UNIVERSITY OF NAPLES FEDERICO II

DEPARTMENT OF VETERINARY MEDICINE AND ANIMAL PRODUCTIONS

PhD programme in Veterinary Sciences

Study on the toxicity induced by polystyrene microplastic in gilthead seabream: focus on intestinal and hepatic homeostasis

PhD student
Filomena Del Piano

Coordinator
Prof.
Paolo de Girolamo

Tutor
Prof.
Maria Carmela Ferrante

XXXVI CYCLE (2020-2023)

SUMMARY

Abstract

Plastic pollution in the natural environment is a threat of growing concern that has drawn the attention of many scientists worldwide. Once discharged into the ecosystems, plastic debris fragment into smaller particles named microplastics (MPs). In aquatic environments, MPs bind to water and sediment phases and undergo bioaccumulation and biomagnification processes along the trophic chains, with health consequences not yet well understood. Polystyrene (PS) is one of the most abundant polymer types contaminating aquatic environments and is frequently encountered in biotic matrices. Ingestion is the main exposure route to MPs in aquatic species, and ingested MPs accumulate in the gastrointestinal tract, causing adverse local effects on the gut. However, MPs may also translocate from the gut to the circulatory system, reaching several tissues and organs where they can cause detrimental effects.

The gut-liver axis is a bidirectional crosstalk between the gut and the liver that may be influenced by several factors, including environmental ones. Disorders of the intestinal barrier may result in an increased portal influx of bacteria or their products, as well as toxic substances to the liver, where they cause or worsen several hepatic diseases. One of these is the non-alcoholic fatty liver disease (NAFLD), which is characterized by an excessive accumulation of lipids in hepatocytes, inflammation, and oxidative stress (OS).

The aim of this study was to assess the effects of MP ingestion on gut-liver axis balance in gilthead seabream (*Sparus aurata* Linnaeus, 1758) fed with a diet enriched with PS-MPs (1–20 μm; 0, 25, or 250

mg /kg b.w./day) for 21 days. Experimental analyses were carried out using histological, molecular, and biochemical methodologies.

PS-MPs differently affected homeostasis of the anterior (AI) and posterior (PI) intestine. Inflammation and immune alterations were revealed in both tracts, but PI showed a greater susceptibility to MP-induced modifications. Specifically, PS-MPs triggered the TLRs-Myd88 signaling pathway with the following augmented release of pro-inflammatory cytokines. Also, PS-MPs raised the expression of other immune-associated genes, such as *Lys*, *CSF1R*, and *ALP*. PS-MP exposure also increased OS and nitrosative stress and impaired the antioxidant defense system, in PI more than in AI. Moreover, the MAPKs (i.e., p38 and ERK) were activated by PS-MPs, and the intestinal barrier integrity was disrupted, as evidenced by the reduced gene expression of tight junctions (i.e., *ZO-1*, *Cldn15*, *Occludin*, and *Tricellulin*), integrins (i.e., *Itgb6*) and mucins (i.e., *Muc2-like* and *Muc13-like*).

On the other hand, an increased synthesis and accumulation of lipids were revealed in the liver of exposed fish. Specifically, PS-MPs induced the up-regulation of genes related to lipid synthesis (i.e., *PPARy*, *Srebp1*, *Fasn*) without modifications of those involved in lipid catabolism or transport (i.e., *PPARα*, *HL*, *Pla2*, *Fabp1*). Moreover, a dose-dependent increase of immune and pro-inflammatory cytokines gene expression was also observed in exposed fish. These findings were confirmed by hepatic histological evaluations reporting evidence of lipid accumulation, inflammation, and necrosis. Additionally, PS-MPs affected the functionality of hepatic antioxidant and detoxifying systems, resulting in OS, as shown by the augmented production of

ROS and MDA. Specifically, the alteration of enzymatic (catalase, superoxide dismutase, and glutathione reductase) and non-enzymatic (glutathione) components of the antioxidant defense system was highlighted in the liver of exposed fish. Similarly, the activity of detoxifying enzymes (cytochrome P4501A and glutathione-S-transferase) was impaired by PS-MP exposure.

Based on the results, PS-MPs affect intestinal and hepatic homeostasis, altering the gut-liver axis balance in gilthead seabreams subchronically exposed via diet. PS-MPs directly damage the intestinal barrier integrity and function, with indirect consequences on liver health. Indeed, the impairment of hepatic lipid metabolism and the following steatosis, the increase of inflammatory response, and the OS induced by PS-MPs cooperate in liver dysfunction. All these alterations may synergistically promote and influence each other in the onset, development, and progression of NAFLD.

Introduction

During the last twenty years, plastic pollution has aroused global concern, and its effects on the environment and living organisms are noteworthy. Due to the advantageous industrial properties, global plastic production and use have exponentially increased over time. The main consequence is the continuous discharge of plastic waste in every ecological niche all over the world (Alimba and Faggio, 2019). Global plastic production has increased from 1.5 million tons in the 1950s to 335 million tons in 2016 and the planet is expected to hold about 33 billion tons of plastic by 2050 (Rochman et al., 2013). Aquatic ecosystems are the most polluted by plastics, whose continuous release into the marine environment is now considered an urgent global issue that requires managerial strategies to mitigate or avoid potential health risks to living organisms (Nawab et al., 2023).

Plastic debris are classified mainly according to their size, shape, and chemical composition (EFSA, 2016). Microplastics (MPs), which are small-size debris, are widely recognized as emerging and ubiquitous pollutants released into the environment by primary or secondary processes. Primary MPs are persistent, chemically inert, raw particles deriving from anthropic activities and directly released into the environment through wastewater, sewage systems, and industrial discharge (Deng et al., 2020). Secondary MPs originate from the fragmentation of bigger plastic debris over time due to biodegradation processes, abrasion by the wind, or UV rays (Andrady, 2017). MPs have been reported to be found across different environmental compartments, food, water, air, as well as human stools (Wang et al., 2021). Due to their low degradability, easy transportability, capability

to accumulate and persist, as well as their small size, MPs can undergo bioaccumulation and, in particular, biomagnification processes within aquatic and terrestrial food webs (Lehel and Murphy, 2021). Therefore, MPs raise concern because of their potential detrimental effects on animal and human health. Indeed, continuous exposure to MPs leads to toxic effects that may be triggered by different pathways related to oxidative stress (OS), inflammation, cytotoxicity, neurotoxicity, and immunotoxicity (Jeong and Choi, 2019; Prata et al., 2020). These mechanisms are strictly intertwined, and one pathway's induction can trigger or exacerbate the others. The onset and size of MP-induced effects are influenced by several factors related to: (I) particle properties, i.e., polymer type, plastic shape and size, superficial functionalization, adsorbed pollutants, and aging processes; (II) exposure conditions, i.e., concentration/dose, and time of exposure; (III) features of the examined organism, i.e., species, developmental stage, and sex (Kogel et al., 2020).

In the last few years, the impact of MPs on aquatic biota has gained attention because aquatic ecosystems (i.e., oceans, seas, rivers, and lakes) are considered primary sinks of these pollutants (Priya et al., 2023). Ingestion, together with retention in gills, are the main exposure routes to MPs in aquatic species that can ingest MPs directly, through both incidental or intentional ways, or indirectly, through the consumption of contaminated prey (Franzellitti et al., 2019). Once ingested, MPs can accumulate in the gastrointestinal (GI) tract (Pereira et al., 2020; de Mesquita et al., 2021), impairing essential physiological functions. To date, GI damage related to MP ingestion has been described in several aquatic species, highlighting histopathological,

molecular, and biochemical alterations in the gut characterized by OS, lipid peroxidation, and inflammation (Hirt and Body-Malapel, 2020). MP size has great relevance since particles smaller than 150 μm can translocate across the gut epithelium, causing systemic effects in addition to local disorders (EFSA, 2016). Indeed, ingested MPs can reach several organs through the circulatory system, and many studies have shown the presence of MPs in the liver, gills, brain, spleen, kidney, and gonads (Ma et al., 2021). MPs induce a wide variety of detrimental effects in both aquatic small invertebrates and large vertebrates, altering physiological functions and causing organ-specific and neurobehavioral toxicity, as well as metabolic, endocrine, and reproductive alterations (Pirsaheb et al., 2020; Ferrante et al., 2022). The harmful effects observed in aquatic species are often similar to those observed in terrestrial organisms. Among the other organs, the liver gains attention because it is strictly connected to the gut by the portal system, and as a central metabolic and detoxifying organ, it is exposed to noteworthy concentrations of xenobiotics. Thus, ingested MPs can first reach the gut and accumulate, then translocate to the liver, with blood circulation, altering its morphology and physiological function (Yin et al., 2022).

Ingested MPs can also translocate to edible tissues, having been detected in the muscle meat of several commercially relevant fish species, crustaceans, and mollusks (Kibria, 2023). This evidence suggests a potential toxicological risk for consumer health. Indeed, consuming a diet consisting of contaminated foods, such as fishery products, can be a way of transferring MPs to humans.

By analysing the scientific literature on the subject, many issues arise related to the adopted experimental conditions and the achieved results. There is a mismatch between the type, size, and concentration of MPs used in experimental conditions and those detected in environmental abiotic and biotic matrices. The studies are mainly carried out on marine species. Most focused on spherical and small MP particles manufactured by chemical companies, adopting short-term exposure conditions to evaluate adverse effects on living organisms. All these conditions are quite different from those most frequently occurring in the environment. From an analytical perspective, other issues emerge. The most relevant are the lack of adequate standard sampling protocols, the need for standardized analytical methods for MP detection as well as the need for uniformity in reporting units for monitoring and toxicity studies. These latter are crucial to perform a useful toxicological risk evaluation for animal and human health.

Thus, these gaps should be filled. On one side, it is necessary to develop strategies to mitigate or prevent MP pollution. On the other side, it is essential to expand the knowledge about MP toxicity by evaluating their effects at tissue/organ, molecular, and cellular levels in target species. Exposure to MPs could result in altered health status and, thus, wellness conditions for fish. The required data will, therefore, constitute helpful elements in the complex risk assessment process for the species and for humans exposed to MPs directly or indirectly through consuming contaminated food, including fishery products. The results could provide scientific support to the request to include MPs in monitoring plans and the definition of appropriate tolerance levels. MP toxicity should also be investigated using wild

biomonitoring species to estimate the occurrence and the effects induced under wild conditions. At the same time, the impact of MPs on living organisms should be studied on animal models in experimentally controlled conditions to obtain clear information to identify the potential risk from a one-health perspective.

GENERAL SECTION

1. Microplastics (MPs): emerging environmental pollutants

1.1 Definition, generation, and distribution of MPs

The rise of using plastic materials in everyday life has led to the emergence of a contaminant that poses a serious concern to the total environment. The discovery of Bakelite, the first synthetic plastic, in 1907 revolutionized polymer science and modern living by introducing several plastic formulations and polymers to our daily lives, many of which still exist in the market (Shashoua, 2008). However, despite the advantageous industrial properties, i.e., inexpensive, lightweight, tensile strength, durability, corrosion-resistance, and thermal and electrical insulation (Thompson et al., 2009), plastics rarely biodegrade when released into the environment.

The greatest amount of plastic is produced in China, followed by USA, Germany, and Brazil. Analogously, the highest quantity of mismanaged plastic waste originated from Asian countries (Lehel and Murphy, 2021). The abundance and spatial distribution of plastic pollution are increasing and affect terrestrial and aquatic ecosystems and air (Petersen and Hubbart, 2021). The most commercially used plastics include polymer types, namely polystyrene (PS), polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), and polyvinyl chloride (PVC). Among these, PE, PP, and PS are the most abundant polymer plastics detected in biotic matrices (EFSA, 2016). PS was the first plastic designed from ethylene and benzene by Eduard Simon in 1839, and given its usefulness and versatility, it led to the invention of the first man-made plastic in 1862 and to the mass

production of plastic products starting in the 1940s (Alimba and Faggio, 2019).

Plastics are classified according to their size into macroplastics, mesoplastics, microplastics (MPs), and nanoplastics (NPs). Macroplastics (>25 mm) and mesoplastics (5–25 mm) are typically responsible for plastic litter visible to the naked eye, and they can cause problems such as entanglements and ingestion in larger animals (Lehel and Murphy, 2021). Instead, MPs and NPs are smaller debris not visible to the naked eye. Specifically, MPs are particles with a size range from 100 nm to 5 mm, while NPs are from approximately 1 to 100 nm (EFSA, 2016). Depending on the shape, plastic debris can also be classified into pellets, fibers, fragments, spheroids, and granules (EFSA, 2016).

MPs are released into the environment by primary or secondary processes, and both primary and secondary MPs tend to fragment in NPs. Primary MPs are small, persistent, chemically inert, raw particles deriving from anthropic activities and already manufactured in the size range of MPs, for example, pellets, nurdles, or fibers. They are employed in several applications and may be present in many consumer products, such as personal care products, drug vectors, and synthetic fabrics. They are released directly into the environment through wastewater, sewage systems, and industrial discharge (Deng et al., 2020). Secondary MPs originate from the fragmentation of macro- and mesoplastics over time by weathering biodegradation or abrasive forces and from cleaning synthetic clothing (Andrady, 2017; De Falco et al., 2018).

To summarize the above, an all-inclusive definition of MPs would be the following proposed by Frias and Nash (2019): “MPs are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water”. This definition includes the most critical descriptive characteristics of MPs, focusing on size and origin, but also considering physical and chemical properties. Other features, such as color, are not considered essential in defining MPs because they relatively contribute to their visual identification (Lusher et al., 2017). However, MP color is deemed crucial for studies regarding aquatic species, as some organisms ingest MPs based on their color preference (Wright et al., 2013).

Recently, in addition to the previously mentioned and well-documented sources of MP pollution, other emerging sources are drawing attention, and efforts are being made to reduce their environmental impact. MP release may also derive from plastic recycling facilities where plastic waste undergoes a process of shredding to reduce their size. Then, plastics are washed to remove any contaminants, and during this process, plastic debris may be left in the wastewater, thus polluting the environment (Guo et al., 2022b; Suzuki et al., 2022). Moreover, recent studies showed that using plastic materials in the construction of tea bags led to MP release when immersed in liquid (Hernandez et al., 2019; Afrin et al., 2022). Finally, the COVID-19 outbreak has strongly contributed to increased plastic waste production because of disposable face masks made in PP, becoming a significant source of MP pollution in our environment (Dey et al., 2023; Mohana et al., 2023).

An overview of MP sources is summarized in Fig. 1.1.

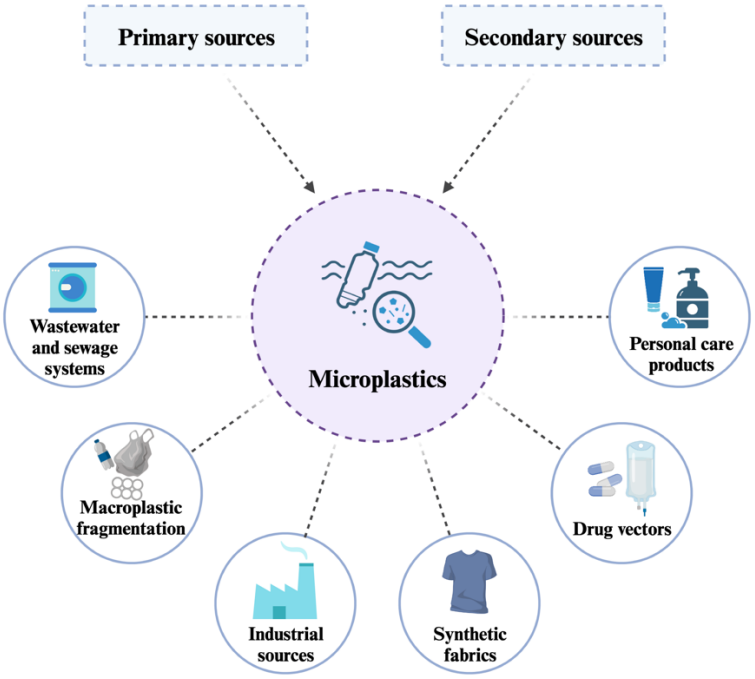

Fig. 1.1 – Primary and secondary sources of MPs.

MPs can be found in various environmental compartments, including soil, wetlands, groundwater, rivers, lakes, and oceans (Priya et al., 2023). The distribution and abundance of MPs across the planet depend on environmental factors, among which wind, tides, gyres, river runoff, tributary inputs, and anthropogenic factors (i.e., water treatment plants that discharge sewage containing MPs). Seven continents and four oceans show the presence of MPs in seawater, surface water, beaches, and sediments. The highest MP concentration was detected in sediments, followed by freshwater and seawater. High MP concentration was frequently linked to urbanization and high population density. This evidence in surface waters has been related to

water treatment plants releasing sewage containing more MPs in areas with high urbanization and dense populations (Frias et al., 2014). However, some studies have reported that MPs may also occur in remote areas with low population density and limited human activities. For instance, MPs have been found in the sediments from remote lakes in Tibet (Zhang et al., 2016). Moreover, MPs have been found in surface waters of the Ross Sea (Antarctica), sediments of Terra Nova Bay (Antarctica), deep-sea sediments of the Arctic, and in benthic organisms from the Arctic (Yu et al., 2020a), confirming that MP pollution also occurs in polar areas.

