freezing process, whether carried out at −40°C or at even lower temperatures, can be considered completed when most of the freezable water at the thermal center of the product has reached a temperature of at least −10°C. The higher the cooling rate, the greater the number of ice crystals that will form and the smaller their size: This is to the full advantage of the quality of the shrimp muscle, which will be less damaged and have a better texture. Furthermore, a higher freezing rate will result in: a lower migration of water from the muscle cells to the extracellular space (with a significant reduction in drip loss during the thawing process), a lower rate of water evaporation from the product (Jiang and Lee, 2005) and, according to Hu et al. (2021), a lower rate of protein hydrolysis and consequent destruction of muscle fiber structure, which is partly due to the action of cathepsins. It should be also noted that moisture loss occurs during pre-freezing and subsequent storage: this is reduced in whole shrimps (due to the resistant exoskeleton) with respect to peeled shrimps.

In agreement with Jiang and Lee (2005), shrimp desiccation during freezing can be significantly reduced by packaging the product in a material (generally plastic trays and/or bags) with low water vapor permeability and reducing headspace between the product and the packaging materials as much as possible. The added value of this technique is shown in the shrimps packed under the skin of Fig. 2.11 compared with Fig. 2.16 where, due to temperature

FIGURE 2.16 A thermo-sealed tray of frozen deepwater rose shrimps with evident signs of frost and desiccation as a consequence of ice that sublimed and condensed on the internal side of the packaging film due to temperature fluctuations during storage.

fluctuations, the ice of shrimps sublimed and consequently condensed on the inside wall of the upper packaging film.

Another important consequence of the temperature variations (or even cold chain interruptions) that can occur during frozen storage is the formation of white spots on the exoskeleton of shrimp, which alter their pigmentation and therefore their appearance.

In conclusion, fast freezing is recommended over slow freezing which, as mentioned above, causes the formation of large extracellular ice crystals with consequent damage to cell membranes and therefore to the muscle. However, recent evidence from two species of bony fish has revealed that ultra-fast freezing with liquid nitrogen can cause tissue breakdown and reduce water-holding capacity (Jiang et al., 2018; Lv & Xie, 2021).

Therefore, according to Hassoun et al. (2020), the freezing method, whether "fast" or "slow", must be carefully defined according to the type, size and structure of the product to be preserved and the potential effects that the chosen technique determines on the quality of muscles.

With regard to the choice of the best storage temperature, if on the one hand the literature of the last few decades has indicated −18°C as a good compromise between maintaining product quality and plant management costs (Blond & Le Meste, 2004), Tolstorebrov et al. (2016) observed that the optimum temperature for long-term storage of fish products is −35°C, and that even lower storage temperatures are uncalled for. However, Ji et al. (2021) argued that storage of greasyback shrimps (*Metapenaeus ensis*) at −60°C can better maintain their quality in terms of water-holding capacity, denaturation and protein oxidation when compared to frozen storage at −18°C.

3.5.1 Freezing techniques

As for the freezing techniques, air blast freezers equipped with suitable coolers and fans can guarantee a moderate freezing rate and a good quality product. However, when using this technique it is important to ensure that each product tray is efficiently and equally exposed to the current of cold air (Jan 2004). This technique is particularly recommended in the case of prawns, whereas the plate freezer lends itself better to other fish products having a greater contact area with the metal freezing plates. Furthermore, considering that freezing the shrimp immediately after capture, i.e., directly on board, guarantees longer shelf-life and therefore product quality, an advantage of this technique is that it can be easily implemented in a small space, therefore also onboard small/medium-sized fishing vessels (even those with around 15 m of LOA).

As the tonnage of the fishing vessel increases, or in the case of land-based plants where farmed shrimps are also processed, the air blast technique can include more effective technologies such as tunnel freezers, belt freezers and fluidized freezers.

In order to further improve the quality of frozen products, new techniques have been developed in recent years: their effectiveness should also be tested on crustaceans and on prawns in particular. These include pressure-shift freezing (PSF), ultrasound-assisted freezing (UAF), electrically assisted freezing, and magnetically assisted freezing (Li et al., 2022; Zhan et al., 2018; Hassoun, Siddiqui, et al., 2022).

3.5.2 Freeze-thaw cycles

Freezing is an excellent method for the long-term preservation (up to 18 months) of fish products and of shrimp in particular. However, the products may undergo temperature variations, for example during transport or during any phases of industrial processing (for example decapitation and/or peeling), or even in restaurants and at home.

Temperature variations during storage and/or transportation, or partial thawing (known as tempering) and refreezing in the case of processed products, induce important changes in the quality of the shrimps such as dehydration, drip-loss (therefore weight loss), fat rancidity, unpleasant taste, etc.

To overcome these chemical-physical, structural and sensorial changes, various solutions/products have been tested in recent years. Although mixtures of phosphates and NaCl have provided good results, their improper use can cause further deterioration as well as health problems. Recent studies therefore aim to overcome these effects with new, lower impact solutions. Among these, by way of example, Wachirasiri et al. (2019) investigated the effects of lysine and $NaHCO_3$ on thawing loss and shrimp texture, with significant results in terms of reduced thawing loss during different freeze-thaw cycles in Pacific white shrimp (*Penaeus vannamei*). Moreover, Zhang et al. (2021) achieved interesting results through radio frequency tempering to inhibit/reduce melanosis and protein oxidation in Pacific white shrimp during freeze-thaw cycles.

3.6 Onboard cooking

Where possible, for example on board medium/large fishing trawlers, the freezing and/or refrigeration of prawns could be combined with pre-cooking, preferably immediately after the catch.

In order to minimize product quality deterioration such as damage to cell structure, drip loss and sensory degradation, the cooking time must be as short as possible (no longer than 5 min): this is possible by keeping the ratio between the weight of the prawns and the volume of the cooking water to around 1:5 (w/v). Cooking must be followed by rapid product cooling under rigorous hygienic conditions that prevent any bacterial contamination.

Several shrimp cookers suitable for small as well as large fishing vessels and onshore production plants are available on the market.