1.2 Sources and fate of MPs in aquatic ecosystem

The aquatic ecosystem is the most polluted with plastics, and the continuous discharge of these pollutants in the marine environment is now considered an urgent global issue. The presence of plastic debris in the marine environment was reported for the first time in the 1970s (Carpenter and Smith, 1972), and nowadays, oceans, seas, rivers, and lakes have been observed to be the primary sinks of MPs (Priya et al., 2023). It has been estimated that about 5 trillion plastic pieces are floating in the oceans, from the Arctic to the Antarctic (Matsuguma et al., 2017), and since plastics are non-biodegradable, they can persist for centuries. Plastic release into marine ecosystems occurs through various pathways, including river and atmospheric transport, beach littering, and directly at sea via aquaculture, shipping, and fishing activities (GESAMP, 2016). The major sources of aquatic MP pollution are considered the secondary ones. Indeed, plastic debris in

the ocean are broken down into MPs due to the actions of wind, waves, microbial degradation, and UV radiation (Alimi et al., 2018). Furthermore, considerable plastic inputs are primary MPs commonly released into domestic and industrial wastewater, which are released into sewage systems, enter rivers and estuaries, and finally flow into the sea (Xu et al., 2020). A global model based on waste management, population density, and hydrological information estimates that between 1.15 and 2.41 million tonnes of plastic waste currently enters the ocean every year from rivers, with over 74% of emissions occurring between May and October, mainly caused by the Asian continent (Lebreton et al., 2017). Consequently, the Northwestern Pacific Ocean is widely polluted by MPs, which reach concentrations ranging from 640 to 42000 items/km^2 depending on ocean currents (Pan et al., 2019). Beyond the Pacific Ocean, the Mediterranean Sea is highly affected by MP pollution due to the large amounts of plastic debris from the surrounding land masses (Cozar et al., 2015; Santini et al., 2022). Strikingly, MPs have even been found in polar regions, such as the Northeast Greenland and in the vicinity of the Antarctic Peninsula (Morgana et al., 2018; Lacerda et al., 2019). Marine MP levels are also related to the closeness of urbanized areas, and waters adjacent to highly urbanized areas are more polluted than waters adjacent to rural areas. In fact, Song et al. (2018) showed that the mean MP abundance in the seawater of South Korea urban coastal areas was much higher than in rural ones. MP abundance and distribution in rivers and lakes are influenced by several factors, such as human population density, closeness to urban centers, as well as hydrological and meteorological conditions (Xu et al., 2020). Firstly, MP distribution shows a positive

correlation with proximity to city, population density, and gross domestic product (Wang et al., 2017; Fan et al., 2019). Moreover, it has been shown that MP distribution may be influenced by wind and water circulation (Fischer et al., 2016) and that MP concentrations increase significantly after rain, mainly in urbanized areas (Faure et al., 2015).

Due to the difficulty of MP degradation, a large quantity of these particles accumulate in sediments, which are the last destination and one of the most important reservoirs of MPs in aquatic environments (Yin, 2023). Generally, high-density MPs easily sink into sediments, while low-density MPs float on the surface or remain suspended in the water column (Li et al., 2020b). However, floating and suspended MPs also fall into the sediment after the aggregation or interaction with other matrices (Leiser et al., 2021). PE, PP, and PS represent more than 75% of all polymer types identified in the sediments, highlighting their extensive use (Klein et al., 2015). In marine sediments, MP abundance can be affected by water flow rate, sediment depth, and distance from the shoreline (Xu et al., 2020). Likewise, MP abundance in freshwater sediments is characterized by spatial variations and can be influenced by physicochemical sediment characteristics, such as the available phosphate content and the specific conductivity (Sarkar et al., 2019). The ubiquitous and abundant presence of MPs in all aquatic ecosystems leads to marine and freshwater biota exposure to these pollutants, which may cause various biological effects. MPs can also change the plankton communities at the ocean surface, and this could exacerbate the deoxygenation driven by climate change, starving marine organisms of oxygen (Kvale et al., 2021). Moreover, MPs in

aquatic sediments alter microbial communities and disrupt nitrogen cycling (Seeley et al., 2020).

Given the current situation, some issues need to be considered to manage better the MP pollution problem in aquatic systems, and measures, including source control, remediation, and cleanup, should be taken as soon as possible.

1.3 Sources and fate of MPs in other ecosystems

MPs have been universally detected in all ecosystems in recent decades. However, while the aquatic environments have raised global attention and have been extensively investigated, terrestrial and atmospheric systems have been largely overlooked (Chen et al., 2020; Li et al., 2020b). Since the extensive usage and poor handling of plastic materials on land, the terrestrial environment is another significant sink for MPs, and has been estimated to have a plastic waste burden of 4 to 23 times higher than that of marine systems (Horton et al., 2017).

MPs can reach the soil directly through landfills, agricultural mulching films, compost, sewage irrigation, flooding, and car tire debris, or indirectly through runoff and diffuse atmospheric deposition (Bläsing and Amelung, 2018). The agroecosystem is the most plastic-polluted terrestrial system due to both direct and indirect plastic inputs, and it has been estimated that more than 700,000 tonnes of MPs enter soil annually in European and North American agricultural lands (Nizzetto et al., 2016). Once into the surface soil, MPs undergo transport processes reaching the deep soil. MPs can cross short distances through bioturbation and agricultural practices, and long

distances through surface runoff and soil erosion. Specifically, they undergo both horizontal and vertical migration that can be potentially influenced by several factors, among these soil biota activities, soil properties, plowing, harvesting, ingestion, and egestion of soil fauna (e.g., earthworms) (Ren et al., 2021).

Due to their poor biodegradability, MPs could reside in the soil for long periods, and this is always accompanied by a series of environmental impacts. Firstly, MPs alter physicochemical parameters of soil, such as bulk density, aggregation, porosity, electrical conductivity, water holding capacity, and pH value, and they can affect the soil nutrient cycle by changing organic matter and available nutrients (Khalid et al., 2020). MPs also pose a potential threat to the survival, growth, and reproduction of soil microbial communities that, in turn, threaten the biodiversity, function, and services of terrestrial ecosystems (Guo et al., 2020). However, their accumulation and fate may, in turn, be influenced by soil physio-chemistry and biota (He et al., 2018). Besides direct effects on soil, MPs can also cause alteration in soil organisms, i.e., earthworms and collembolans (Chang et al., 2022a). In addition, they have negative impacts on seed germination and plant growth (Huang et al., 2022). However, the influence of MPs on soil and soil organisms is highly influenced by plastic type, size, and properties, and the combined effects with other pollutants may exacerbate the effect. Indeed, MPs are subject to aging processes that change their properties such as color, chemical composition, and sorption capability, and aged MPs tend to adsorb other pollutants and toxicants (i.e., heavy metals and organic contaminants) that can, in turn, affect soil balance (Ren et al., 2021).

MPs were observed for the first time in the atmosphere by Dris et al. (2015), who showed their presence in the atmospheric fallout of urban areas in Greater Paris, with an average of 118 particles/m^2/day. In recent years, some studies have shown that MPs are ubiquitous in the atmosphere. PP, PE, PS, and PET are the most abundant polymer types, and fibers are the dominant shape of MPs in airborne (Chen et al., 2020). MPs have been found in both indoor and outdoor environments, but the indoor concentrations are higher than outdoor ones. This may be due to the high and prolonged production of MPs in indoors and the lower rates of air renovation than outdoors (Dris et al., 2017). The main contributors to airborne MPs are synthetic textiles, but other possible sources are the degradation of large plastics, waste in landfills or incineration, industrial emissions, particles released from traffic, dust re-suspension, and wind transport (Chen et al., 2020).

Atmospheric MP abundance may be influenced by several factors, and among these, meteorological conditions play a significant role. Indeed, rainfall, snowfall and, wind occurrences and intensities, rather than duration, showed a positive correlation with MP deposition (Allen et al., 2019). Moreover, significant differences in MP concentrations were observed between urban and sub-urban areas, and this may be due to the higher population density and more frequent anthropogenic activities (Chen et al., 2020). However, even if the urban areas are the most polluted, MPs have also been found in remote mountain and polar regions. For instance, Allen et al. (2019) detected MPs in atmospheric fallout from the Pyrenees mountains, inhabited areas with limited development. This is due to the wind that can transport MPs through the atmosphere over a long distance. Finally, altitude and geographical

environment may also influence MP abundance, i.e., at increasing altitudes, the concentrations of airborne MPs decrease (Liu et al., 2019a).

MPs in the air can be directly inhaled by humans or animals and accumulate in the lungs, potentially inducing acute and chronic inflammation. Moreover, airborne MPs are also a source of contamination in both terrestrial and aquatic environments (Chen et al., 2020). Therefore, the continuous exchange of MPs among air, terrestrial, and aquatic ecosystems, represents a dynamic cycle of MPs in the environment.

1.4 Levels of MPs in aquatic biota

MP occurrence in aquatic ecosystems is widely recognized as a significant environmental issue, and it is crucial to understand their levels in aquatic biota for ecological risk assessment. Many studies on MP contamination have recently been published, highlighting their hazard to biota in wild conditions. A high abundance of MPs was found in aquatic ecosystems by numerous studies conducted all around the world, and the highest quantity was observed in Asian countries, followed by European countries, South America, and Oceania (Nawab et al., 2023). In addition, several studies have shown that MPs are ingested by many aquatic species in a wide range of aquatic ecosystems (Lusher, 2015; Foley et al., 2018).

From the analysis of studies reporting MP abundance in biotic and abiotic samples, a great variation emerged depending on the considered sample. Specifically, MP quantity ranging from 0 to 153

items/gastrointestinal (GI) tract in fish, 0.07 ± 0.19 to 12.83 ± 1.47 items/g in mollusks, 0.00009 ± 0.00002 to 2.66 × 10^3 particles/L in water, and 0 to 30,890 ± 11,560 particles/kg in sediments (Ma et al., 2023). Moreover, a positive correlation was revealed between the abundance of MPs in marine mussels and sediments, suggesting mussels as a possible bioindicator of MP pollution in sediment. However, only few data on MP occurrence in freshwater mussels were reported, reflecting the lack of studies on freshwater species (Ma et al., 2023).

The presence of MPs was revealed in several tissues of aquatic invertebrates and vertebrates, and the GI tract is the most investigated tissue. The presence of MPs was shown in the GI tract of sediment-feeders holothurians (mean: 12.67 ± 7.31 MPs/individual) and fish (mean: 3 ± 4.44 MPs/individual), and in the whole soft tissue of bivalves (mean: 4.83 ± 5.35 MPs/individual), from a coastal pristine area in Western Mediterranean Sea (Rios-Fuster et al., 2022). However, MP presence was observed not only in the GI tract of aquatic biota but also in other tissues, including the edible ones. A study conducted on fish from the Northeast Atlantic Ocean showed the presence of MPs in GI tracts (48%), gills (30%), and muscle (22%), with the most prominent percentage of fragments (45%), followed by fibers (54%), and pellets (1%) (Barboza et al., 2020). Recently, Mistri et al. (2022) showed high abundance of PE (57%), PP (32.9%), PET (6.3%), and PS (1.3%) in muscle and GI tracts of fish from the Northern Adriatic Sea, with a mean concentration of particles ranging between 4.11 ± 2.85 items/individual in Adriatic soles, and 1.75 ± 0.71 items/individual in pilchards. Ugwu et al. (2021) reviewed 132 articles

reporting MP presence in different groups of marine vertebrates, i.e., turtles, mammals, birds, and fish. The authors report that most of the analyzed articles have been made in the Atlantic, Mediterranean, and Pacific areas and studied the complete GI system of considered species. From the analysis of these articles, it was found that turtles were the species most affected by MP pollution, as it is the group with the highest percentage of individuals affected by MPs (88% turtles, 59.5% marine mammals, 50.4% sea birds, 42% fish), and with the highest mean number of MP particles found in individuals (121.7 items in turtles, 2.6 items in fishes, 9.7 items in marine mammals, 7.0 items in sea birds). Moreover, they highlighted that the predominant type of MPs found in all groups were fibers (71.1% in fish, 72.7% in marine mammals, 45.5% in seabirds, and 50.0% in turtles) and this is in accordance with data reporting fibers as the most abundant MP shape in the marine environment. Nonetheless, the predominant MP size was <2 mm (73.6%), while the predominant color was blue (32.9%), due to the ease of consumption and the resemblance to common prey, respectively.

The presence of MP particles was also evidenced in tissues of freshwater biota (Bhatt and Chauhan, 2023). MP occurrence was revealed in freshwater invertebrates even if these species are less investigated than marine ones. For instance, studies on annelids were restricted to only one study that showed the presence of MPs in *Tubifex tubifex* with a mean value of 129 ± 65.4 MPs/g weight (Hurley et al., 2017). MPs were also found in freshwater mollusks, and the highest numbers of MPs were found in *Anadara granosa*, which was 180.6 ± 21.22 MPs per individual (Fitri and Patria, 2019), while 72.5 MPs per

individual were found in *Gammarous setosu* (Iannilli et al., 2020). In both studies, fibers were among the most dominant MP types. Similarly, fibers are the dominant shape found also in freshwater fish, while the most dominant colors are blue, black, and white (Bhatt and Chauhan, 2023). Moreover, as for marine fish, the GI tract is the most investigated tissue also in freshwater fish, while studies focusing on the muscles and other edible parts are lacking.

Overall, the available data suggest that MPs are ingested by aquatic biota, but there is an urgent need to carry out more studies also considering less analyzed organisms and tissues, as well as poorly investigated areas, and optimizing the used methodology to not underestimate the impact of contamination.

1.5 Exposure routes to MPs

The ubiquitous presence of MPs in the environment leads to the continuous exposure of living organisms to these pollutants, with health consequences that are not yet well understood. The primary exposure routes of organisms to MPs are: (I) ingestion of contaminated food and water, (II) inhalation through the air or retention in gills, (III) and dermal contact with MPs contained in products, cosmetics, textiles, or dust (Fig 1.2) (Sangkham et al., 2022).

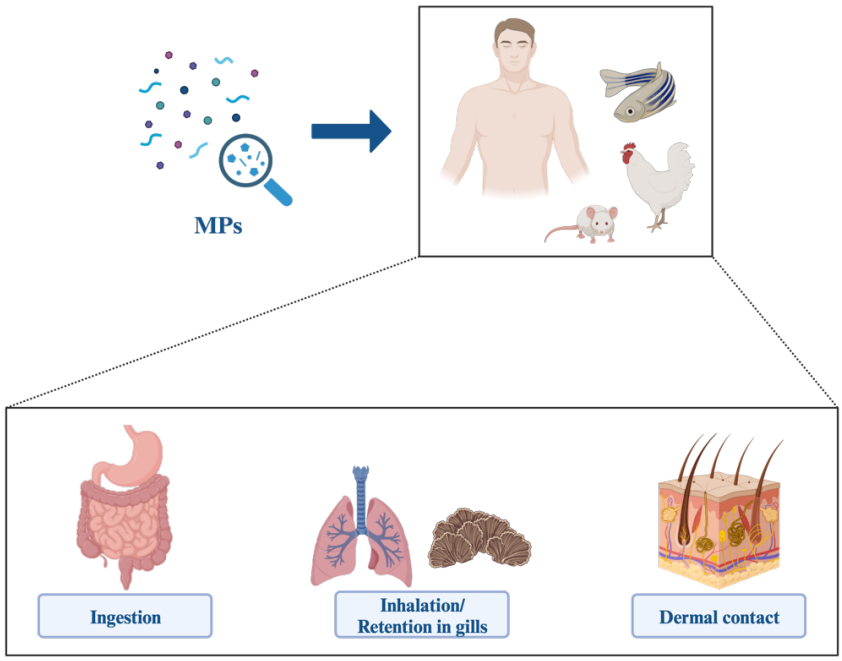

Fig. 1.2 – Exposure routes of terrestrial and aquatic organisms to MPs.

1.5.1 Ingestion

Ingestion is the main exposure route to MPs for both aquatic and terrestrial organisms, including humans (Franzellitti et al., 2019; Sangkham et al., 2022). Specifically, MP ingestion can be differentiated into two sub-categories: direct and indirect. In aquatic species, direct ingestion is highly related to the uptake by filter-, suspension- and deposit- feeders, because they cannot discriminate food from MPs since their size is in the same range (Franzellitti et al., 2019). However, direct ingestion also occurs by consuming foods and drinking liquids contaminated through the passage of MPs from the packaging. In fact, MP presence has been reported in table salt, sugar, honey, and beer (Jin et al., 2021). Moreover, Schymanski et al. (2018)

showed the presence of polyester and PP in mineral waters from single-use plastic bottles made up of these polymer types. On the other hand, the indirect ingestion of MPs refers to their trophic transfer through the consumption of organisms that have ingested MPs (Miller et al., 2020).