4. Quality changes

4.1 Physicochemical changes

4.1.1 pH changes during fresh and frozen storage

Immediately after capture, namely in the case of a product not yet treated with antioxidants, the pH of both wild and farmed shrimp remains around neutral (7.0) (Bono, Badalucco, et al., 2012; Goncalves et al., 2003; López-Caballero et al., 2007). During refrigerated storage the pH may slightly increase due to microbial and enzymatic reactions (Basiri et al., 2015; Nirmal & Benjakul, 2011; Rezaei et al., 2023), leading to the formation of some basic substances (TMA, therefore TVB-N, and biogenic amines).

Freezing seafood leads to a change in pH (usually a reduction) due to the precipitation of salts such as phosphate and other modifications in dissolved and colloidal substances (Jiang and Lee 2005). In the case of shrimp, after the usual anti-melanotic treatment to prevent blackening, the pH tends to increase slightly during storage due to the high alkalinity (pH 8.5) of the salts used (e.g., sodium sulfite) (Na_2SO_3) as antioxidants. This increase in pH is also due to the production of dimethylamine (DMA), which is considered another important component of TVB-N, especially in frozen seafood (Bono, Badalucco, et al., 2012). Tsironi et al. (2009) and Jin et al. (2018) observed a similar increase in pH during the frozen storage of two other species of shrimp.

4.1.2 Changes in color, texture and water content during fresh and frozen storage

One of the main problems encountered during the handling and storage of fresh and frozen shrimp is the rapid formation of black pigments (i.e., melanosis) after catching. At ambient temperatures (18−25°C) and in contact with atmospheric oxygen, melanosis occurs no later than 2 h after death (Fig. 2.9). During the product processing phase (grading, handling, etc., both on board and ashore), the only remedy to slow down this process − prior to antioxidant treatment − is to quickly lower the product temperature to about 0°C degrees. To definitively stop the blackening, the shrimp must then be treated with antioxidants (currently the most effective ones are chemical, although some interesting natural products are emerging) or be frozen and packaged in an oxygen-free environment, as in the case of vacuum/skin or modified atmosphere packaging (Bono et al., 2016; Bono & Badalucco, 2012). For instance, Fig. 2.17 shows a specimen of giant red shrimp packed for 6 months under modified atmosphere packaging (100% nitrogen). The sample shows no sign of oxidation or external dehydration: the tail appears very shiny and translucent compared to the GRS specimens on the right, which are dehydrated and oxidized, with signs of blackspot due to poor preservation techniques.

Frozen shrimp, like most fish products, undergoes a series of muscle changes caused by the formation of dimethylamine (Bono, Badalucco, et al., 2012)

FIGURE 2.17 (A) GRS specimens packed under modified atmosphere packaging (100% N_2); after 6 months of storage at −18°C the shrimps show no sign of oxidation, external dehydration or blackspot; the tail meat is shiny and bright; (B) dehydrated and oxidized GRS specimens with signs of blackspot due to poor preservation; the meat tail is opaque and shows clear signs of dehydration.

and formaldehyde (from the breakdown of trimethylamine oxide), which gradually modify the texture (firmness/tenderness, cohesiveness and juiciness) and therefore the palatability of the product by binding to the proteins. In this regard, a study by Tsironi et al. (2009) on Mediterranean shrimp stored at different temperatures (−5, −8, −12 and −15°C) found small variability among frozen storage times (up to 350 days), suggesting that changes in texture parameters are not good indicators of quality deterioration.

Considering the role of environmental oxygen in the physiochemical degradation of shrimp, cheaper techniques generally adopted in the case of frozen products is glazing, which retards both dehydration and lipid oxidation.

Moreover, according to Jiang and Lee (2005), the main physiochemical changes in frozen shrimps occur at temperatures between the freezing point and −10°C. Shrimps should therefore be kept within this temperature range as little as possible, both during freezing and thawing.

The described physical-chemical changes are obviously less evident in fresh shrimps, which maintain their palatability, smell and taste for three-four days when stored at temperatures of 2−4°C, and for a few more days (max 5−6) under superchilling conditions (near the freezing point). After this time psychrophic bacterial growth can no longer be controlled and the product undergoes rapid deterioration.

As for melanosis, this process can be kept under control in refrigerated products without resorting to the use of chemical additives. In this regard, Fig. 2.18 is an interesting example of deep-water rose shrimps packed under nitrogen (100%) and stored for 6 days (T = 1°C) without chemical additives (authors' unpublished data), compared to the control sample (shrimps in a petri dish) kept at the same temperature but under atmospheric air.

In addition, both fresh and frozen shrimps have a longer shelf-life than the peeled and/or minced product, as such processing causes tissue damage and subsequent deterioration.

FIGURE 2.18 A tray of deep-water rose shrimps packed under a modified atmosphere (100% N_2) and stored at 1°C for 6 days compared to a sub-sample (in the petri dish) of the same lot kept at 1°C under air.

Other techniques used to curb melanosis and other degradative phenomena in shrimp during their shelf-life is rapid cooking, as mentioned in Section 3.6. In this regard, a recent study by Gringer et al. (2020) on changes occurring in steamed cold water shrimps (*Pandalus borealis*) compared to uncooked samples, revealed that the steaming process (90 s at 100°C) increased the springiness and decreased both the hardness and chewiness of products.

4.2 Biochemical changes

4.2.1 Changes in nutritional composition

Overall, both whole shrimps and tails have a high water content. In wild deep red shrimps, it ranges between 68% and 75%. Proteins are more concentrated in the tails (22%) and lower in the cephalothorax (12%). This difference is mainly attributed to the high fat content (about 10% between triglycerides and polar lipids) present in the hepatopancreas and in female gonads (Fig. 2.19), especially during the reproductive season which generally coincides with summer. In contrast, the tails of males and females have a relatively low lipid content (2%) and are therefore an excellent seafood for balancing nutrients; they are also rich in proteins and contain a good level (about 35%) of polyunsaturated fatty acids. Among the fatty acids, a study carried out on Mediterranean red shrimps (Bono, Gai, et al., 2012) detected the highest values for palmitic acid (15%−18%), oleic acid (20%−28%), eicosapentaenoic acid (EPA, 9%−15%) and docosahexaenoic acid (DHA, 16 27%). The lower linoleic acid values may be related to the omnivorous habit of wild shrimp. High LA contents are generally found in the flesh of cultivated shrimp such as *Fenneropenaeus indicus* and *Litopenaeus vannamei* (Ju et al., 2009; Ouraji et al., 2009).

4.2.2 Spoilage mechanisms during fresh and frozen storage

4.2.2.1 TVB-N

Total volatile basic nitrogen (TVB-N) is widely used to detect the bacterial and enzymatic breakdown of nitrogen compounds (dimethylamine, trimethylamine, ammonia and other minor volatile basic nitrogen compounds) present in seafood products.

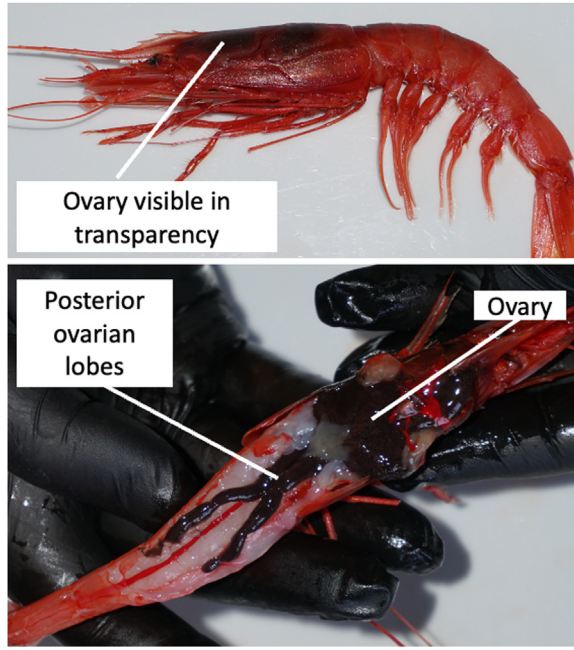

FIGURE 2.19 Above: an example of a red shrimp with a mature gonad visible through the carapace. Below: detail of the gonad after partial removal of the carapace.

TVB-N has been studied extensively in shrimp, although results have not always converged. Overall, according to the valuable literature on the subject (Angel et al., 1981), a TVB-N value higher than 30 mg/100 identifies serious product deterioration. As for trimethylamine, a value of 5 mg/100 g is the threshold limit for spoilage of seafood and refrigerated shrimp in particular (Zeng et al., 2005), while dimethylamine production is considered the major compound of TVB-N in frozen seafood (Botta, 1995). It is also undisputed that this parameter is strongly influenced by storage temperature, i.e., the lower the temperature the better. However, in some case studies, values higher than the TVB-N threshold mentioned above have been detected (López-Caballero et al., 2002; Mendes et al., 2005), for example in deep-water rose shrimp and giant red shrimp after 3 days of rapid freezing performed on board a fishing trawler (Bono & Badalucco, 2012; Bono et al., 2016). Bono et al. (2016) therefore argued that TVB-N in shrimp must be studied further to define the limit of acceptability, especially in frozen products treated with sulfite.

Remaining in the field of shrimp stored in a modified atmosphere, Wang et al. (2016) reported good results in the control of TVB-N in Pacific white shrimp (*L. vannamei*); however it would seem that an increase in CO_2 in the adopted gas mixture leads to an increase in TVB.

Other similar studies such as that of Zhang et al. (2015), always on Pacific white shrimp, found TVB-N values lower than 20 mg/100 g after 16 days of refrigerated storage in slurry ice.

4.2.2.2 K-value

The K-value is frequently used as an indicator of shrimp freshness. It is determined by quantifying adenosine triphosphate (ATP) and its associated compounds such as adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine 5′-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx). As reviewed by Pan et al. (2019), a K-value of about 20% is an indication of good product quality, whereas a higher value indicates an increase in ATP degradation (60% is the rejection point).

According to Nirmal and Benjakul (2009), natural plant extracts such as catechin could be excellent antioxidants, working especially against the degradation reactions that lead to an increase in K value.

While frozen storage of shrimps can slow down the degradation of ATP-related compounds, these increase significantly after one 24 h at 25°C (Huang et al., 2016).

4.2.2.3 Lipids

Due to the high presence of unsaturated fatty acids, aquatic products (and crustaceans in particular) undergo oxidative phenomena (otherwise known as rancidity) faster than meat products of terrestrial origin.

Lipid degradation in meat products occurs in two phases: the first, primary oxidation phase starts with the loss of polyunsaturated fatty acids and leads to the formation of hydroperoxides (products which are generally measured through the Peroxide Value, PV), whereas the second phase starts with the formation of carbonyls and leads to the formation of malondialdehyde (measured through the TBARS test).

Fat degradation processes eventually lead to the production of aldehydes, ketones and inferior fatty acids, which, as they react with proteins, phospholipids and DNA, produce off-flavors and odors and, in extreme cases, are responsible for harmful effects on human health (Pan et al., 2019).

However, according to Tsironi et al. (2009) and Bono et al. (2016), the TBARS value in shrimp fluctuates without any clear tendency during frozen storage, so that this value alone cannot be used as an indicator of lipid oxidation.

4.3 Microbial

The autochthonous microbial community (microbiota) of aquatic products is qualitatively and quantitatively correlated with the environment in which the same products were fished or farmed. The autochthonous bacterial flora of fish

can be divided into Gram-negative (Photobacterium, Acinetobacter, Vibrio, Flavobacterium, Moraxella, Shewanella, Aeromonas, Pseudomonas, etc.) and Gram-positive organisms (Brochothrix, Bacillus, Clostridium, Micrococcus, Lactobacillus, etc.) (Anagnostopoulos et al., 2022; Odeyemi et al., 2018). During the farming, fishing and processing phases (decapitation, evisceration, filleting, etc.) the fishery products can be contaminated by pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, Shigella spp., Salmonella spp, especially when these are processed in unhygienic conditions. Based on the optimal growth temperature, the bacterial community can be divided into mesophiles (20−45°C) and psychrophiles (<0−20°C).