There is a lack of information on the fate of MPs in the GI tract, and the available data on their toxicokinetic only include absorption and distribution, whereas no information is available on their metabolism and excretion (EFSA, 2016). Many studies have shown that ingested MPs accumulate in the GI tract of various species, causing local effects on the gut (Deng et al., 2017; Hirt and Body-Malapel, 2020; Alomar et al., 2021). Additionally, ingested plastic particles could be internalized in the intestinal epithelial cells through several processes, i.e., endocytosis, transcytosis, persorption, and paracellular uptake, and the Peyer's patches are the most involved cells in the intestinal MP absorption (Hirt and Body-Malapel, 2020). However, these processes are influenced by the characteristics of both particles and organisms and result in their translocation into the circulatory system and systemic distribution, followed by several health consequences (Prata et al., 2020). Specifically, only MPs smaller than 150 μm may migrate across the gut epithelium, causing systemic distribution (EFSA, 2016).

1.5.2 Inhalation

Inhalation is considered the second major exposure route to MPs due to their ubiquitous occurrence in the air. Since their small size, atmospheric MPs can be inhaled by both animals and humans, and synthetic fibers have been detected in biopsies of human lungs (Chen

et al., 2020). MPs larger than 5 μm are inhaled and subjected to mucociliary clearance, such as sneezing, mucociliary escalator, phagocytosis by macrophages, and lymphatic transport. On the contrary, inhaled smaller and less dense MPs may avoid the clearance mechanisms reaching and accumulating in the deep lungs, where they trigger several biological responses (i.e., inflammation and fibrosis), causing acute and chronic respiratory problems (Gasperi et al., 2018). The risk associated to MP inhalation is higher in industry workers, who are exposed to elevated concentrations of plastic particles for several hours daily. In fact, occupational exposure is commonly related to respiratory symptoms such as throat irritation, shortness of breath, cough, and chest pain (Prata, 2018). Analogously to the inhalation through the air for terrestrial organisms, MP exposure also occurs through retention in the gills in aquatic species, and several studies showed their presence in the gills of several fish (Huang et al., 2021; Ma et al., 2021).

1.5.3 Dermal contact

In addition to ingestion and inhalation, dermal contact is a further exposure route to MPs for both terrestrial and aquatic organisms. In aquatic environments, floating MPs can interact with fish not only through ingestion and respiratory exposure but also through skin absorption (Huang et al., 2021). On the other hand, MPs deriving from the air fallout may result in deposition on both human and animal skin, resulting in dermal exposure (Prata, 2018). Furthermore, primary MPs are contained in skin care products such as creams, sunscreens, and facial and body cleaners. The most used MPs in cleaning products are

PE, followed by PP and PS (Lei et al., 2017). However, the absorption risk and health consequences via dermal exposure are not yet completely understood. In fact, MP physiochemical properties make crossing of the stratum corneum unlikely under normal conditions, suggesting that MPs may not reach the deeper layers of skin, but they may accumulate in the epidermis (Sangkham et al., 2022). Schirinzi et al. (2017) showed that cutaneous contact with MPs may induce oxidative stress (OS) in epithelial cells. Moreover, it is known that plastic use in medicine may be responsible for low inflammatory and foreign body reactions (Prata et al., 2020). However, compromised skin because of injury or illness is more permeable, making possible MP migration also in the skin's deeper layers (Zoabi et al., 2021). Nevertheless, other chemical compounds adsorbed on the MP surface may lead to skin alterations and MP deeper absorption with unknown health consequences (Sangkham et al., 2022).

1.6 Accumulation of MPs along trophic chains

MPs are known to be everywhere in the environment and are growing cause of concern for their potential hazards to humans and animals within the ecosystems (Lehel and Murphy, 2021). Plastics have several properties, among which durability, corrosion resistance, recalcitrance to degradation. These characteristics, on one hand explain the reasons for their widespread use in industry and daily life; on the other hand, represent the cause of their persistence for centuries. Therefore, plastics are released on a large-scale into aquatic and terrestrial environments, and due to their small size, MPs undergo

bioaccumulation and biomagnification processes within the food chains (Lehel and Murphy, 2021).

MPs can be directly introduced by primary species (i.e., zooplankton), which integrate and transfer them to their natural predators, cascading through the food chain and biomagnifying (Latchere et al., 2021). The potential bioaccumulation and biomagnification of these particles along marine, freshwater, and terrestrial food webs lead to human exposure, with yet unknown consequences.

In aquatic environments, MPs can be ingested by different organisms intentionally or unintentionally because their size is in the same range of plankton and sand grains. This makes them accessible to organisms with different feeding strategies, who often mistake them for food sources (Carbery et al., 2018). Both marine and freshwater organisms can ingest plastic particles directly through filtration or adsorption or indirectly through the consumption of polluted prey (Latchere et al., 2021). MP consumption is highly influenced by both the feeding strategy and the trophic level of the species. They have been detected in many marine and freshwater organisms from different trophic levels, including zooplankton, barnacles, bivalves, decapod crustaceans, fish, marine mammals, freshwater birds, and seabirds (Carbery et al., 2018; Huang et al., 2021). One of the first studies that investigated the trophic transfer of MPs in marine organisms was carried out in 2013 by Farrell and Nelson, who showed that PS-MPs were transferred from mussel *Mytilus edulis* that filter-feed MPs to the tissues and hemolymph of crab *Carcinus maenas* (Farrell and Nelson, 2013). Further, Santana et al. (2017) showed MP transfer between a

prey and two predator species even if preys had already cleared their guts when offered to predators, suggesting that the impact could be more harmful when predators ingest their prey immediately after their exposure to MPs.

MP trophic transfer does not exclusively imply that the prey species have previously ingested them. In fact, even if ingestion is the main exposure route to MPs in aquatic environments, it has been shown that they can also adhere to the surfaces of exposed organisms, and other absorption routes are possible (i.e., absorption by gills) (Au et al., 2017). Since the trophic transfer of MPs occurs from prey to predators, this process may be influenced by their concentration in prey as well as predator depuration capacity and rate (Santana et al., 2017). Indeed, organisms accumulate MPs when their egestion amount is less than the ingested or absorbed ones (Au et al., 2017). However, the surface characteristics and shape of MPs may also influence their uptake and residence time in prey species. For instance, Watts et al. (2015) showed that fiber-shaped MPs resulted in the formation of aggregates in the gut of exposed species and in a reduction of egestion time.

Ingested particles can pass through the gut, be absorbed, or accumulate in the digestive tract. Consequently, the greatest quantity of MPs is contained in the digestive tract of aquatic organisms. Removing this tract from larger fish partially limits the risk to human consumers. Conversely, the digestive tract is not removed from bivalves, oysters, and several small fish species, which poses a risk to human consumers who eat contaminated seafood (Lehel and Murphy,

2021). Nevertheless, MPs can also translocate from the gut to muscles (Kibria, 2023), which represent the main edible fraction of fish.

Compared to marine ecosystems, freshwater environments have been less examined from the perspective of trophic transfer in food webs, despite the evidence of MP ingestion by freshwater biota. MP bioaccumulation occurs in freshwater organisms in the same way as marine ones, and with an increasing trend of the particle number of MPs from herbivorous to carnivorous and omnivorous, confirming that MPs are also transferred along the freshwater trophic levels (Bhatt and Chauhan, 2023).

Analogously to the aquatic environments, MPs can accumulate in terrestrial and continental food webs, but more research are needed in this area. Bioaccumulation of MPs in yeasts and filamentous fungi has been observed to be a widespread phenomenon in the terrestrial environment (Lehel and Murphy, 2021). In addition, MPs have been detected in the GI tracts or feces of multiple terrestrial animals, among these earthworms, herpetofauna (i.e., tropical house gecko and Amazon lava lizard), and birds (Wang et al., 2022d). One of the first studies regarding the trophic transfer of MPs in terrestrial organisms was conducted by Huerta Lwanga et al. (2017), who showed an increase in MP concentrations from home garden soil to earthworm casts and chicken feces, suggesting that trophic transfer of MPs might occur in this short terrestrial food chain. In terrestrial ecosystems, plants represent the basis of food webs. MPs can enter the terrestrial trophic chains through the internalization in plants via root or foliar uptake, and consequently, MPs transfer to the primary consumers of plant structures may occur. In fact, Chae and An (2020) found that 20

nm PS could be absorbed in mung bean (*Vigna radiata*) through root uptake and then transferred to the African giant snail (*Achatina fulica*) that fed on mung bean leaves. Ingested MPs are mainly retained in the GI tract or excreted through feces, but there are also evidences of their translocation from the gut to other organs (Banerjee and Shelver, 2021). Furthermore, animals like small soil invertebrates are usually eaten entirely by their predators, leading to MP passage along the trophic levels, which is influenced by predator-prey interactions. Moreover, MP internalization is affected by their physicochemical properties (i.e., particle size, polymer type, and surface chemistry), exposure concentrations, and species physiology (Wang et al., 2022d).

Thus, there are still gaps in knowledge of MP bioaccumulation and biomagnification along the food webs. Moreover, the impact of adsorbed pollutants responsible for bioaccumulation and biomagnification within ecosystems (i.e., persistent organic pollutants - POPs) should be considered in the future.

2. MPs: a threat to animal and human health

2.1 Factors influencing MP exposure and toxicity

Assessment of plastic particle toxicity can be very complex because it is influenced by several factors related to particle properties (i.e., polymer type, plastic shape and size, superficial functionalization, adsorbed pollutants, and aging processes), exposure conditions (concentration/dose, and time of exposure) and organism characteristics (species, developmental stage, and sex) (Fig. 2.1).

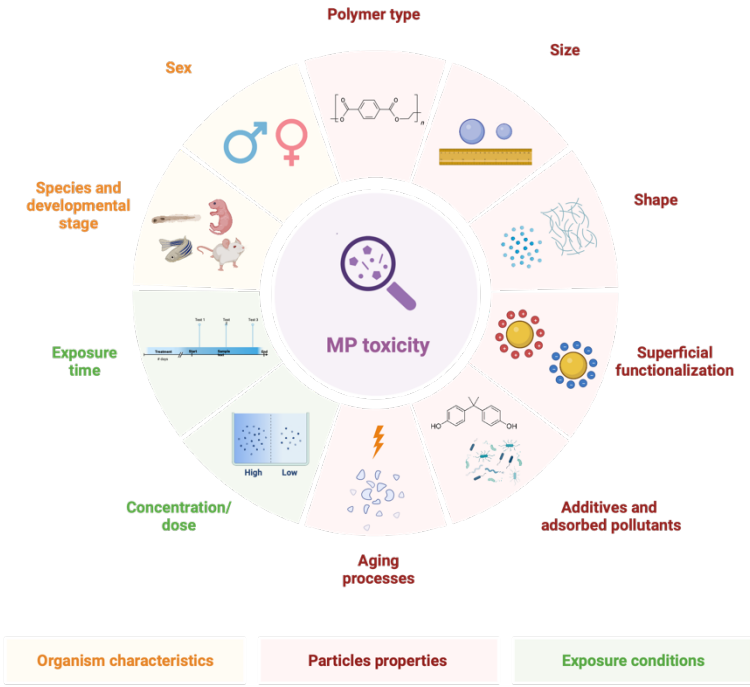

Fig. 2.1 – The main plastic particle toxicity determining factors.

2.1.1 Characteristics and properties of MP particles

MP toxicity may vary based on the polymer properties. Firstly, plastic polymer type plays a significant role in toxicity. Lithner et al.

(2011) reported polyurethane, polyacrylonitriles, PVC, epoxy resins, and styrene-based copolymers as the most hazardous polymer types, classifying them as mutagen or carcinogen (category 1A or 1B). Currently, the most investigated plastic polymers in terms of toxicity are PS, PE, PVC, PP, and PET, and all of them showed more sublethal toxic effects than lethality (Pelegrini et al., 2023).

Moreover, plastic shape and size may influence their toxicity. It has been reported that non-spherical MPs, like fragments and fibers, have higher toxicity on organisms than spherical ones (Jung et al., 2021). The effects of microspheres, fragments, and fibers on zebrafish gut were compared in a study by Qiao et al. (2019a), showing that fibers accumulate more and are more damaging to the gut than either spheres or fragments. Also, an inverse relationship between particle size and toxicity has been reported. In fact, smaller particles can be more easily internalized by cells through endocytic or passive uptake processes, while larger particles require phagocytosis by specialized cells (Alberts et al., 2002). Thus, size reduction may facilitate MP absorption through their contact site (i.e., intestine or respiratory tract), impacting their cellular fate and distribution. Consequently, it has been shown that larger particles exert local effects in the gut, while smaller particles induce toxic effects also in other organs and tissues (EFSA, 2016).

Several studies also investigate the impact of superficial functionalization on MP uptake and toxicity. The cellular uptake of PS is positively influenced by positive charge, and it is higher for positively charged particles compared to negatively charged ones (Yacobi et al., 2010). Similarly, *in vivo* studies showed greater uptake and toxicity associated with cationic-charged particles (Kogel et al.,

2020). MP toxicity can also be enhanced by plastic additives, which can constitute 4% of MP content and include stabilizers, plasticizers, lubricants, dyes, and flame retardants (EFSA, 2016). Among these, bisphenol A, phthalates, and brominated flame-retardants are of high concern because of their capability to disrupt endocrine function (Campanale et al., 2020). Nonetheless, MPs can adsorb and act as carriers of other pollutants such as POPs, heavy metals, or pathogens (Yu et al., 2019), which may influence their toxicity.

Finally, aging processes over time (i.e., photodegradation, thermal degradation, biodegradation, and mechanical fragmentation) change MP characteristics (i.e., shape and size), enhance their surface area, affect the mechanism of interaction with other pollutants, and stimulate the release of additives and leachate. Together, these factors influence and enhance MP toxicity (Luo et al., 2023).

2.1.2 Exposure conditions

Along with particle properties, exposure conditions are crucial factors in MP toxicity. In fact, although it is known that smaller MPs are more toxic than larger ones, this is not universally confirmed depending on the influence of other elements, such as exposure mode/time and dose/concentration used (Kogel et al., 2020; Banerjee and Shelver, 2021). Indeed, it has been shown that prolonged exposure and high dose/concentration may amplify the MP effect on the organism or cell systems. Blarer and Burkhardt-Holm (2016) exposed amphipods (*Gammarus fossarum*) to polyamide (PA) and PS for different time periods. They showed that both polymer types were ingested and egested by amphipods, but only the longer exposure to

PA fibers exerted an effect on their health and ecological functions. Similarly, PVC exposure reduced in a time-dependent manner the antioxidant enzyme activities in the liver of exposed catfish (Iheanacho and Odo, 2020). On the other hand, MP-induced dose-dependent damage was evidenced in different species. For instance, OS resulting from a dose-dependent increase in reactive oxygen species (ROS) and malondialdehyde (MDA) content was revealed in the testis of mice after chronic oral exposure to PS (Xie et al., 2020). Similarly, a time and dose-dependent effect was also evidenced by using *in vitro* systems (Banerjee and Shelver, 2021). Among the others, Xu et al. (2019) showed a positive correlation between uptake and toxic effects of PS particles and exposure duration and concentration in human alveolar epithelial A549 cells.

2.1.3 Physiological characteristics of exposed organism

Another critical aspect to consider in evaluating of MP toxicity is the specificity of the exposed organism. Although nearly all investigated organisms are found to be affected by plastic debris, specific characteristics, such as species, developmental stage, and sex, may influence the toxicity (Kogel et al., 2020).

Species dependency of MP toxicity was demonstrated in studies conducted by exposing different fish species to MPs with similar characteristics. In fact, while it has been reported that ingestion of PET, PVC, PS or PE induced toxic effects in planktivorous fish (*Acanthochromis polyacanthus*) and white sturgeon (*Acipenser transmontanus*), no effects were observed in gilthead seabream

(*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*) exposed to MPs in the same size range (Rochman et al., 2017; Asmonaite et al., 2018; Critchell and Hoogenboom, 2018; Jovanovic et al., 2018). Moreover, there are evidence for a species dependency of MP uptake. A study on blue mussel showed that PS 30 and 90 µm were not taken up (Browne et al., 2008). On the contrary, Van Cauwenberghe et al. (2015) found that PS up to 30 µm was taken up in lugworms. Thus, species characteristics may influence MP uptake, kinetics, and distribution in the organism and, consequently, also their toxicity (Kogel et al., 2020).

Additionally, the developmental stage of the organisms may regulate MP uptake into tissues. In oysters, it has been shown that younger larvae took up only smaller PS particles, while older larvae also took up the larger ones (Cole and Galloway, 2015).

Finally, the toxic effects of MPs may also be related to sex. Wei et al. (2022) compared the effects of PS exposure on reproduction and fertility in male and female mice. They showed that, even if PS exposure caused damages in both testes and ovaries, females appear more susceptible to PS-induced reproductive alteration than male mice. Similarly, the impairment of hepatic lipid metabolism was more pronounced in female mice than male ones (Luo et al., 2019b).

2.2 Potential mechanisms of toxicity induced by MPs

The exposure of living organisms to MPs leads to the onset of widespread toxic effects, but the underlying mechanisms are still unclear. Studies conducted across different taxonomic groups have

shown that different adverse outcome pathways are involved in MP-induced toxicity, mainly involving OS, cytotoxicity, inflammation, neurotoxicity, and immunotoxicity (Jeong and Choi, 2019; Prata et al., 2020). However, all the mechanisms are strictly intertwined, and the induction of one pathway can trigger or power the others (Fig. 2.2).