From a microbial standpoint, a recent study by Parlapani et al. (2020) revealed the presence of bacterial species, such as Staphylococcus, Legionella, Acinetobacter, Bacillus, Escherichia, Shigella, Enterococcus, Enterobacter, not typically associated with shrimp. This kind of contamination can therefore be attributed to environmental contamination and must be kept in check through the introduction of good hygiene practices.

5. Conclusions

For some decades now, shrimp fishing, farming and consumption have grown significantly, revolutionizing the relationship between man and seafood. While from the standpoint of food availability (in agreement with some of the recent 17 UN Sustainable Development Goals) this can be considered good news, from an ecological perspective the huge increase in the production of shrimp and its unregulated exploitation (heavy in many waters of the world), may have important consequences on the overall availability and conservation of marine resources, possibly leading to habitat degradation and biodiversity loss. The intensive use of land and freshwater resources (i.e., rice fields and mangroves) for shrimp farms, which seriously impact agricultural activity and the environment, should come under greater scrutiny.

The handling processes and storage conditions, methods and applications presented in this chapter clearly demonstrate that many tools and techniques are available to maintain the high quality of shrimp and extend its shelf-life, even in an increasingly globalized world in which it is now possible to consume in a large European city live prawns caught on the other side of the world.

This chapter has shown how shrimp is handled and stored, especially on board fishing vessels. In addition, some sections discuss the potential use of relatively new packaging techniques, such as protective atmospheres, to substitute the chemical additives widely used to combat melanosis.

Lastly, in a globalized food system where seafood products often travel faster than other goods, traceability is very important and must be improved so as to adequately inform consumers about the identity and quality of the product they are eating. The introduction of new techniques will improve the quality and image of these valuable aquatic products in the years to come.

References

Anagnostopoulos, D. A., Parlapani, F. F., & Boziaris, I. S. (2022). The evolution of knowledge on seafood spoilage microbiota from the 20th to the 21st century: Have we finished or just begun? *Trends in Food Science and Technology, 120*, 236–247. https://doi.org/10.1016/j.tifs.2022.01.004

Angel, S., Basker, D., Kanner, J., & Juven, B. J. (1981). Assessment of shelf-life of freshwater prawns stored at 0 C. *International Journal of Food Science & Technology, 16*, 357–366.

Banerjee, R., & Maheswarappa, N. B. (2019). Superchilling of muscle foods: Potential alternative for chilling and freezing. *Critical Reviews in Food Science and Nutrition, 59*(8), 1256–1263. https://doi.org/10.1080/10408398.2017.1401975

Basiri, S., Shekarforoush, S. S., Aminlari, M., & Akbari, S. (2015). The effect of pomegranate peel extract (PPE) on the polyphenol oxidase (PPO) and quality of Pacific white shrimp (*Litopenaeus vannamei*) during refrigerated storage. *LWT - Food Science and Technology, 60*(2), 1025–1033. https://doi.org/10.1016/j.lwt.2014.10.043

Blond, G., & Le Meste, M. (2004). Principles of frozen storage. In Y. H. Hui, P. Cornillon, I. G. Legaretta, M. H. Lim, K. D. Murrell, & W. K. Nip (Eds.), *Handbook of frozen foods* (pp. 25–54). New York: Marcel Dekker.

Bono, G., & Badalucco, C. (2012). Combining ozone and modified atmosphere packaging (MAP) to maximize shelf-life and quality of striped red mullet (*Mullus surmuletus*). *LWT, 47*(2), 500–504. https://doi.org/10.1016/j.lwt.2012.02.014

Bono, G., Badalucco, C., Corrao, A., Cusumano, S., Mammina, L., & Palmegiano, G. B. (2010). Effect of temporal variation, gender and size on cuticle polyphenol oxidase activity in deep-water rose shrimp (*Parapenaeus longirostris*). *Food Chemistry, 123*(2), 489–493. https://doi.org/10.1016/j.foodchem.2010.04.055

Bono, G., Gai, F., Peiretti, P. G., Badalucco, C., Brugiapaglia, A., Siragusa, G., & Palmegiano, G. B. (2012). Chemical and nutritional characterisation of the Central Mediterranean Giant red shrimp (*Aristaeomorpha foliacea*): Influence of trophic and geographical factors on flesh quality. *Food Chemistry, 130*(1), 104–110. https://doi.org/10.1016/j.foodchem.2011.07.004

Bono, G., Badalucco, C. V., Cusumano, S., & Palmegiano, G. B. (2012). Toward shrimp without chemical additives: A combined freezing-MAP approach. *LWT - Food Science and Technology, 46*(1), 274–279. https://doi.org/10.1016/j.lwt.2011.09.020

Bono, G., Okpala, C. O. R., Alberio, G. R. A., Messina, C. M., Santulli, A., Giacalone, G., & Spagna, G. (2016). Toward shrimp consumption without chemicals: Combined effects of freezing and modified atmosphere packaging (MAP) on some quality characteristics of Giant Red Shrimp (*Aristaeomorpha foliacea*) during storage. *Food Chemistry, 197*, 581–588. https://doi.org/10.1016/j.foodchem.2015.10.146

Bono, G., Okpala, C. O. R., Vitale, S., Ferrantelli, V., Noto, A. D., Costa, A., Di Bella, C., & Monaco, D. L. (2017). Effects of different ozonized slurry-ice treatments and superchilling storage (−1°C) on microbial spoilage of two important pelagic fish species. *Food Science and Nutrition, 5*(6), 1049–1056. https://doi.org/10.1002/fsn3.486

Botta, J. R. (1995). *Evaluation of seafood freshness quality.* VCH Publishers Inc.

Carbonell, A., Carbonell, M., Demestre, M., Grau, A., & Monserrat, S. (1999). The red shrimp *Aristeus antennatus* (Risso, 1816) fishery and biology in the Balearic Islands, western Mediterranean. *Fisheries Research, 44*(1), 1–13. https://doi.org/10.1016/s0165-7836(99)00079-x

Cartes, J. E., Maynou, F., Abelló, P., Emelianov, M., de Sola, L. G., & Solé, M. (2011). Long-term changes in the abundance and deepening of the deep-sea shrimp *Aristaeomorpha foliacea* in

the Balearic Basin: Relationships with hydrographic changes at the Levantine Intermediate Water. *Journal of Marine Systems, 88*(4), 516−525. https://doi.org/10.1016/j.jmarsys.2011.07.001

Chan, T. Y. (1998). Shrimps and prawns. The living marine resources of the western central Pacific. In *Cephalopods, Crustaceans, Holothurians, and Sharks. FAO fisheries identification guide for fishery purposes* (Vol. 2, pp. 851−971). FAO.

Chen, J., Huang, J., Deng, S., & Huang, Y. (2016). Combining ozone and slurry ice to maximize shelf-life and quality of bighead croaker (*Collichthys niveatus*). *Journal of Food Science and Technology, 53*(10), 3651−3660. https://doi.org/10.1007/s13197-016-2331-8

Duarte, A. M., Silva, F., Pinto, F. R., Barroso, S., & Gil, M. M. (2020). Quality assessment of chilled and frozen fish—mini review. *Foods, 9*(12), 1739. https://doi.org/10.3390/foods9121739

Edmunds, W. J., & Lillard, D. A. (1979). Sensory characteristics of oysters, clams, and cultured and wild shrimp. *Journal of Food Science, 44*(2), 368−373. https://doi.org/10.1111/j.1365-2621.1979.tb03791.x

Erickson, M. C., Bulgarelil, M. A., Resurreccion, A. V. A., Vendetti, R. A., & Gates, K. A. (2007). Consumer differentiation, acceptance, and demographic patterns to consumption of six varieties of shrimp. *Journal of Aquatic Food Product Technology, 15*(4), 35−51. https://doi.org/10.1300/J030v15n04_04

European Food and Safety Authority. (2015). Scientific and technical assistance on the evaluation of the temperature to be applied to pre-packed fishery products at retail level. *EFSA Journal, 13*(7). https://doi.org/10.2903/j.efsa.2015.4162

FAO. (2022). *The state of world fisheries and aquaculture 2022: Towards Blue transformation*.

Fiorentino, F., Garofalo, G., Bono, G., Vitale, S., Fiorentino, F., Garofalo, G., & Bono, G. (2024). VitLearning from the history of red shrimp fisheries in the Mediterranean to improve sustainability of deep-water bottom trawling. *ICES Journal of Marine Science*. https://doi.org/10.1093/icesjms/fsae031. fsae031.

Gökoğlu, N. (2021). *Shellfish processing and preservation*. Springer International Publishing. https://doi.org/10.1007/978-3-030-60303-8

Gonclalves, A. C., Lopez-Caballero, M. E., & Nunes, M. L. (2003). Quality changes of deepwater pink shrimp *(Parapenaeus longirostris*) packed in modified atmosphere. *Journal of Food Science, 68*(8), 2586−2590. https://doi.org/10.1111/j.1365-2621.2003.tb07065.x

Gringer, N., Skytte, J. L., Dang, T. T., Orlien, V., Olsen, K., Schlippè-Steffensen, K., & Jessen, F. (2020). Effect of ice maturation, freezing and heat treatment on the peelability and quality of cold water shrimps (*Pandalus borealis*). *LWT, 134*, 110139. https://doi.org/10.1016/j.lwt.2020.110139

Hassoun, A., Prieto, M. A., Carpena, M., Bouzembrak, Y., Marvin, H. J. P., Pallarés, N., Barba, F. J., Punia Bangar, S., Chaudhary, V., Ibrahim, S., & Bono, G. (2022). Exploring the role of green and Industry 4.0 technologies in achieving sustainable development goals in food sectors. *Food Research International, 162*, 112068. https://doi.org/10.1016/j.foodres.2022.112068

Hassoun, A., Shumilina, E., Di Donato, F., Foschi, M., Simal-Gandara, J., & Biancolillo, A. (2020). Emerging techniques for differentiation of fresh and frozen−thawed seafoods: Highlighting the potential of spectroscopic techniques. *Molecules, 25*(19), 4472. https://doi.org/10.3390/molecules25194472

Hassoun, A., Siddiqui, S. A., Smaoui, S., Ucak, İ., Arshad, R. N., Garcia-Oliveira, P., Prieto, M. A., Aït-Kaddour, A., Perestrelo, R., Câmara, J. S., & Bono, G. (2022). Seafood processing,

preservation, and analytical techniques in the age of industry 4.0. *Applied Sciences, 12*(3), 1703. https://doi.org/10.3390/app12031703

Heu, M.-S., Kim, J.-S., & Shahidi, F. (2003). Components and nutritional quality of shrimp processing by-products. *Food Chemistry, 82*(2), 235−242. https://doi.org/10.1016/s0308-8146(02)00519-8

Holthuis, L. B. (1980). *FAO species catalogue. Volume 1-shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries*.

Hu, L., Ying, Y., Zhang, H., Liu, J., Chen, X., Shen, N., Li, Y., & Hu, Y. (2021). Advantages of liquid nitrogen freezing in long-term frozen preservation of hairtail (*Trichiurus haumela*): Enzyme activity, protein structure, and tissue structure. *Journal of Food Process Engineering, 44*(9). https://doi.org/10.1111/jfpe.13789

Huang, Y.-R., Zelaya, M. F. G., & Shiau, C.-Y. (2016). Changes in biochemical compositions and quality of white shrimp (*Litopenaeus vannamei*) during storage. *Journal of Aquatic Food Product Technology, 25*(1), 35−45. https://doi.org/10.1080/10498850.2013.824944

Ji, W., Bao, Y., Wang, K., Yin, L., & Zhou, P. (2021). Protein changes in shrimp (*Metapenaeus ensis*) frozen stored at different temperatures and the relation to water-holding capacity. *International Journal of Food Science and Technology, 56*(8), 3924−3937. https://doi.org/10.1111/ijfs.15009

Jiang, S. T., & Lee, T. C. (2005). Freezing seafood and seafood products: Principles and applications. In *Handbook of food science, technology, and engineering - 4 volume set* (pp. 695−729). CRC Press. https://www.taylorfrancis.com/books/e/9781466507876.