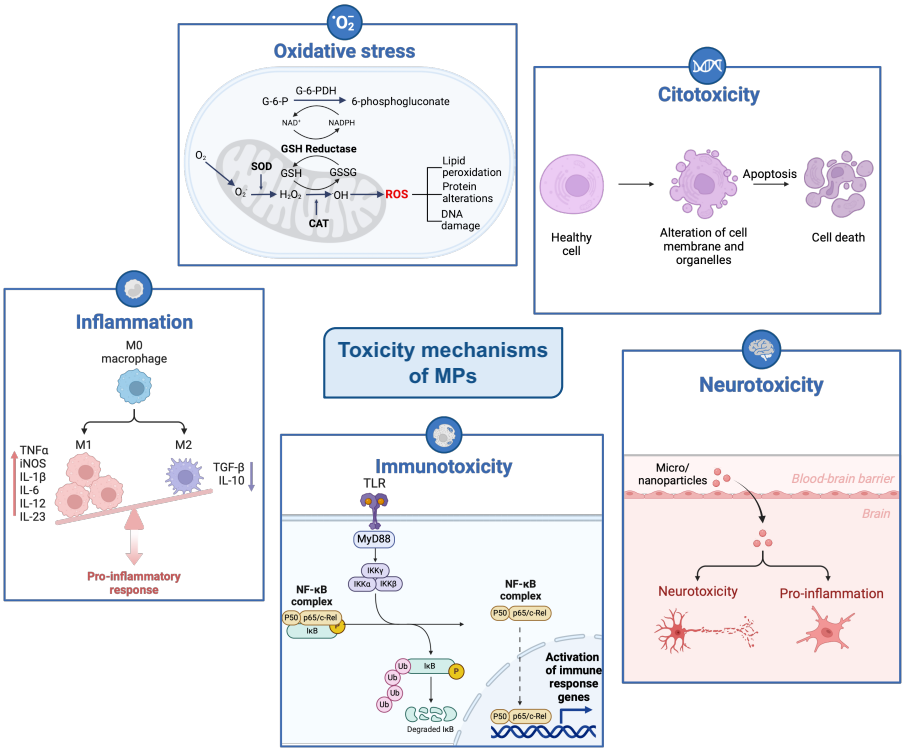

Fig. 2.2 – Mechanisms of toxicity involved in the onset of MP-induced effects.

The OS is defined as the main mechanism responsible for MP-induced damage. In physiological conditions, ROS are produced in low amounts that are required to maintain cell homeostasis, and the antioxidant defense systems, such as the antioxidant enzymes and glutathione (GSH), counteract their overproduction. OS occurs when there is an unbalance between ROS production and antioxidant system activity. Several studies showed that MP exposure induces an oxidative

unbalance caused by ROS overproduction and/or antioxidant system impairment (Geremia et al., 2023). It has been suggested that the overproduction of ROS (i.e., hydrogen peroxide, singlet oxygen, superoxide anion, ozone, hydroxyl radicals, and nitric oxide) is an early molecular key event in MP toxicity (Hu and Palic, 2020). ROS can cause damage to cell components (i.e., lipids, proteins, and DNA), and lipid peroxidation and DNA strand damage are consequences of MP-induced ROS overproduction (Ribeiro et al., 2017; Barboza et al., 2018). Moreover, MP exposure also activated redox sensitive signaling pathways such as mitogen-activated protein kinases (MAPKs) and impacted the nuclear factor erythroid 2-related factor 2 (Nrf2) transcription, suggesting its involvement in the regulation of antioxidant enzymes gene expression (Jeong et al., 2017).

As regards cytotoxicity, MPs may trigger adverse cellular effects through membrane damage, mitochondrial dysfunction, and lysosome disruption. Amongst these, the cytotoxicity of MPs has been mostly attributed to a loss of plasma membrane integrity because of particle capability to impair membrane structure and function (Banerjee and Shelver, 2021). Mitochondrial dysfunctions are, in turn, related to MP-induced OS. In fact, the increased ROS generation may be responsible for mitochondrial membrane damage and dysfunction and for the release of pro-apoptotic and pro-inflammatory mediators, resulting in cell death (Hu and Palic, 2020; Banerjee and Shelver, 2021). Similarly, lysosomal damage may be due to excessive production of ROS induced by MPs, but also to a direct damage because of the lysosomal attempts to digest the foreign particles (Hu and Palic, 2020).

Inflammation, together with OS, is another significant mechanism responsible for MP-induced damage. An increase in pro-inflammatory cytokines was evidenced at molecular level in several experimental models after MP exposure (Jin et al., 2018; Dong et al., 2020; Shen et al., 2022). Inflammation is also associated with the activation of immune response, as showed by infiltration and activation of immune cells in mice liver, along with the increase in pro-inflammatory cytokines, after oral exposure to MPs (Zhao et al., 2021a). Moreover, inflammation is likely related to OS and lysosome dysfunction induced by MPs (Hu and Palic, 2020).

Another key event triggered by MP exposure is the inhibition of acetylcholinesterase (AChE), a critical enzyme for proper nervous system function. This effect has been shown in different organisms after MP exposure (Avio et al., 2015; Deng et al., 2017). The mechanisms involved in the MP interference with AChE are still unknown, but an involvement of MP-induced OS is possible. In fact, the direct relationship between H_2O_2 and modifications of AChE metabolism and activity was established in SH-SY5Y cells, confirming that the ROS increase could promote disturbances in the cholinergic system of neural cells (Garcimartín et al., 2017). Simultaneous detection of MP-induced OS and AChE inhibition further supports such possibility (Deng et al., 2017).

Finally, immunotoxicity is another critical mechanism underlying the effects of MPs. The innate immune system is the first line of defense against external stimuli, acting as an interface between the environment and organisms (Suzuki et al., 2020). Phagocytes, especially neutrophils, are scavenging machines of innate immunity.

Once environmental chemicals reach the body, these cells engulf and "digest" the particles and transfer the information to the surface of the scavenger cells, activating the immune system cascade. After MP uptake, immune cells activate the modulation of several transcriptional factors, impacting aspects ranging from enzymatic activities to cytokine release. Thus, endocytosed MPs can alter the signal pathways as well as stimulate the autophagy process, activating the innate immune response and leading to immune system dysfunction (Yang et al., 2022a). Several studies have linked the MP exposure to impairment of the immune system. After PS exposure, hemocyte alterations with higher mortality and decreased phagocytosis activity were found in mussels and bivalve mollusks. Moreover, variations in parameters related to OS (i.e., increased ROS production), apoptosis, and inflammatory response were also revealed in hemocytes (Paul-Pont et al., 2016; Shi et al., 2020; Tang et al., 2020). An impacted phagocytic capacity was also shown in the leucocytes of fish exposed to MPs (Espinosa et al., 2017; Espinosa et al., 2019). In sea urchin, exposure to PS microbeads increased the coelomocyte count and the intracellular levels of ROS and reactive nitrogen species (RNS), indicating a stress-related impact on these circulating immune cells (Murano et al., 2020). Additionally, the hemolymph activity of several enzymes related to the immune system (i.e., acid phosphatase, alkaline phosphatase – ALP, lysozyme – Lys, and phenoloxidase) was found modified after MP exposure in crab and mussel (Liu et al., 2019b; Revel et al., 2019). Similarly, exposure to MPs affected the activity of immunity-related enzymes in the plasma of carp, also causing an impairment of the complement system (Banaee et al., 2019). Moreover, PE exposure

increased pro-inflammatory cytokine interleukin (IL)-1α levels in mice serum and decreased regulatory T cell count in splenocytes (Li et al., 2020a).

2.3 Experimental models used for studying the uptake and toxicity of MPs

Nowadays, living organisms are encountered with MPs every day and everywhere, but there are still gaps about their effects on health and environment. During the last few years, the number of studies examining MP effects has increased exponentially. While before 2010 only 10 articles per year were published on the subject, since 2011 the number continues to rise, and from 2017 there were more than 300 publications per year (Zhang et al., 2020). As MP-induced adverse effects may be influenced by several factors (i.e., polymer type and size, environmental factors, and considered organism), different experimental models and experimental conditions were used. Current available data on the effects of MPs on biological systems come mainly from *in vivo* studies in model organisms and, to a lesser extent, from *in vitro* studies. *In vivo* studies represent an opportunity to mimic the environmental conditions and have been conducted on both simple and complex organisms (Fig. 2.3). In fact, they allow the assessment of the whole organism at different exposure times (i.e., acute, sub-acute, sub-chronic, chronic, or whole life) as well as at different life stages (i.e., embryonic, postembryonic development, juvenile, fertile period, adulthood, senile stage). Prokic et al. (2021) highlighted that 60.16% of articles published from January 2011 to May 2020 on MP effects

were laboratory-based studies, while only 39.84% were field investigations. Moreover, they reported a significant difference in the ratio between laboratory and field research for different animal groups. In fact, while for vertebrates there is a 1:1 ratio between laboratory and field studies, for invertebrates it is needed to expand future research also under natural conditions. On the contrary, there is a lack of studies about the impact of MPs on birds and reptiles in laboratory conditions.

Studies on MP impact on invertebrate organisms are slightly more than studies on vertebrate ones (Fig. 2.3).

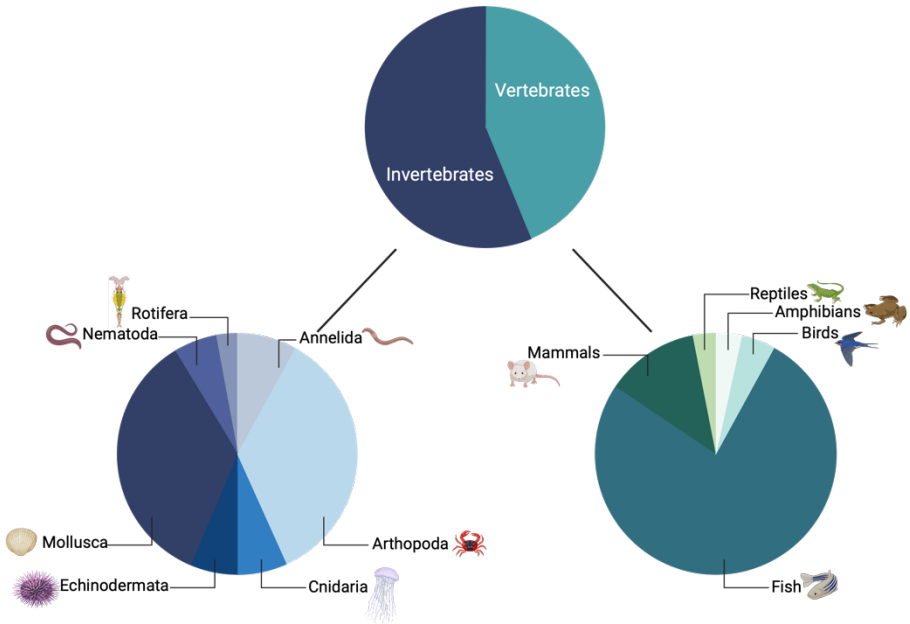

Fig. 2.3 – Model organisms used in *in vivo* toxicity studies on MPs.

Among the invertebrates, Mollusca and Arthropoda are the most investigated taxonomic groups because they are filter-feeders, most of them are primary consumers, and they give a significant contribution to the human diet. Other less investigated groups are Annelida, Nematoda, Echinodermata, Cnidaria, and Rotifera (Prokic et al., 2021).

Specifically, the most studied species under laboratory conditions are mussels from the genus *Mytilus* (i.e., *M. galloprovincialis* and *M. edulis*) for marine environments, *Daphnia* (i.e., *D. magna* and *D. pulex*) for freshwater ecosystems, and *Caenorhabditis elegans* for terrestrial one. Performing studies on invertebrates offers several advantages, such as their small size and easy maintenance, and few legal restrictions (Burgos-Aceves and Faggio, 2017; Hunt, 2017). On the other hand, the complexity of vertebrates and their high position in the food chain ensure they provide more information about MP effects and bioaccumulation (Lillicrap et al., 2016).

Regarding vertebrates, fish are the most investigated species, followed by mammals, birds, amphibians, and finally, reptiles (Prokic et al., 2021). Zebrafish (*Danio rerio*) is one of the most used model organisms because of its advantages, such as small size, ease of breed, short life cycle, inexpensive maintenance, fewer legal restrictions, and genetic analogies with humans (Bhagat et al., 2020). Moreover, since zebrafish embryos and larvae are transparent, they are highly used for studies of fluorescent-labeled MP localization (Bhagat et al., 2020). Other fish species belonging to high trophic levels in both marine and freshwater environments are used as experimental models in the evaluation of MP-caused damage; among these, *Oryzias melastigma*, *Tigriopus japonicas*, *Dicentrarchus labrax*, *Sparus aurata*, *Perca fluviatilis,* and *Cyprinus carpio* (Yin et al., 2021).

For mammalian species, most of the conducted studies used standard laboratory model organisms, largely mice and rats, to examine the possible MP mechanisms of action and their effects, providing predictions for human health (Prokic et al., 2021). Studies on higher

vertebrate species are scarce due to ethical and legal limitations (i.e., endangered species, monkeys) and are usually limited to MP exposure and/or ingestion (Prokic et al., 2021).

Finally, since there is obviously a lack of toxicity data for humans, *in vitro* studies are used to evaluate the MP effects on human cell cultures. GI cells (i.e., the human colorectal adenocarcinoma Caco-2 cells), airway cells (i.e., the adenocarcinoma human alveolar basal epithelial A549 cells), and immune cells (i.e., the human monocytic THP-1 cells) are the most investigated to assess the uptake and biological effects in body districts that are directly exposed to MPs and/or contribute to the removal of xenobiotics. However, since MPs can be systemically absorbed reaching several organs, their harmfulness has also been investigated in blood, cerebral, endothelial, epithelial, hepatic, kidney, melanoma, ovarian, and placental mammalian cells (Banerjee and Shelver, 2021). Despite the increase in research about MP occurrence and toxicity, several questions are still unanswered, and there is a need to increase knowledge also considering other helpful animal models.

2.4 Toxic effects of MPs on aquatic organisms

Exposure of aquatic biota to MPs causes their accumulation mainly in tissues that come into direct contact with plastic particles, such as the gut and gills. However, it is widely believed that MPs may reach other tissues (i.e., liver, muscle, kidney, brain, ovary, and testis), impacting their physiological functions (Franzellitti et al., 2019).

Firstly, histological alterations associated with infiltration of immune cells were evidenced in both aquatic invertebrates and vertebrates after MP exposure (Paul-Pont et al., 2016; Jovanovic, 2017). In recent years, many studies have focused on the toxicity of MPs in freshwater and marine invertebrates under laboratory conditions evidencing they are among the most susceptible organisms. Their feeding, growth, survival, and reproduction were negatively affected by MP exposure (Foley et al., 2018). Long-term exposure to PS altered the feeding capacity and decreased the reproductive capability of the pelagic copepod *Calanus helgolandics* (Cole et al., 2015). Similarly, exposure to PET negatively affected the reproductive output of the calanoid copepod *Parvocalanus crassirostris*, leading to population decline during the experimental period (Heindler et al., 2017). In another study, Jeyavani et al. (2022) reported changes in swimming behavior and increased mortality in *Artemia salina* exposed to PP. Also, ingestion of PET caused a rise in mortality in freshwater crustacean *Daphnia magna* (Jemec et al., 2016). Recent data indicate that MP exposure can affect fish health, reducing growth performance and increasing mortality (Naidoo and Glassom, 2019).

Ingested MPs can accumulate in the gut, causing intestinal damage associated with the impairment of oxidative and inflammatory balance, perturbations in microbial communities, and epithelial disruption (Qiao et al., 2019b; Montero et al., 2022). Moreover, they can create a blockage in the digestive tract that may lead to nutritional imbalance and growth inhibition in fish (Jabeen et al., 2018). Yin et al. (2018) showed that the exposure of juvenile jacopever (*Sebastes schlegelii*) to PS causes a weakened feeding activity, influencing fish growth, energy

reserve, and nutrient quality. Moreover, an impairment of hunting behavior was highlighted by reduced swimming speed and range of movement.

Behavioral changes were also evidenced in other fish species after exposure to MPs. PE exposure caused a decrease in predatory performance in discus fish (*Symphysodon aequifasciatus*) (Wen et al., 2018). Recently, Chen et al. (2022) highlighted that PE influenced swimming speed, acceleration, and social behavior in carp (*Cyprinus carpio*). Similarly, a negative impact on the social behavior of juvenile perch (*Perca fluviatilis*) was evidenced after chronic ingestion of poly(L-lactide) particles (Konig Kardgar et al., 2023).

MP intake could alter the metabolic function and the proper immune function. It was found that PS can alter the glucose, lipid, and amino acid metabolism, as well as energy metabolism in the liver of exposed fish (Lu et al., 2016; Lu et al., 2022; Zhang et al., 2022b). In addition, MPs may induce hemato-biochemical alterations, causing anemia and affecting the immune system of fish (Hamed et al., 2019).

Finally, it has been shown that MPs can impair fish reproductive capability by causing alterations in the ovary and testis, often related to OS damage (Ferrante et al., 2022).