Jiang, Q., Okazaki, E., Zheng, J., Que, T., Chen, S., & Hu, Y. (2018). Structure of northern snakehead (*Channa argus*) meat: Effects of freezing method and frozen storage. *International Journal of Food Properties, 21*(1), 1166−1179. https://doi.org/10.1080/10942912.2018.1437631

Jin, L., Ding, G., Li, P., Gu, J., & Zhang, X. (2018). Changes in quality attributes of marine-trawling shrimp (*Solenocera crassicornis*) during storage under different deep-frozen temperatures. *Journal of Food Science and Technology, 55*(8), 2890−2898. https://doi.org/10.1007/s13197-018-3207-x

Ju, Z. Y., Forster, I. P., & Dominy, W. G. (2009). Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vannamei*). *Aquaculture, 292*(3−4), 237−243. https://doi.org/10.1016/j.aquaculture.2009.04.040

Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Bekaert, K., Cropotova, J., García, M. R., … Bover-Cid, S. (2021). The use of the so-called 'superchilling' technique for the transport of fresh fishery products. *EFSA Journal, 19*(1). https://doi.org/10.2903/j.efsa.2021.6378

López-Caballero, M. E., Martínez-Alvarez, O., Gómez-Guillén, M. del C., & Montero, P. (2007). Quality of thawed deepwater pink shrimp (*Parapenaeus longirostris*) treated with melanosis-inhibiting formulations during chilled storage. *International Journal of Food Science and Technology, 42*(9), 1029−1038. https://doi.org/10.1111/j.1365-2621.2006.01328.x

López-Caballero, M., Gonçalves, A., & Nunes, M. (2002). Effect of CO_2/O_2-containing modified atmospheres on packed deepwater pink shrimp (*Parapenaeus longirostris*). *European Food Research and Technology, 214*(3), 192−197. https://doi.org/10.1007/s00217-001-0472-z

Li, T., Kuang, S., Xiao, T., Hu, L., Nie, P., Ramaswamy, H. S., & Yu, Y. (2022). The effect of pressure−shift freezing versus air freezing and liquid immersion on the quality of frozen fish during storage. *Foods, 11*(13), 1842. https://doi.org/10.3390/foods11131842

Li, Y., She, Q., Han, Z., Sun, N., Liu, X., & Li, X. (2018). Anaesthetic effects of eugenol on grass shrimp (*Palaemonetes sinensis*) of different sizes at different concentrations and temperatures. *Scientific Reports, 8*(1). https://doi.org/10.1038/s41598-018-28975-w

Liu, L., Lan, W., Pu, T., Zhou, Y., & Xie, J. (2021). Combining slightly acidic electrolyzed water and slurry ice to prolong the shelf-life of mackerel (*Pneumatophorus japonicus*). *Journal of Food Processing and Preservation, 45*(9). https://doi.org/10.1111/jfpp.15762

Lv, Y., & Xie, J. (2021). Effects of freeze−thaw cycles on water migration, microstructure and protein oxidation in cuttlefish. *Foods, 10*(11), 2576. https://doi.org/10.3390/foods10112576

Mendes, R., Gonçalves, A., Pestana, J., & Pestana, C. (2005). Indole production and deepwater pink shrimp (*Parapenaeus longirostris*) decomposition. *European Food Research and Technology, 221*(3−4), 320−328. https://doi.org/10.1007/s00217-005-1171-y

Mills, S. (2001). *Let them eat seafood*. Restaurants USA Magazine.

Monsalve, E. R., & Quiroga, E. (2022). Farmed shrimp aquaculture in coastal wetlands of Latin America — a review of environmental issues. *Marine Pollution Bulletin, 183*, 113956. https://doi.org/10.1016/j.marpolbul.2022.113956

Nirmal, N. P., & Benjakul, S. (2009). Melanosis and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) treated with catechin during iced storage. *Journal of Agricultural and Food Chemistry, 57*(9), 3578−3586. https://doi.org/10.1021/jf900051e

Nirmal, N. P., & Benjakul, S. (2011). Retardation of quality changes of Pacific white shrimp by green tea extract treatment and modified atmosphere packaging during refrigerated storage. *International Journal of Food Microbiology, 149*(3), 247−253. https://doi.org/10.1016/j.ijfoodmicro.2011.07.002

Odeyemi, O. A., Burke, C. M., Bolch, C. C. J., & Stanley, R. (2018). Seafood spoilage microbiota and associated volatile organic compounds at different storage temperatures and packaging conditions. *International Journal of Food Microbiology, 280*, 87−99. https://doi.org/10.1016/j.ijfoodmicro.2017.12.029

Okpala, C. O. R. (2014). Investigation of quality attributes of ice-stored Pacific white shrimp (*Litopenaeus vannamei*) as affected by sequential minimal ozone treatment. *LWT - Food Science and Technology, 57*(2), 538−547. https://doi.org/10.1016/j.lwt.2014.02.007

Okpala, C. O. R., Bono, G., Abdulkadir, A., & Madumelu, C. U. (2015). Ozone (O_3) process technology (OPT): An exploratory brief of minimal ozone discharge applied to shrimp product. In *Energy procedia* (pp. 2427−2435). Elsevier Ltd. https://doi.org/10.1016/j.egypro.2015.07.206

Okpala, C. O. R., Bono, G., Cannizzaro, L., & Jereb, P. (2016). Changes in lipid oxidation and related flesh qualities of white shrimp (*Litopenaeus vannamei*) during iced storage: Effects on the use of increasing ozone exposures. *European Journal of Lipid Science and Technology, 118*(12), 1942−1951. https://doi.org/10.1002/ejlt.201500347

Okpala, C. O. R., Bono, G., & Gancitano, V. (2015). Consumer propensity to purchase non-chemical treated crustacean product: A case study of Italy. *AACL Bioflux, 8*(6), 839−845. http://www.bioflux.com.ro/docs/2015.839-845.pdf.