2.5 Toxic effects of MPs on terrestrial animals and humans

MP exposure may impact the health status of both small and large terrestrial animals in many ways. Since the critical ecological function of soil fauna, organisms such as earthworms, nematodes, and arthropods are considered biological indicators of soil pollution and

have been extensively studied for MP toxicity (Chang et al., 2022b). Many studies highlighted that exposure of soil fauna to MPs causes several adverse effects with the consequent impairment of growth, development, behavior, reproduction, and survival (Huerta Lwanga et al., 2016; Yu et al., 2020b; Sun et al., 2021a). Moreover, MP exposure can cause intestinal microflora dysbiosis (Ju et al., 2019) and barrier impairment (Yu et al., 2020b) in exposed earthworms, and a correlation with OS has been established (Yu et al., 2020b). In addition, MPs can directly or indirectly damage cellular DNA in soil invertebrates, resulting in mutagenic and carcinogenic effects (Chang et al., 2022b).

Regarding large terrestrial animals, there is still little knowledge of MP-induced toxic effects. MPs have been reported to accumulate in sheep (Beriot et al., 2021) and chickens (Huerta Lwanga et al., 2017; Zhang et al., 2022a). However, while no information is available on the potential effects of ingested MPs in sheep, it has been found that they cause pathological damage and ultrastructural changes in chicken hearts, inducing myocardial pyroptosis, inflammation, and mitochondrial damage (Zhang et al., 2022a).

The harmful effects of MPs on rodents have been instead more largely investigated. *In vivo* studies showed the presence of MPs in several tissues of rodents accompanied by local inflammation, OS, and metabolic disruption, leading to widespread toxic effects (da Silva Brito et al., 2022). Firstly, MP exposure caused their accumulation in the gut and impaired intestinal function and structure. Specifically, intestinal dysbiosis, barrier dysfunction, and metabolic disorders were evidenced in MP-exposed mice (Jin et al., 2019; Li et al., 2020a).

Accumulation and metabolic dysfunction were also observed in the liver after the exposure to MPs. In fact, many studies reported alteration of mice hepatic lipid metabolism in response to MPs (Lu et al., 2018; Luo et al., 2019a; Luo et al., 2019b; Zheng et al., 2021).

Recently, a MP-induced neurotoxic effect was also reported. Chronic exposure to PS-MPs disrupted the blood-brain barrier, accumulating in the brains of exposed mice and resulting in cognitive and memory deficits through the induction of OS (Jin et al., 2022; Wang et al., 2022c). In addition, an induction of anxiety-like behaviors was observed in mice after oral PS-MP treatment (Chen et al., 2023).

OS-mediated reproductive dysfunction was demonstrated in both male and female rodents exposed to MPs, and adverse effects were reported also in the progeny (Ferrante et al., 2022). PS-MPs induced an increase in OS biomarkers (i.e., ROS and MDA) in testes, resulting in a decreased quantity and quality of sperm in mice (Xie et al., 2020). Similarly, PS could induce a decrease in ovarian reserve capacity, causing fibrosis via Wnt/β-Catenin signaling pathway activation and granulosa cell apoptosis through OS in exposed rats (An et al., 2021).

The potential effects of MPs on other tissues are still poorly investigated in rodent models. However, it has been shown that MPs can induce apoptosis of cardiomyocytes and cause cardiac fibrosis and dysfunction in rats (Li et al., 2020c). Nonetheless, it has been recently found that MP exposure may lead to injury also in the lung and kidneys of exposed mice, inducing an inflammatory response, OS, cell apoptosis, and promoting fibrosis (Cao et al., 2023; Xiong et al., 2023). Finally, MPs can impair lymphoid organ function, impacting immunological functions. In fact, MP exposure causes spleen

alteration, resulting in the induction of lupus-like symptoms in C57BL/6 mice and in the exacerbation of existing lupus symptoms in MRL/lpr mice (Chen et al., 2024).

Human exposure to MPs has raised public concerns. Recently, compelling evidence has been provided that exposure to MPs poses hazards to human health (Noventa et al., 2021; Vethaak and Legler, 2021). The potential risks for individuals occur at cellular, tissue, organ, and system levels, and may involve digestive, respiratory, endocrine, reproductive, and immune systems (Yang et al., 2022b). The results of a monitoring study presented at the 26th Meeting of the European Federation of Gastroenterology showed the presence of 20 MP particles per 10 g of fecal sample on average (Chang et al., 2022b). Moreover, it has been shown that fecal MP concentration is significantly higher in patients with inflammatory bowel disease than in healthy people, suggesting that exposure to MPs may be associated with the occurrence and development of intestinal disorders (Yan et al., 2022). MP occurrence was also found in human urine samples and placenta (Ragusa et al., 2021; Pironti et al., 2022), opening a new scenario to understand the toxicity and evaluate the health risks induced by these pollutants.

3. Impact of MPs on gut-liver axis

3.1 Gut-liver axis: pathophysiological concepts and health implications

The gut-liver axis is a bidirectional communication system between the gut, along with its microbiota, and the liver. It may be influenced by several factors, including the environmental conditions (Albillos et al., 2020). The reciprocal interaction between these organs is established by the portal vein, which transports gut-derived products to the liver and, in the opposite direction, the bile and antibodies from the liver to the gut. The intestinal barriers are functional and anatomical structures limiting the systemic dissemination of microbes and toxins, while allowing nutrients to access circulation and reach the liver. Indeed, just a slight amount of potentially pathogenic or toxic compounds are carried from the gut to the liver in healthy conditions, and the healthy liver, with its immune system, can deal with such stressors. On the contrary, the intestinal barrier can be disrupted under compromised conditions. Then the translocation of inflammatory microbial metabolites, microbes, or toxic compounds to the liver occurs, promoting several disorders, such as non-alcoholic fatty liver disease (NAFLD) (Albillos et al., 2020; Tilg et al., 2022) (Fig. 3.1). NAFLD is a disease that can be associated with the exposure to many environmental pollutants (Zheng et al., 2021). Its development and progression are characterized by several features, among which an excessive accumulation of lipids, mainly triglycerides, in hepatocytes cytoplasm, inflammation, as well as OS (Karkucinska-Wieckowska et al., 2022).

MPs can damage the epithelial architecture and intestinal function, also altering the gut microbiota composition (Hirt and Body-Malapel, 2020; Souza-Silva et al., 2022). As the main metabolizing and detoxifying organ, the liver is equally highly susceptible to chemical xenobiotics, and MPs can impair its homeostasis (Yin et al., 2022). Thus, the exposure to MPs may contribute to intestinal and hepatic disorders, disrupting the balance of the gut-liver axis.

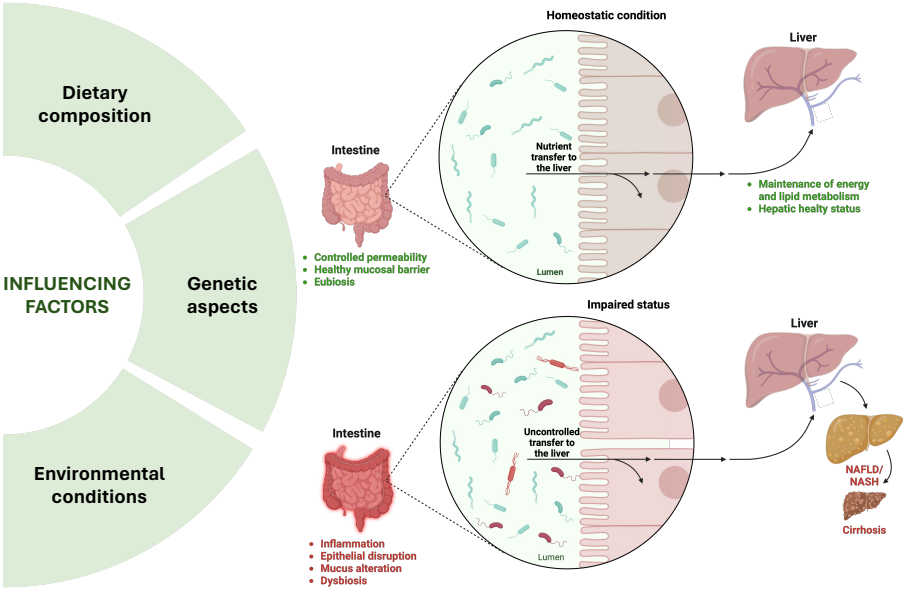

Fig. 3.1 – The gut-liver axis in healthy and disease conditions.

3.2 Effects of MPs on intestinal integrity, function, and microbiota

The intestine is among the most important organs directly communicating with the external environment. Thus, the intestinal immune system constantly interacts with xenobiotics in ecosystems and food, including plastic particles. MPs with greater size are not

absorbed and stay bound to the intestinal mucus layer, determining gut inflammation and local effects on the immune system. On the contrary, particles smaller than 150 μm can cross the mucus barrier, and most of them are absorbed by M cells in the Peyer patches (Hirt and Body-Malapel, 2020). Recently, Yuan et al. (2023) revealed that exposure to PE activated the mucosal immune pathway in zebrafish gut. Specifically, PE impacted the intestinal goblet cell coverage, increased the complement C3 and C4 content, and activated the pathway for mucosal immunoglobulin production. Altered recruitment of immune cells was observed in the proximal portion of the small intestine and colon of mice orally exposed to PE (Djouina et al., 2022).

Moreover, the increased levels of immune-related cytokines evidenced in the gut of animals exposed to MPs were related to the activation of intestinal inflammatory response (Huang et al., 2020; Djouina et al., 2022; He et al., 2022). The involvement of Toll-like receptors (TLRs) pathway was highlighted by some authors in in the gut of exposed fish and mice (Gu et al., 2020; Huang et al., 2020; Li et al., 2020a). Notably, exposure to MPs may even worsen intestinal damage in organisms with pre-existing intestinal immune imbalance (Liu et al., 2022a).

MPs may also impact gut epithelium, disrupting epithelial homeostasis and integrity (Hirt and Body-Malapel, 2020). Histopathological alterations and epithelial deconstruction were revealed in the gut of different species after MPs exposure, among these: epithelial detachment, disordered cellular arrangement, increased lipid droplet numbers, alteration of the villi and crypt, and vacuolation of enterocytes (Peda et al., 2016; Jabeen et al., 2018;

Limonta et al., 2019; Wang et al., 2019; Ahrendt et al., 2020). Increased intestinal permeability may be a consequence of epithelium modifications. Indeed, Usman et al. (2021) showed that the exposure of medaka fish to PS induced histological alterations in the gut, associated with increased permeability. The intestinal permeability augments when the tight junctions (TJs) break (Camilleri, 2019), and reduced expression of intestinal tight junction proteins was reported in the gut of aquatic and terrestrial species exposed to MPs (Chen et al., 2022; Jia et al., 2023). MPs can also impair the intestinal production of mucus, which plays a critical role in immune and intestinal barrier function. The modulation of genes codifying for factors involved in mucus production and properties maintenance, such as mucins (Muc), was revealed in the gut of exposed fish and mice (Lu et al., 2018; Jin et al., 2019; Chen et al., 2022; Hoseini et al., 2022).

An increasing body of evidence suggests that MPs can induce OS in the gut. In fact, several studies reported increased levels of ROS and MDA in the gut after the exposure (Kang et al., 2021; Zhao et al., 2021b; Hou et al., 2022), as well as an impairment of antioxidant system, due to the modification of antioxidant enzymes activity induced by MPs (Paul-Pont et al., 2016; Qiao et al., 2019a; Qiao et al., 2019b; Revel et al., 2019; Kang et al., 2021).

Gut microbiota is known to provide essential functions to its host, and current studies have shown that environmental pollutants can affect its structure, leading to host physiological dysfunction and health disorders (Jin et al., 2017). Variations of intestinal microbiota following MPs *in vivo* exposure have been investigated in several experimental conditions. It has been shown that MPs are able to induce

dysbiosis and alter bacterial diversity, resulting in host health damage (Fackelmann and Sommer, 2019). Many studies highlighted a relationship between MP exposure and alteration of intestinal microbial communities in both aquatic and terrestrial species (Lu et al., 2018; Ju et al., 2019; Qiao et al., 2019b). Significant modifications were observed in the relative abundance of specific phyla, affecting more often *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Lu et al., 2018; Jin et al., 2019; Qiao et al., 2019b; Wan et al., 2019). A reduced alpha diversity, described as richness, diversity, and evenness of microbial communities, was also reported in MP-exposed organisms (Ju et al., 2019; Qiao et al., 2019b; Wan et al., 2019).

3.3 Effects of MPs on hepatic homeostasis

The liver of vertebrates plays a key role in the detoxification processes of drugs and environmental contaminants; thus, the hepatic cells are exposed to noteworthy concentrations of chemicals. Several studies have shown that MPs can accumulate in the liver of fish and mice (Lu et al., 2016; Deng et al., 2017; Ye et al., 2021). MPs were recently detected also in the human liver (Horvatits et al., 2022). Specifically, six different polymer types were found in the liver of patients with cirrhosis but not in the liver of healthy individuals, suggesting a possible link between exposure to MPs and the development of this chronic, progressive liver disease (Horvatits et al., 2022). The presence and accumulation of MPs in the liver can cause histological modifications, affecting normal liver function. Morphological alterations of hepatocytes, including necrosis, edema-

type degeneration, hypertrophy, and hyperplasia, were evidenced in the liver of some exposed species (Lu et al., 2016; Araújo et al., 2020; Iheanacho and Odo, 2020). Additionally, inflammatory infiltration, hepatic ballooning, and increased lipid droplets were displayed in the liver of both fish and mice, suggesting that MPs can cause hepatic inflammation and lipid accumulation (Lu et al., 2016; Luo et al., 2019a; Araújo et al., 2020; Li et al., 2021; Pan et al., 2021).

The exposure of goldfish to PS-MPs induced mitochondrial vacuolation in hepatocytes (Yang et al., 2020), indicating that these organelles are a potential target of MPs toxicity. Similarly, Pan et al. (2021) showed that MP exposure induced mitochondrial dysfunction modulating dynamin-related protein 1 and mitochondrial fusion protein levels in L02 hepatocytes. Since these proteins regulate mitochondrial fission and mitophagy, the authors inferred that MPs could trigger these processes. Moreover, the effects on mitochondria were reverted by inhibiting the endoplasmic reticulum stress, implying its involvement in MP-induced dysfunction (Pan et al., 2021).

MPs also impact the hepatic enzymes impairing their function. The levels and/or activities of ALP, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) measured in the plasma were modified by MP exposure (Banaei et al., 2022; Mu et al., 2022; Choi and Kim, 2023).

Studies showed that MPs also affect the hepatic detoxifying system, altering the activity of Phase I and/or Phase II enzymes. Cytochrome (CYP) P450 enzymes are the critical enzymes that act in Phase I of metabolization, and MPs modulate their activity in the liver (Pannetier et al., 2020; Martyniuk et al., 2022). Similarly, the hepatic

activity of glutathione s-transferases (GST), as enzymes involved in Phase II reactions, can be modulated by MPs (Cohen-Sanchez et al., 2023; Jeyavani et al., 2023; Sahabuddin et al., 2023).

Similarly, MPs impact the hepatic antioxidant systems by modulating the activity of involved enzymes, thus contributing to the onset of oxidative unbalance in fish and mice (Deng et al., 2017; Solomando et al., 2021; Banaei et al., 2022; Mu et al., 2022; Choi and Kim, 2023; Tao et al., 2024). Also, an impact on hepatic GSH content was observed after the exposure to MPs (Yu et al., 2023a).

MP-induced liver damage is also related to lipid and glucose metabolism impairment. Alterations of metabolites involved in lipid metabolism, such as metabolites of triglycerides and fatty acids (FA) as well as choline, phosphorylcholine, and cholesterol, were revealed in zebrafish liver after 7 days of exposure to PS (Lu et al., 2016). Moreover, it has been shown that MP exposure affects the levels of proliferator-activated receptors (PPARs), which are involved in the regulation of FA signaling (Zhang et al., 2021b; Liu et al., 2022b), as well as the gene expression of factors related to lipid synthesis and catabolism (Zhao et al., 2020; Zhang et al., 2021b; Du et al., 2023). Similarly, MP exposure can alter pathways involved in carbohydrate metabolism. Indeed, the exposure of rare minnow to PS resulted in perturbation of most monosaccharide pathways in the liver, including galactose, fructose and mannose metabolism, pentose phosphate pathway, pentose and glucuronic acid interconversion, and glycolysis/gluconeogenesis (Wang et al., 2022a). Moreover, long-term exposure to MPs resulted in impaired glucose tolerance in mice (Wang et al., 2022b; Li et al., 2024), also affecting key signaling pathways,

such as the AMP-activated protein kinase (AMPK) pathway in the liver (Li et al., 2024).

Finally, MP exposure altered ATP/ADP/AMP metabolites in the liver of zebrafish exposed to PS, indicating the disruption of energy metabolism in fish (Lu et al., 2016). Similarly, MPs decreased ATP concentration and increased LDH activity in mice, confirming an impairment of hepatic energy metabolism (Deng et al., 2017).

EXPERIMENTAL SECTION

4. Aim of the research

The ubiquitous occurrence of MPs in aquatic environments poses a real threat to aquatic biota. It is now well known that aquatic organisms live in plastic-polluted waters and are exposed to MPs from embryonal to adult life stages, with health consequences not fully understood (Xu et al., 2020). As mentioned above, ingestion is the main exposure route to MPs and the gut is a crucial target organ for their effects (Hirt and Body-Malapel, 2020). However, once absorbed, MPs may translocate from the gut to the blood circulatory system, reaching tissues and organs far from the site of introduction, among which the liver, directly connected to the gut itself (Ma et al., 2021).