Okpala, C. O. R., & Bono, G. (2016). Investigating the biometric and physicochemical characteristics of freshly harvested Pacific white shrimp (*Litopenaeus vannamei*): A comparative approach. *Journal of the Science of Food and Agriculture, 96*(4), 1231−1240. https://doi.org/10.1002/jsfa.7211

Okpala, C. O. R., Bono, G., Pipitone, V., Vitale, S., & Cannizzaro, L. (2016). Toward crustacean without chemicals: A descriptive analysis of consumer response using price comparisons. *Food & Nutrition Research, 60*. https://doi.org/10.3402/fnr.v60.30955

Ouraji, H., Shabanpour, B., Kenari, A. A., Shabani, A., Nezami, S., Sudagar, M., & Faghani, S. (2009). Total lipid, fatty acid composition and lipid oxidation of Indian white shrimp (*Fenneropenaeus indicus*) fed diets containing different lipid sources. *Journal of the Science of Food and Agriculture, 89*(6), 993−997. https://doi.org/10.1002/jsfa.3545

Pan, C., Chen, S., Hao, S., & Yang, X. (2019). Effect of low-temperature preservation on quality changes in Pacific white shrimp, *Litopenaeus vannamei*: A review. *Journal of the Science of Food and Agriculture, 99*(14), 6121−6128. https://doi.org/10.1002/jsfa.9905

Parlapani, F. F., Ferrocino, I., Michailidou, S., Argiriou, A., Haroutounian, S. A., Kokokiris, L., Rantsiou, K., & Boziaris, I. S. (2020). Microbiota and volatilome profile of fresh and chill-stored deepwater rose shrimp (*Parapenaeus longirostris*). *Food Research International, 132*, 109057. https://doi.org/10.1016/j.foodres.2020.109057

Rezaei, F., Hosseinzadeh, S., Basiri, S., Golmakani, M.-T., Gholamhosseini, A., & Shekarforoush, S. S. (2023). The effects of Shirazi thyme (*Zataria multiflora*) oil nanoemulsion on the quality of shrimp (*Litopenaeus vannamei*) during refrigerated storage. *Journal of Food Science and Technology, 60*(2), 710−719. https://doi.org/10.1007/s13197-022-05657-0

Rusanova, P., Bono, G., Dara, M., Falco, F., Gancitano, V., Lo Brutto, S., Okpala, C. O. R., Nirmal, N. P., Quattrocchi, F., Sardo, G., & Hassoun, A. (2022). Effect of different packaging methods on the free amino acid profiles of the deep-water rose shrimp (*Parapenaeus longirostris*) during frozen storage. *Frontiers in Nutrition, 9*. https://doi.org/10.3389/fnut.2022.955216

Sipahutar, Y. H., Suryanto, M., Ramli, H. K., Pratama, R. B., & Panjaitan, T. F. C. (2020). Organoleptic quality of whiteleg shrimp (*litopenaeus vannamei*) cultivated from intensive and traditional pond at Bulukumba District, South Sulawesi. *IOP Conference Series: Earth and Environmental Science, 564*(1), 012040. https://doi.org/10.1088/1755-1315/564/1/012040

Srinivasan, P., Robinson, J., Geevaretnam, J., & Rayappan, J. B. B. (2020). Development of electronic nose (Shrimp-Nose) for the determination of perishable quality and shelf-life of cultured Pacific white shrimp (*Litopenaeus Vannamei*). *Sensors and Actuators B: Chemical, 317*, 128192. https://doi.org/10.1016/j.snb.2020.128192

Stevens, B. G., & Kotwicki, S. (2021). The ups and downs of traps: Environmental impacts, entanglement, mitigation, and the future of trap fishing for crustaceans and fish. *ICES Journal of Marine Science, 78*(2), 584−596. https://doi.org/10.1093/icesjms/fsaa135

Tolstorebrov, I., Eikevik, T. M., & Bantle, M. (2016). Effect of low and ultra-low temperature applications during freezing and frozen storage on quality parameters for fish. *International Journal of Refrigeration, 63*, 37−47. https://doi.org/10.1016/j.ijrefrig.2015.11.003

Tsironi, T., Dermesonlouoglou, E., Giannakourou, M., & Taoukis, P. (2009). Shelf life modelling of frozen shrimp at variable temperature conditions. *LWT - Food Science and Technology, 42*(2), 664−671. https://doi.org/10.1016/j.lwt.2008.07.010

Wachirasiri, K., Wanlapa, S., Uttapap, D., Puttanlek, C., & Rungsardthong, V. (2019). Effects of multiple freeze−thaw cycles on biochemical and physical quality changes of white shrimp (*Penaeus vannamei*) treated with lysine and sodium bicarbonate. *Journal of Food Science, 84*(7), 1784−1790. https://doi.org/10.1111/1750-3841.14635

Wang, L., Liu, Z., Yang, H., Huang, L., & Zeng, M. (2016). Optimal modified atmosphere for packaging and its effects on quality and shelf-life of Pacific white shrimp (*Litopenaeus vannamei*) under controlled freezing-point storage at −0.8°C. *Food Science and Technology Research, 22*(2), 173−183. https://doi.org/10.3136/fstr.22.173

Zeng, Q. Z., Thorarinsdottir, K. A., & Olafsdottir, G. (2005). Quality changes of shrimp (*Pandalus borealis*) stored under different cooling conditions. *Journal of Food Science, 70*(7), s459−s466. https://doi.org/10.1111/j.1365-2621.2005.tb11493.x

Zhan, X., Sun, D.-W., Zhu, Z., & Wang, Q.-J. (2018). Improving the quality and safety of frozen muscle foods by emerging freezing technologies: A review. *Critical Reviews in Food Science and Nutrition, 58*(17), 2925−2938. https://doi.org/10.1080/10408398.2017.1345854

Zhang, B., Deng, S., & Wang, Q. (2015). Chemical changes related to loss of quality in Pacific white shrimp (*Litopenaeus vannamei*) during chilled storage under slurry ice conditions. *Journal of Food Processing and Preservation, 39*(6), 2507−2515. https://doi.org/10.1111/jfpp.12500

Zhang, R., Cheng, Z., Ding, F., Hua, L., Fang, Y., Han, Z., Shi, J., Zou, X., & Xiao, J. (2022). Improvements in chitosan-based slurry ice production and its application in precooling and storage of *Pampus argenteus*. *Food Chemistry, 393*, 133266. https://doi.org/10.1016/j.foodchem.2022.133266

Zhang, Y., Li, F., Yao, Y., He, J., Tang, J., & Jiao, Y. (2021). Effects of freeze-thaw cycles of Pacific white shrimp (*Litopenaeus vannamei*) subjected to radio frequency tempering on melanosis and quality. *Innovative Food Science and Emerging Technologies, 74*, 102860. https://doi.org/10.1016/j.ifset.2021.102860

Zhao, Y., Lan, W., Shen, J., Xu, Z., & Xie, J. (2022). Combining ozone and slurry ice treatment to prolong the shelf-life and quality of large yellow croaker (*Pseudosciaena crocea*). *LWT, 154*, 112615. https://doi.org/10.1016/j.lwt.2021.112615

Main funding and final remarks

"The first law of ecology is that everything is connected to everything else."
Aldo Leopold

In marine science, and particularly in fisheries research, the term 'sustainability' is often associated with the protection and conservation of aquatic resources and, in particular, with safeguarding their biodiversity. The easiest and perhaps simplest way to ensure this is to reduce fishing in areas of particular ecological importance (e.g. nursery areas of target species) and to bring the indices of resource exploitation within the MSY range.

However, sustainability approaches should protect not only the marine environment but also the fishermen, i.e. those who, over the millennia, have used these resources initially for subsistence and later for enterprise/development. An overall vision is required, one that considers the human component of "sustainability". In this case, it is important to ensure the survival of natural resources as well as of the communities that feed and live off them.

In this context, my thesis addresses two main issues that all stakeholders in the fisheries sector must consider in order to preserve this ecosystem: emerging pollutants (e.g. microplastics, which are a problem for aquatic resources and for those who consume them) and, with regard to the balance between resource exploitation and renewal, valorizing the catch in response to the call to fish less.

Emerging Contaminants in fishery resources

This chapter addresses the issue of pervasive marine pollution, focusing on microplastics and other contaminants. Research has shown that contamination of marine species represents a substantial health hazard to marine life and may also pose a risk to humans who consume contaminated seafood. Findings from this study help understand the risks posed by different kinds of pollutants. This is particularly important, as awareness of the potential risks and prevalence of pollutants in commonly consumed commercial species enables informed decision-making, minimizing or avoiding the risks associated with the ingestion of contaminated seafood or fish.

According to our studies, particularly those on *Raja clavata*, the probability of finding significant quantities of microplastics in the muscle of the aforementioned elasmobranchs is increasing. This finding, which will soon be further explored (also with the help of other international experts in the field), raises concern about the transport of such pollutants from the intestine and/or gills to the edible tissues of fish: given the global increase in the consumption of fish products, this is not only an environmental problem (*sensu lato*) but also a human health issue.

Postharvest Technologies and Enhancing the Quality of Seafood

This section focuses on two other important aspects concerning the seafood sector, namely (1) the quality and nutritional value of the species of greatest interest to fishermen and consumers, one of which is the Mediterranean deep water rose shrimp, and (2) the origin of the Norway lobsters found in fish retailers, restaurants and supermarkets. With regard to the first point - the quality and safety of pink shrimp - our work «The effects of storing shrimp in a protective atmosphere (i.e. without the known chemical additives such as sulphites) on the free amino acid (FAA) composition of the deep water rose shrimp (*Parapenaeus longirostris*)» not only demonstrated the high nutritional value of these crustaceans in terms of free amino acids (especially the essential ones, but also those such as argine, glycine and proline that typically make these products sweet) but also revealed that it is possible to reduce the use of chemical additives such as sulphites while maintaining high quality standards. As for the Norway lobster, by comparing specimens caught in the Mediterranean with those from Northern European waters (often fraudulently marketed as Mediterranean), we showed that, where classical genetics does not work well (both populations resulting homogeneous), it is possible to identify the origin (fishing area) of this highly prized crustacean by analysing its gut microbiome. The differences found in the gut microbiota can also be used for ecological studies.

In addition, this section examines how postharvest technologies can enhance seafood quality, diminish spoilage, and possibly provide ways to optimize catch value and revenue. This research highlights the impact of different storage methods, particularly freezing techniques, on quality in high-value commercial species such as shrimp. Findings indicate the importance of rapid freezing and optimal cold chain management to limit waste and safeguard the structural integrity of seafood. These technologically enhanced methodologies not only guarantee the security and quality of seafood products but also promote the advancement of sustainable practices within the fishing industry.

As part of my doctoral research programme, I also completed an internship with the United Nations Industrial Development Organization (UNIDO) within the framework of the project Cambodia Programme for Sustainable and Inclusive Growth in the Fisheries Sector: Capture Component (CaPFish Capture). The internship focused on the comprehensive exploration of innovative strategies to maximize the quality and economic potential of fish products in Cambodia. The internship programme placed particular emphasis on the valorisation of low-value fish species, the development of generic commercial fish products, and the exploration of alternative products, including bioactive products and zoo animal feed. The internship also entailed the evaluation of quality parameters for fermented food products characteristic of the region and analysis of their compliance with EU standards and norms. This work is a practical demonstration of the relevance and applicability of PhD research.

In conclusion, adopting the slogan "less fishing and better fish quality", the aim of my thesis is to draw attention to a more holistic, sustainable use of fisheries and aquaculture resources. New ideas and innovative strategies for better preserving seafood products will make it possible to fish less (thereby tackling overfishing) and compensate for the decline in resources (as well as in fishermen's profits). By improving the quality of fish and guaranteeing its origin and safety, it will be easier to guarantee environmental, economic and social sustainability.