To date, little is known about the effects of MP exposure via contaminated diet on different intestinal tracts and, therefore, on liver health due to existence of the gut-liver axis.

The aim of the present research was to investigate the adverse effects deriving from the ingestion of PS, one of the most widespread plastic polymers in the aquatic environment, on a predatory fish with elevated commercial diffusion, such as the gilthead seabream (*Sparus aurata* Linnaeus, 1758). Specifically, it was examined the effect of a subchronic oral treatment with different doses of PS-MPs on adaptive modifications of intestinal immune response and barrier integrity. Indeed, the gut not only digests food and adsorbs nutrients but also provides a defense barrier against pathogens and ingested noxious agents (Cain and Swan, 2010). The study was conducted by examining two different intestinal portions, the anterior (AI) and posterior (PI) intestine, knowing that they play different physiological and functional roles. In fact, a transcriptomic study on gilthead seabream reported that

AI is mostly involved in the absorption of lipids and proteins, while PI can take up macromolecular proteins (Perez-Sanchez et al., 2015). The PI is also the primary site where antigen uptake occurs and the immune response starts (Cain and Swan, 2010). Toxicity related to the immune system is relevant. Indeed, in the absence of acute effects and under conditions of chronic exposure to MPs, impairment of the immune system could have repercussions on the health status of organisms. Moreover, synergistic interaction with other immunosuppressive xenobiotics and stressors can occur. However, little is known about the potentially different effects of xenobiotics, and in particular of MPs, on the fish gut. In both tracts, the attention was focused on TLRs pathway-mediated immune activation, tight junction expression, and modification of the mucosal layer and submucosa thickness, which represent the targets of stressor and/or inflammatory insult after fish exposure to MPs.

It was also investigated the potential induction of oxidative damage to lipids and proteins in the gut of gilthead seabreams, measuring the production of ROS and MDA, as well as the levels of nitrosylated proteins. The modulation of gene expression of some antioxidant enzymes and heat shock proteins (HSPs) was also considered as further biomarkers of oxidative unbalance.

Finally, the effects of PS-MPs on hepatic homeostasis were evaluated. As reported above, the intestine and liver interact through a close bidirectional connection of the portal vein, bile duct, and systemic circulation. The gut-liver axis has recently been widely used in toxicology to analyze the connection between the gut and the liver. Specifically, it was evaluated the involvement of PS-MPs in the onset

and development of NAFLD. The impact on lipid metabolism was studied by examining the levels of key biomarkers involved in lipid synthesis and catabolism. Moreover, since inflammation and OS play a crucial role in the onset and progression of this disease, inflammatory cytokines levels, oxidative damage, and alterations of antioxidant and detoxifying systems were also analysed.

Histological evaluations were also performed to assess the intestine and liver health status and confirm the results obtained from molecular and biochemical analysis.

4.1. Selection of the experimental model: focus on gilthead seabream (Sparus aurata)

Gilthead seabream (*Sparus aurata* Linnaeus, 1758) is a carnivorous sparid widely distributed in the Atlantic European coasts from Portugal to the United Kingdom, including the Mediterranean and the Black Seas (Seginer, 2016). This teleost has a high commercial interest since it is the most farmed species in the Mediterranean Basin and the third in Europe, mainly in Southern Europe (Stankus, 2021). Gilthead seabream is a top predatory and voracious fish that can bioaccumulate and biomagnify chemical pollutants (Espinosa et al., 2017; Capó et al., 2022). Thus, it is considered a suitable sentinel species for ecotoxicological and bioaccumulation studies in the natural environment (Seginer, 2016), providing integrated and useful information on the types, concentrations, availability, and specific responses to diverse stressors, among which environmental pollutants. Moreover, gilthead seabream is a well-recognized bioindicator for

toxicity testing (Espinosa et al., 2017) and endocrine studies (Forner-Piquer et al., 2018). Gilthead seabream was chosen as experimental model also because its metabolism and responses to diets are well recognized, and it has ease of maintenance.

Fish bred in sea cages are exposed to the xenobiotics present in the aquatic environment but also to those potentially deriving from the equipment and facilities used in the aquaculture plant. Plastics have surrounded these fishes throughout their life cycle as most of the materials are plastic-derived (i.e., juvenile tanks, nets, ropes, pipes, buoys, and the parts that form the cages) (Zhu et al., 2019; Wu et al., 2020). Moreover, continuous care and cleaning to prevent fouling organisms on aquaculture equipment also contribute to releasing of MPs (fibers and particles) into the aquatic environment (ACTION, 2020). All features, taken together with the fact that the Mediterranean Sea is highly polluted by plastic debris (Cozar et al., 2015; Santini et al., 2022), explain the usefulness of studying the presence and the effects of these pollutants on farmed fish, such as gilthead seabreams. Thus, the use of the gilthead seabream allows not only for monitoring the state of environmental pollution by MPs but also for studying the potentially harmful effects of MPs on the species, widely eaten by humans. Finally, the effects found can help in the development of predictive data on the effects of MPs on human health.

4.2 Choice of MP polymer, size, and dose

PS was the first plastic designed and is a crucial material for modern plastic manufacturers (Alimba and Faggio, 2019). Indeed, PS

is one of the most widely used plastic polymers owing to its low cost, chemical stability, and properties. It is employed mainly in the production of scrubs, handwashing soaps, cleansers, toothpaste, biomedical products, as well as disposable products (Siddiqui et al., 2023). Consequently, the extensive use of PS is followed by its massive discard into the environment and the continuous exposure of living organisms. Indeed, PS is among the most abundant plastic polymers detected in biotic matrices (EFSA, 2016). Taking into account all these aspects, PS was selected for the present research.

Specifically, PS particles with a diameter between 1 and 20 μm were used for the experiment. The size range was chosen considering that the study aimed to investigate not only the direct impact on the intestine but also the effects deriving from PS-MP intestinal absorption as well as distribution in other tissues, such as the liver. Indeed, given the small dimension of used PS-MPs, these particles could be biologically available but also phagocytized or translocated across the gut barrier. Moreover, to evaluate the possible correlation of observed effects with different concentrations of PS-MPs in the diet, two different doses were used in the experiment: the lowest one of 25 mg/kg b.w./day and the highest one of 250 mg/kg b.w./day. These PS-MP doses were chosen based on the analysis of previous papers reporting similar doses, polymer type, and time of exposure in teleost fish (Asmonaite et al., 2018; Jovanovic et al., 2018).

5. Materials and methods

5.1 Dietary preparation

Three different treatment diets were formulated for the experiment: (I) the standard diet composed of pellets of commercial feed for aquaculture gilthead seabream; (II) the experimental diet constituted by pellets of commercial feed enriched with 0.5 % of hyperdispersed spherical PS-MPs particles in an odorless powder.; (III) the experimental diet constituted by pellets of commercial feed enriched with 5 % of the same hyperdispersed spherical PS-MPs (Fig. 5.1). The used PS particles were additive-free with a concentration of 100% and a diameter between 1 and 20 μm (microparticles GmbH, Berlin, Germany). The pellets constituting the standard and experimental diets had a 4–4.5 mm diameter and were made by the same feed company using a cold extrusion machine. (VRM S.r.l., NaturAlleva, Verona, Italy). The ingredient composition of the standard diet is reported in Table S1. The pellets were stored in airtight bags in a cool and dry place during the experimental time.

Fig. 5.1 – The standard and experimental diets used during the experiment.

5.2 In vivo experimental procedures

Eighty-one juvenile gilthead seabreams (172,6 g ± 4,036) were obtained from a local fish farm (Soc. Coop. Acquamarina, Villa Literno, Caserta, Italy). Fish were housed in three tanks of 1000 L and acclimated for 7 days to monitor their health status. Then, animals were weighted, randomly distributed into 6 fiberglass tanks of 250 L, and acclimated for another 14 days before the start of the experimental exposure. For each treatment, 27 animals from two different tanks were used collectively. The experimental groups were: (I) the control group fed on the standard diet (CON), (II) the experimental group fed on the diet containing the lowest PS-MPs dose (25 mg/kg b.w./day, PS1), and (III) the experimental group fed on the diet containing the highest PS-MPs dose (250 mg/kg b.w./day, PS2). The gilthead seabreams were following a rate of 1% body weight/tank/day. Fish were fed twice a day, once in the morning and once in the evening. The CON group received the standard diet both times, while the PS1 and PS2 groups received the diet containing PS-MPs in the morning and the standard diet in the evening. The choice to administer the contaminated diet in the morning ensured the consumption of the entire ration due to the ravenousness of the fish after the night fasting. The distribution of the feed ration to fish in each tank was gradually done and visually checked to ensure that all animals were able to gain access to the pellets, reducing the effects of feeding hierarchy and avoiding that some pellets were not ingested and lost.

During the experimental time, the use of plastic material was avoided and replaced by glass and metal tools to minimize the risk of contamination. Moreover, tanks were siphoned daily (changing around

10–15% of the water) immediately after the morning meal to remove feces or feed residue, which, in any case, was low because of the adopted food administration protocol. The exposure lasted 21 days, and at the end of the experimental time, fish were euthanatized by overexposure to tricaine methanesulfonate-MS222 (Sigma Aldrich, St. Louis, MO, USA). Then, biometric parameters, such as body and liver weight and total length, were recorded for each fish. Subsequently, fish were dissected, and the AI, PI, and liver were collected on ice and immediately stored at −80 °C until molecular and biochemical analysis were performed. In particular, the AI was identified as the tract just after the pyloric ceca, while the PI was considered as the portion just before the rectum. The experimental design is reported in Fig. 5.2.

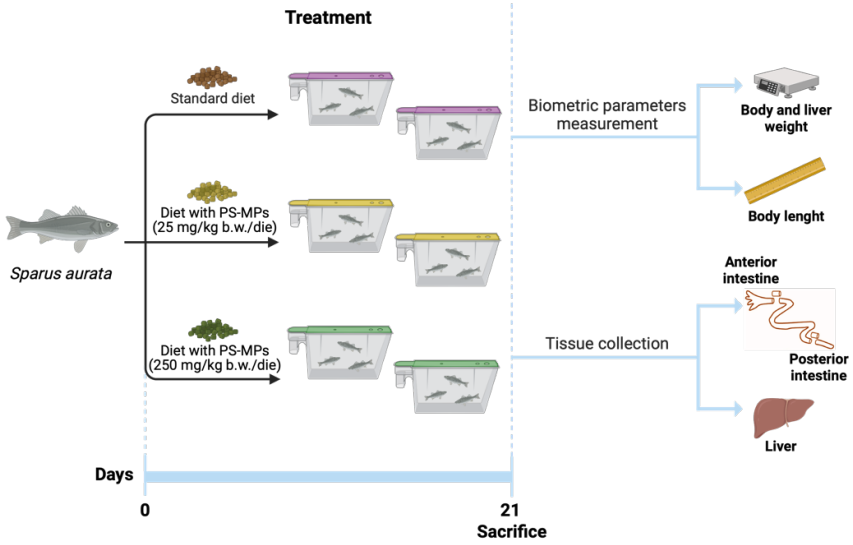

Fig. 5.2. **Experimental design.** Gilthead seabreams (*Sparus aurata*) were divided into three groups: (I) control group (CON) fed on a standard diet; (II) PS1 group fed on a diet containing the lowest PS-MP concentration (25 mg/kg b.w./day); and (III) PS2 group fed on a diet containing the highest PS-MP concentration (250 mg/kg b.w./day). The exposure started after an acclimation period of 14 days and lasted 21 days.

All experimental procedures involving fish were performed under license number 1057/2020-PR, released by the Italian Ministry of Health and approved by the Ethics Committee of the "Federico II" University of Naples (Italy). All procedures related to the handling of animals, treatment, euthanasia, and removal of organs were carried out in the indoor Recirculating Aquaculture System (RAS) at the Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Italy (Italian Ministry of Health authorization n. 25/2019-UT), following the guidelines of the European Communities Council (2010/63/UE). The system was provided with thermostatic control and regulation of water temperature, mechanical sand filter, skimmer, cartridge filters, biological filter, and UV lamp apparatus.

5.3 Biometric parameters

The body weight (g), the liver weight (g), and the total length (cm) were recorded for each animal. Specifically, total length was considered as the length from the front end of the snout to the rear end of the tail fin (Fig. 5.3).

Fig. 5.3 – Collection of biometric parameters at the end of experimental time.

The weight gain (WG) expressed as percentage (%) and the hepatosomatic index (HSI) were calculated adopting the following formula:

- WG% = [(final body weight − initial body weight) / initial body weight] x 100
- HSI = (liver weight in g / body weight in g) x 100

Moreover, Fulton's condition factor (CF) was estimated to evaluate the possible impact of PS-MPs on fish welfare and physical condition. This factor is widely used in fisheries and general fish biology studies. It is calculated from the relationship between the weight and length of a fish to describe the "condition" of that individual (Nash et al., 2006). Precisely, CF was calculated for each fish with the following formula:

- CF = [total weight in g / (total length in cm)^3] x 100

5.4 Intestinal evaluations

Histological analysis

5.4.1 Gut morphological studies

Samples of AI and PI (n=3) were collected and fixed in 10% neutral buffered formalin (code no. 05-01007Q, Bio-Optica, Milan, Italy). Then, samples were dehydrated, embedded in paraffin (code no. 06-7920, Bio-Optica, Milan, Italy), and cut into 4 µm thick sections before being stained with hematoxylin & eosin (H&E) for morphological evaluations (De Biase et al., 2020). Images were obtained by using an optical microscope (Nikon E600; Nikon, Tokyo, Japan). For each intestinal tract, the sections were histomorphometrically analyzed to

perform a semi-quantitative evaluation using a score system ranging from 1 to 5. Specifically, score 1 was given to the tissue with a normal appearance or minimal changes, and subsequent scores (up to 5) were given to tissues with increasing histomorphology alterations (Castro et al., 2019). The evaluated parameters were: (I) widening and shortening of intestinal folds; (II) increased cellularity of connective tissue and widening of lamina propria and submucosa; (III) leucocyte infiltration (namely intraepithelial lymphocytes and eosinophilic granular cells) in the lamina propria and submucosa. The overall score of histomorphology alterations was calculated by averaging scores of all evaluated criteria. Moreover, intestinal villi height was measured from the five highest intact and randomly selected intestinal villi and at least for fifteen sections of both intestinal tracts for each sample.

Alcian Blue-Periodic Acid Schiff's (AB-PAS) double staining was performed to determine the modifications of Muc layer. Goblet cells were identified and categorized through AB-PAS, which identifies neutral and acid (pH 2.5) Muc (Takeyama et al., 2000). Their number was calculated using a high-power field (HPF; 400x magnification; goblet cell numbers/HPF) (Kord et al., 2021).

Molecular analysis

5.4.2 Real Time semi-quantitative PCR analysis

Total RNA was extracted from AI and PI samples using TRIzol Reagent (Bio-Rad Laboratories, Hercules, CA, USA) and following the instructions of RNA extraction kit (NucleoSpin®, MACHEREY-NAGEL GmbH & Co, Düren, Germany). cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Bio-

systems, Foster City, CA, USA) from 4 µg total RNA. All Real-Time PCR analyses were performed with a Bio-Rad CFX96 Connect Real-Time PCR System instrument and software (Bio-Rad Laboratories, Hercules, CA, USA). The PCR conditions were 15 min at 95 °C followed by 40 cycles of two-step PCR denaturation at 94 °C for 15 s, annealing extension at 55-65 °C (depending on primer) for 30 s, and extension at 72 °C for 30 s. Each sample contained 500 ng cDNA in 2X QuantiTect SYBRGreen PCR Master Mix and primers pairs (IDT Technologies, Coralville, IA, USA) to a final volume of 50 µL. The relative amount of each studied mRNA was normalized to Rps18 as a housekeeping gene, and data were analyzed according to the $2^{-\Delta\Delta CT}$ method. Specific primers for target and housekeeping genes are listed in Table S2.

5.4.3 Western blot analysis

AI and PI samples were homogenized on ice in lysis buffer (0,3 M Mannitol, 0,2 mM EDTA, 10 mM HEPES buffer, 0,1 % Bovine Serum Albumin (BSA), 1mM phenylmethylsulfonyl fluoride, 1mM Na_3VO_4, 10 µg/mL leupeptin, and 10 µg/mL trypsin inhibitor). Cytosolic protein concentration was spectrophotometrically measured using BSA as standard in a Bradford reagent assay. 50 µg of proteins were subjected to SDS-PAGE, transferred to nitrocellulose membranes using a Bio-Rad Transblot Turbo (Bio-Rad Laboratories, Hercules, CA, USA), and probed at 4 °C overnight with the following primary antibodies: anti-Phospho-p38 mitogen-activated protein kinase (MAPK, 1:500, Cell Signaling Technology, Massachusetts, USA), anti-p38 MAPK (1:1000, Cell Signaling Technology, Massachusetts, USA), anti-Phospho-Extracellular signal-regulated kinase (ERK, 1:1000, Santa

Cruz Biotechnology, Inc., Santa Cruz, CA), anti-ERK (1:1000, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-nitrotyrosine (Nox-Tyr, 1:1000, Merck Millipore, Billerica, MA, USA). Western Blot for anti-GAPDH (1:8000, Sigma-Aldrich, Milan, Italy) was performed to ensure equal sample loading. Bands were detected by ChemiDoc Imaging System (Bio-Rad Laboratories, Hercules, CA, USA) and densitometrically analyzed with the Image Lab software (Bio-Rad Laboratories, Hercules, CA, USA).

Biochemical analysis

5.4.4 ROS assay

ROS levels were determined as previously reported by Pirozzi et al. (2016). Briefly, freshly prepared homogenates of AI and PI were diluted in 100 mM potassium phosphate buffer (pH 7.4) and incubated with a final concentration of 5 μM dichloro-fluorescein diacetate (Sigma-Aldrich, Milan, Italy). Samples were kept for 15 min at 37 °C and then centrifuged at 12,500 g per 10 min at 4 °C. The obtained pellet was suspended at ice-cold temperatures in 100 mM potassium phosphate buffer (pH 7.4) and then incubated for 60 min at 37 °C. The fluorescence measurement was performed with a GloMax® Explorer plate reader spectrofluorometer (Promega, Milan, Italy) at 488 nm and 525 nm for excitation and emission wavelengths, respectively. ROS were quantified from the dichloro-fluorescein standard curve in dimethyl sulfoxide (0-1 mM) and normalized on protein content of tissue homogenate.

5.4.5 MDA measurement

MDA was quantified as described by Pirozzi et al. (2016). Briefly, AI and PI were homogenized in 1.15% KCl solution and then added to a reaction mixture containing 8.1% SDS, 20% acetic acid (pH 3.5), 0.8% thiobarbituric acid, and distilled water. The supernatant absorbance was spectrophotometrically measured at 550 nm with a GloMax® Explorer plate reader spectrofluorometer (Promega, Milan, Italy). A standard curve was prepared using MDA bis (dimethyl acetal) as MDA source. The concentration of MDA in each sample was expressed as micromoles of MDA normalized on protein content of tissue homogenate.

5.5 Hepatic evaluations

Histological analysis

5.5.1 Liver morphological studies

Liver samples (n=5) were fixed in 10% neutral buffered formalin, dehydrated, and then embedded in paraffin. Subsequently, 4 µm sections were stained with H&E for morphological evaluations and with Periodic Acid-Schiff (PAS) (code no. 04-130802, Bio-Optica, Milan, Italy) for glycogen detection (Lu et al., 2013). Livers were subjected to histomorphometric analysis to perform semi-quantitative evaluations. Specifically, inflammation and necrosis were investigated by H&E stain and glycogen storage by PAS stain. Inflammation was scored as follows: <5% (score 0), 5-33% (score 1), >33-66% (score 2), >66% (score 3), based on the amount of inflammatory cells in the

parenchyma; necrosis was scored as absent (score 0) or present (score 1); glycogen vacuoles accumulation was classified as: absent (score 0), mild (score 1), moderate (score 2) and severe (score 3).

Other liver samples (n=5) were collected and snap-frozen in isopentane precooled in liquid nitrogen and then stored at -80 °C. Frozen transverse sections 8 µm thick were stained with Oil Red O (ORO) (# 04-220923, Bio Optica, Milan, Italy) to evaluate the accumulation of intracytoplasmic lipid vacuoles (Turola et al., 2015). In that case, the histomorphometric analysis assessed the lipid accumulation, and steatosis was classified as: absent (score 0), mild (score 1), moderate (score 2), or severe (score 3).

Molecular analysis

5.5.2 Real Time semi-quantitative PCR analysis

Total RNA was extracted from the liver using RNeasy Mini Kit (Qiagen, Hilden, Germany), and cDNA was obtained using the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA) from 1 µg total RNA. All Real-Time PCR analyses were performed with a Bio-Rad CFX96 Connect Real-Time PCR System instrument and software (Bio-Rad Laboratories, Hercules, CA, USA). The PCR conditions were 2 min at 95 °C followed by 40 cycles of two-step PCR denaturation at 95 °C for 5 s, annealing extension at 55–65 °C (depending on primer) for 30 s, finalizing in a melting curve (5 s at 95 °C and 5 s from 65 to 95 °C in 0.5 °C increments). Each sample contained 100 ng cDNA in 2x SYBR® Green Master Mix (Bio-Rad Laboratories, Hercules, CA, USA) and primers pairs (IDT Technologies, Coralville, IA, USA) to a final volume of 10 µL. The

relative amount of each studied mRNA was normalized to Rps18 as a housekeeping gene, and data were analyzed according to the $2^{-\Delta\Delta CT}$ method. Specific primers for target genes and housekeeping genes are listed in Table S2.

Biochemical analysis

5.5.3 Enzymatic activities

Liver samples were homogenized on ice using 0.1 M Na/K-phosphate and 0.15 M KCl buffer (pH 7.5). Then, they were centrifuged at 10000 g for 20 min at 4 °C, and the S9 fraction was collected and centrifuged again to obtain the microsomal and cytosolic fractions.

Catalase (CAT) activity was measured in the cytosolic fraction using hydrogen peroxide as a substrate, as previously reported by Aebi (1974). Briefly, a reaction mixture containing 50 mM K-phosphate buffer and 50 mM H_2O_2 diluted in 80 mM K-phosphate buffer (pH 6.5) was incubated at 25°C. After recording the baseline, the decrease in absorbance was spectrophotometrically measured at 240 nm for each sample.

Glutathione reductase (GR) activity was determined as described by Cribb et al. (1989). Samples cytosolic fraction was mixed to 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and NADPH. Then, the reaction was initiated by adding of 4 mM L-Glutathione oxidized, and absorbance was read at 415 nm.

GST activity was measured in the cytosolic fraction by using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate, as previously reported by Habig et al. (1974), with some modifications (Stephensen

et al., 2000). Specifically, samples were incubated with a reaction mixture containing 2 mM CDNB, 1 mM L-Glutathione reduced, and 0.1 M Na phosphate buffer (pH 7.5), and the absorbance was measured at 350 nm.

Ethoxyresorufin-O-deethylase (EROD) activity was determined in the microsomal fraction using rhodamine as standard and ethoxyresorufin as a substrate. NADPH was used to provide reducing equivalents and to start the reaction (Förlin et al., 1994). The fluorescence was measured at 530 and 590 nm for excitation and emission, respectively.

According to Lowry et al. (1951), total protein content was determined in cytosolic and microsomal fractions using BSA as a standard to normalize all the analyzed enzymatic activities.

5.5.4 Glutathione measurements

Total (tGSH) and oxidized glutathione (GSSG) levels were measured according to Sturve et al. (2017). Briefly, liver samples were homogenized in 5-sulfosalicylic acid dihydrate and left on ice for 15 min. After protein precipitation, the homogenates were centrifuged at 10000 g for 20 min at 4 °C, and the supernatant was collected. To measure tGSH quantity, the supernatant was added to a reaction mixture containing 143 mM sodium phosphate buffer with 6.3 mM EDTA (pH 7.4), 1 mM DTNB and 2 mM NADPH. Then, the reaction was started by adding 17I U/ml of GR and the absorbance for each sample was spectrophotometrically measured at 415 nm. Otherwise, for GSSG quantification, GSH was firstly derivatized with 2-vinylpyridine, and then the reaction was started as reported for tGSH. The amounts of tGSH and GSSG were normalized on the weight of

liver sample. Moreover, GSSG percentage was calculated for each sample with the following formula: (GSSG/tGSH) x 100.

5.5.5 ROS and MDA measurement

ROS levels were determined following the same protocol previously reported for the intestinal evaluations.

MDA quantity was determined by using the Lipid Peroxidation (MDA) Assay Kit (Sigma-Aldrich, Milan, Italy). Briefly, 10 mg of liver were homogenized using MDA lysis buffer and centrifugated at 13,000 g for 10 min at 4 °C to remove insoluble material. Then, the supernatant was collected and added to the thiobarbituric acid solution. After the incubation at 95 °C for 60 min, the absorbance was spectrophotometrically measured at 532 nm. MDA was quantified from linear regression analysis of the MDA standard curve (0-20 nmole). Values were expressed as nmole MDA/ml of tissue homogenate.

5.5.6 Quantification of protein carbonyls

Levels of protein carbonyls were measured using a colorimetric assay, as described by Levine et al. (1990). Liver was homogenized in 50 mM sodium phosphate buffer (pH 7.4) containing 1 mM EDTA, 0.1% digitonin, and antiproteases cocktail. Then, proteins were derivatized with 10 mM 2,4-Dinitrophenylhydrazine (DNPH) in 2M HCl, precipitated with 20% trichloroacetic acid, and rinsed with a 1:1 ethanol-ethyl acetate solution. Thus, the obtained pellet was dissolved in 20 mM potassium phosphate buffer (pH 2.3) containing 6 M guanidine hydrochloride and stored at 4 °C overnight. Thereafter, samples were incubated 60 min at 37 °C, gently vortexed, and

centrifugated at 3000 g for 5 min at 20 °C to remove every insoluble material. The absorbance was spectrophotometrically measured at 360 nm and the obtained results were normalized on mg of protein determined according to Lowry et al. (1951).

5.6 Data and statistical analysis

Data are presented as mean ± SEM. All experiments were analyzed using analysis of variance (ANOVA) for multiple comparisons, followed by Bonferroni's post hoc test, using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Normality was tested using the Shapiro-Wilk test, and the Brown–Forsythe's test was used to evaluate whether the variances across groups were homogeneous. Statistical significance was set at $p < 0.05$.

6. Results

6.1 Effects of PS-MPs on growth and physical conditions of S. aurata

Over the entire experimental time, no mortality or evidence of suffering occurred in fish of each treatment group. Formulated diets were well accepted, and fish of all experimental groups properly grew throughout 21 days of exposure. The subchronic exposure to PS-MPs had no significant effects on fish weight gain (WG%: 13,79 ± 2,281 for CON; 16,69± 1,721 for PS1; and 15,59± 1,897 for PS2) the wellbeing and physical conditions (CF: 1,668 ± 0,037 CON; 1,578 ± 0,032 PS1; and 1,659 ± 0,035 PS2). Likewise, HSI did not vary among the experimental groups (HSI: 2,054 ± 0,087 for CON group; 2,258 ± 0,112 for PS1; 1,918 ± 0,052 for PS2).

6.2 Impact of PS-MPs on gut architecture and physiology

6.2.1 PS-MPs induce intestinal histological alterations

Fish fed on the CON diet did not show pathological changes in AI and PI (Fig. 6.2.1.1 A and D). PS1 and PS2 groups showed morphological differences compared to the CON group, without significant differences between them (Fig. 6.2.1.1 B, C, E, F). Intestinal folds were shortened and appeared multifocally blunted and necrotic at both doses. Specifically, for PS1 group, the mean score for intestinal folds shortening and widening was 1,3 ± 0,09 for AI and 2,3 ± 0,11 for PI. As regards PS2 group, the mean score was 1,7 ± 0,12 for AI and 2,6 ± 0,09 for PI. Moreover, in both intestinal portions, lamina

propria and submucosa were diffusely expanded by mild to moderate leukocyte infiltration in PS1 and PS2 groups, respectively. The mean score for lamina propria and submucosa widening in AI were 1,3 ± 0,09 in PS1 group and 1,6 ± 0,13 in PS2 group; regarding the PI, the mean score for the same parameter were 1,8 ± 0,14 and 2,1 ± 0,14 in PS1 and PS2 group, respectively. Finally, the inflammation and cellular infiltration score were 1,8 ± 0,13 for AI and 2,3 ± 0,12 for PI in PS1 group; 2 ± 0,16 for AI and 2,6±0,09 for PI in PS2. Considering the above data, it follows that the severity of damage was significantly higher in PI than in AI for all evaluated parameters (Fig. 6.2.1.1 A–F). Finally, the height of intestinal villi was reduced in a dose-dependent manner by PS-MPs in both intestinal tracts (Fig. 6.2.1.1 G).

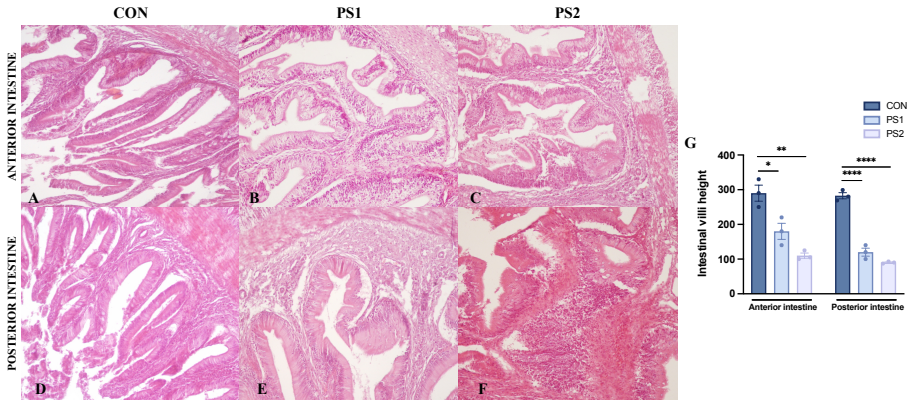

Fig. 6.2.1.1. Impact of PS-MPs on AI and PI morphology in *S. aurata*. Representative H&E stained sections from AI and PI portions of gilthead seabream (original magnification, 20×). (A) and (D) CON group without relevant pathological lesions, (B) and (E) PS1 group, (C) and (F) PS2 group, (G) average value of intestinal villi height (μm) (n= 3 for each group). All data are shown as mean ± S.E.M. *p < 0.05, **p < 0.01, **** p < 0.0001.

The presence of intestinal goblet cells containing acid Muc was determined by AB-PAS double staining (Fig. 6.2.1.2 A-F). The average number of intestinal goblet cells was reduced by PS in a dose-dependent manner in both AI and PI of exposed fish (Fig. 6.2.1.2 G).

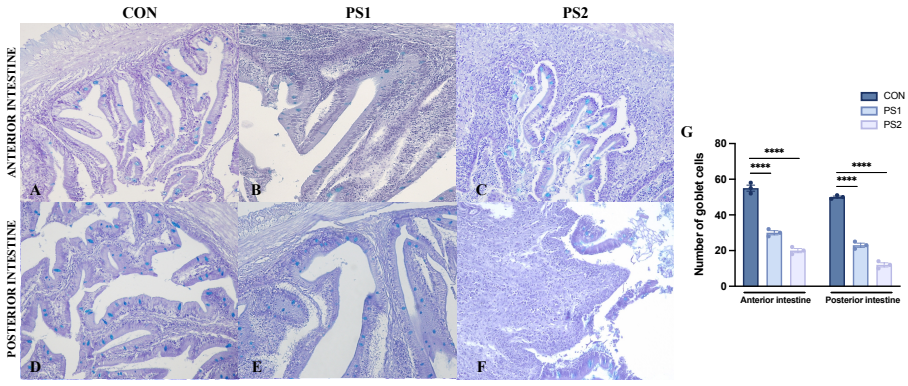

Fig. 6.2.1.2. Impact of PS-MPs on mucin layer in AI and PI of *S. aurata*. Representative AB-PAS stained sections from AI and PI tissues of gilthead seabream (original magnification, 20×). (A) and (D) CON group, (B) and (E) PS1 group, (C) and (F) PS2 group, (G) the average number of goblet cells (n= 3 for each group). All data are shown as mean ± S.E.M. **** $p < 0.0001$.

6.2.2 PS-MPs induce the activation of immune response and inflammation in the gut

The signaling pathway of TLRs-Myeloid differentiation primary response 88 (MyD88) was impacted by PS-MPs in both AI and PI portions. Specifically, *TLR2* levels were increased at the highest PS-MP dose in AI but were not modified in PI (Fig. 6.2.2.1 A). Meanwhile, *TLR5* was increased by both PS-MP doses in AI and only by PS1 in PI (Fig. 6.2.2.1 B). Moreover, the main downstream molecule of TLRs, *MyD88*, was augmented for PS2 group in AI, and for both PS1 and PS2 groups, in a dose-dependent manner, in PI (Fig. 6.2.2.1 C). The levels

of *Lys*, well recognized as an important defense molecule of innate immune system in *S. aurata*, were increased for PS1 in AI and PS2 in PI (Fig. 6.2.2.1 D). Contextually, *CSF1R* was raised by the highest PS dose in AI and by both doses in PI (Fig. 6.2.2.1 E). Finally, the levels of *ALP*, a constituent enzyme of the nonspecific immune system in fish, were augmented for PS2 in AI, and for both experimental groups in PI (Fig. 6.2.2.1 F).

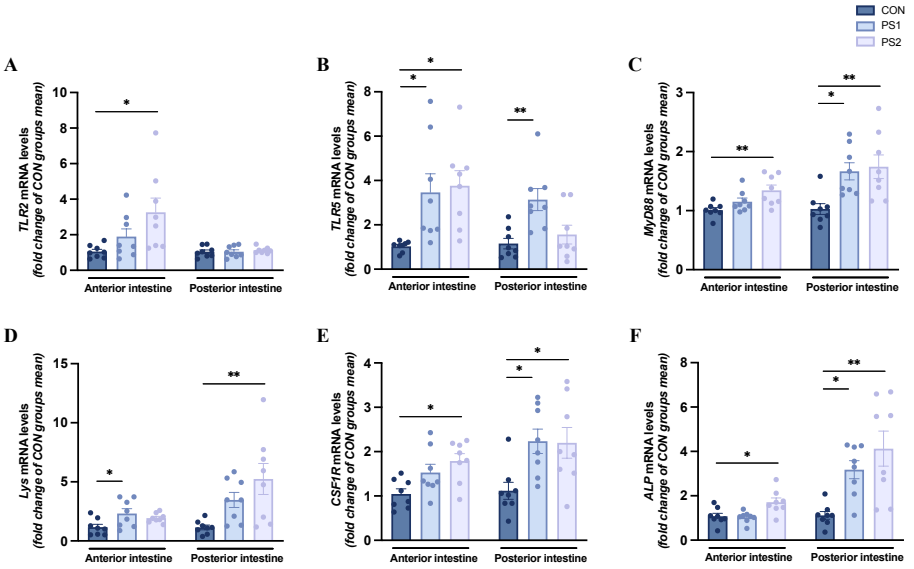

Fig. 6.2.2.1. Impact of PS-MPs on the innate immune response in the AI and PI of *S. aurata*. The mRNA levels of *TLR2* (A), *TLR5* (B), *MyD88* (C), *Lys* (D), *CSF1R* (E) and *ALP* (F) in the AI and PI of gilthead seabreams (n= 8 for each experimental group). All data are shown as mean ± S.E.M. *$p < 0.05$, **$p < 0.01$.

Overall, PS-MP exposure modified the gene expression of pro- and anti-inflammatory cytokines in both intestinal tracts. As regards AI, it was observed the increase of *TNF-α*, *IL-6*, and *IL-1β* levels for PS2 group (Fig. 6.2.2.2 A–C), while *COX-2* levels were increased by both PS-MP doses (Fig. 6.2.2.2 D). *IL-10* was not modified among the

groups (Fig. 6.2.2.2 E), and *TGF-β1* was increased by PS2 (Fig. 6.2.2.2 F). At the same time, while *TNF-α* levels in PI were not changed by PS-MPs, a significant increase was evidenced at both doses in gene expression of pro-inflammatory cytokines *IL-6* and *IL-1β* (Fig. 6.2.2.2 A–C) and *COX-2* (Fig. 6.2.2.2 D). However, the levels of anti-inflammatory cytokine *IL-10* were reduced by PS2 (Fig. 6.2.2.2 E), while *TGF-β1* did not differ among the groups (Fig. 6.2.2.2 F).

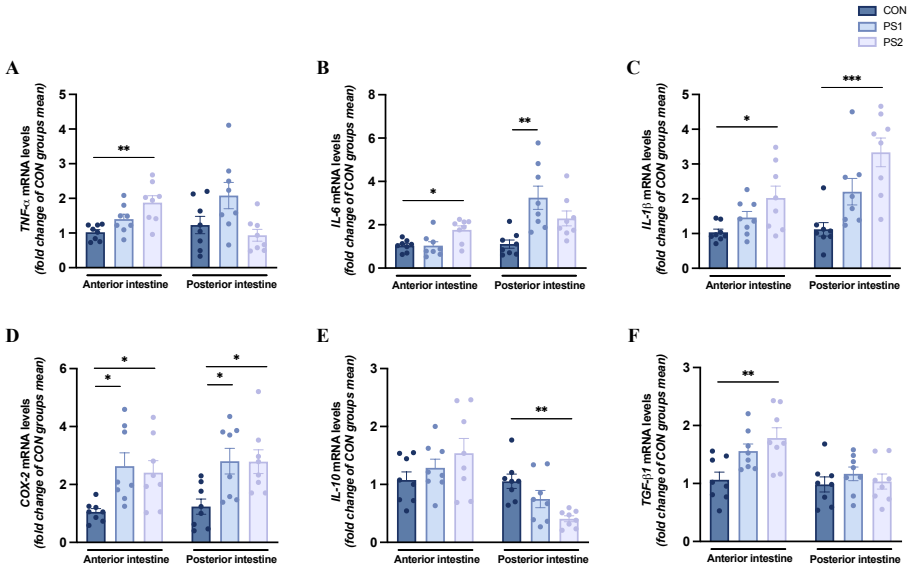

Fig. 6.2.2.2. Impact of PS-MPs on pro- and anti-inflammatory cytokines in the AI and PI of *S. aurata*. The mRNA levels of *TNF-α* (A), *IL-6* (B), *IL-1β* (C), *COX-2* (D), *IL-10* (E), *TGF-β1* (F) in the AI and PI of all experimental groups (n= 8 for each group). All data are shown as mean ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001.

6.2.3 PS-MPs alter intestinal biomarkers of oxidative and nitrosative stress

The exposure to PS-MPs caused an increase in oxidative and nitrosative stress biomarkers in AI and PI of *S. aurata*. In AI, ROS production was increased in PS2 group (Fig. 6.2.3.1 A), without modifications in MDA production (Fig. 6.2.3.1 B). On the contrary, ROS and MDA levels in PI increased at the highest PS-MP dose (Fig. 6.2.3.1 A and B). However, higher protein nitrosylation levels (Nox-Tyr) were revealed at the highest dose in AI and at the lowest dose in PI (Fig. 6.2.3.1 C and D), suggesting a PS-MP induced nitrosative stress.

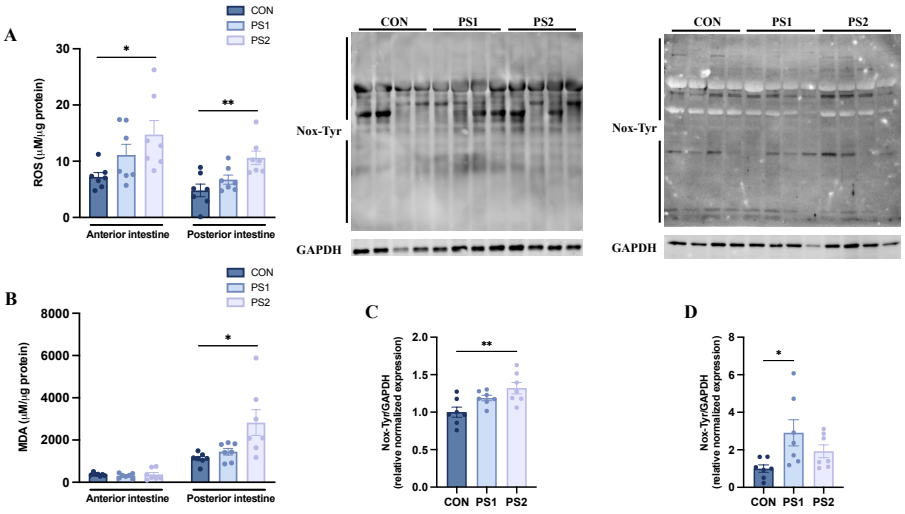

Fig. 6.2.3.1.1 Impact of PS-MPs on the oxidative and nitrosative stress in AI and PI of *S. aurata*. Levels of ROS (A) and MDA (B) in the AI and PI of gilthead seabream (n= 7 for each group). Western blot analysis for Nox-Tyr in AI (C) and PI (D) of all experimental groups (n= 7 for each group). All data are shown as mean ± S.E.M. *$p < 0.05$, **$p < 0.01$.

6.2.4 PS-MPs modify intestinal antioxidant and repair systems

Antioxidant enzymes and chaperones were impacted by PS-MP exposure in AI and PI of *S. aurata*. In particular, the *CuZn/SOD* gene expression was increased, and *CAT* was decreased at the highest PS-MP dose in AI (Fig. 6.2.4.1 A and B), whereas *GR* was not modified (Fig. 6.2.4.1 C). Similarly, no change was revealed for *Nrf2*, *hsp70*, and *hsp90* gene levels in AI (Fig. 6.2.4.1 D-F). However, *CuZn/SOD* and *GR* were not impacted by PS-MPs in PI (Fig. 6.2.4.1 A and C), while *CAT* level decreased in PS2 group (Fig. 6.2.4.1 B). Moreover, also *Nrf2* level was reduced in PS2 group (Fig. 6.2.4.1 D). *hsp70* and *hsp90* levels increased at the lowest PS-MP dose and both doses in PI, respectively (Fig. 6.2.4.1 E and F).

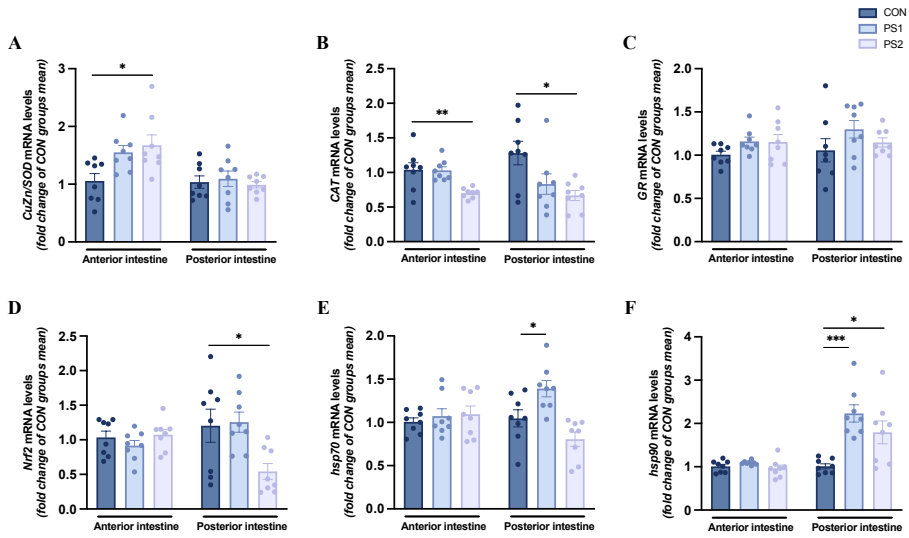

Fig. 6.2.4.1. Impact of PS-MPs on the antioxidant defense system in AI and PI of *S. aurata*. The mRNA levels of *CuZn/SOD* (A), *CAT* (B), *GR* (C), *Nrf2* (D), *hsp70* (E), *hsp90* (F) in the AI and PI of all experimental groups (n= 8 for each group). All data are shown as mean ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001.

6.2.5 PS-MPs modulate MAPK signaling pathway in the intestine

The activation of the MAPK pathway was investigated by evaluating ERK and P38 phosphorylation levels after exposure to PS-MPs. In AI, this pathway was not affected by PS-MP exposure since the phosphorylation levels of ERK and P38 were comparable among the experimental groups (Fig. 6.2.5.1 A and C). Interestingly, PS-MP exposure differentially impacted on PI. Indeed, there was an increase of p-ERK and p-P38 at both doses and at highest dose, respectively, in this portion (Fig. 6.2.5.1 B and D).

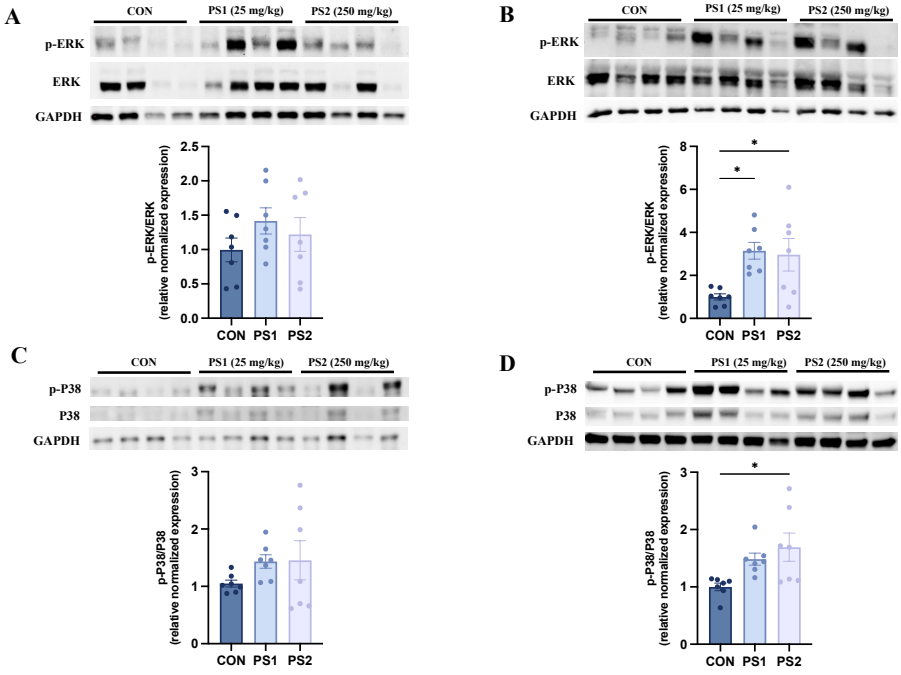

Fig. 6.2.5.1. Impact of PS-MPs on the phosphorylation levels of ERK and P38 in AI and PI of *S. aurata*. Western blot analysis for p-ERK (A, B) and p-P38 (C, D) in the AI and PI of fish (n= 7 for each treatment group). All data are shown as mean ± S.E.M. *p < 0.05.

6.2.6 PS-MPs disrupt the intestinal barrier integrity

PS-MPs differently impacted the barrier integrity in AI and PI. Specifically, the gene expression levels of TJs, namely *ZO-1*, *occludin*, *cldn15*, *tricellulin*, and *Itgb6*, were not modified in AI after PS treatment (Fig. 6.2.6.1 A-E). Similarly, *Muc2-like* and *Muc13-like* did not vary (Fig. 6.2.6.1 F and G). Instead, in PI, PS-MP exposure decreased, in a dose-dependent manner, the mRNA expression of *occludin*, *cldn15*, and *tricellulin* (Fig. 6.2.6.1 B, C and D), and that of *ZO-1* and *Itgb6* at the highest dose (Fig. 6.2.6.1 A and E). Moreover, TJs reduction in PI was accompanied by a dose-dependent decrease of *Muc2-like* and *Muc13-like* (Fig. 6.2.6.1 F and G).

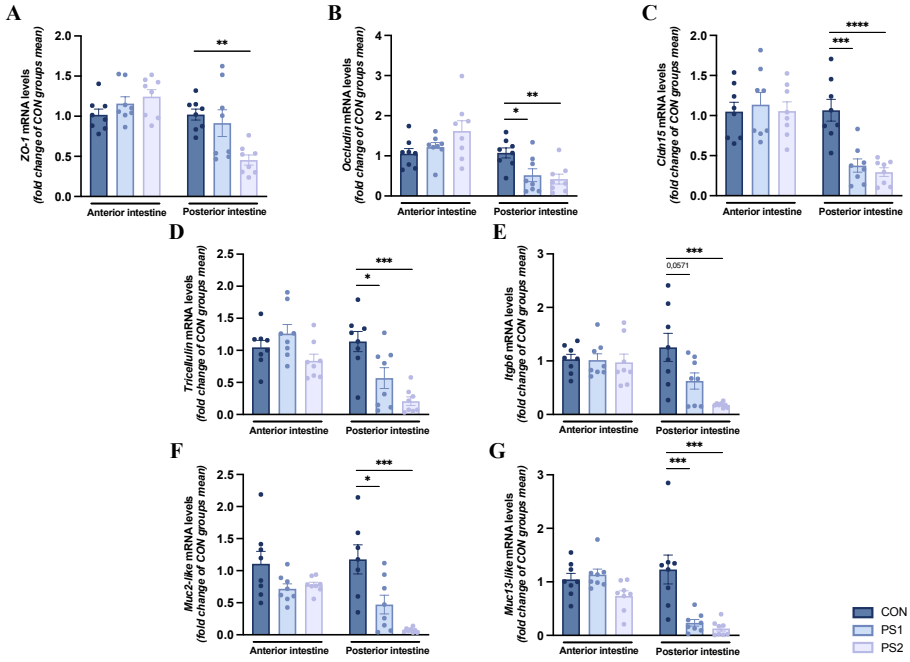

Fig. 6.2.6.1. Impact of PS-MPs on tight junctions and mucins in AI and PI of *S. aurata*. The mRNA levels of *ZO-1* (A), *Occludin* (B), *Cldn15* (C), *Tricellulin* (D), *Itgb6* (E), *Muc2-like* (F), *Muc13-like* (G) of all experimental groups (n= 8 for each group). All data are shown as mean ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

6.3 Effects of PS-MP exposure on liver metabolism and function

6.3.1 PS-MPs affect hepatic glycolipid metabolism

For the CON group, PAS staining didn't detect intracellular glycogen storage (Fig. 6.3.1.1 A and D), and ORO staining revealed moderate accumulation of lipid vacuoles (Fig. 6.3.1.1 E and H). PS1 group showed mild to moderate accumulation of intracellular glycogen (Fig. 6.3.1.1 B and D), and moderate accumulation of intracellular lipid vacuoles, with the nucleus partially located at the periphery (Fig. 6.3.1.1 F and H). Instead, PS2 group exhibited a moderate to severe accumulation of intracellular glycogen (Fig. 6.3.1.1 C and D), and a moderate to severe accumulation of lipid vacuoles with nucleus partially or completely located at the periphery (Fig. 6.3.1.1 G and H).

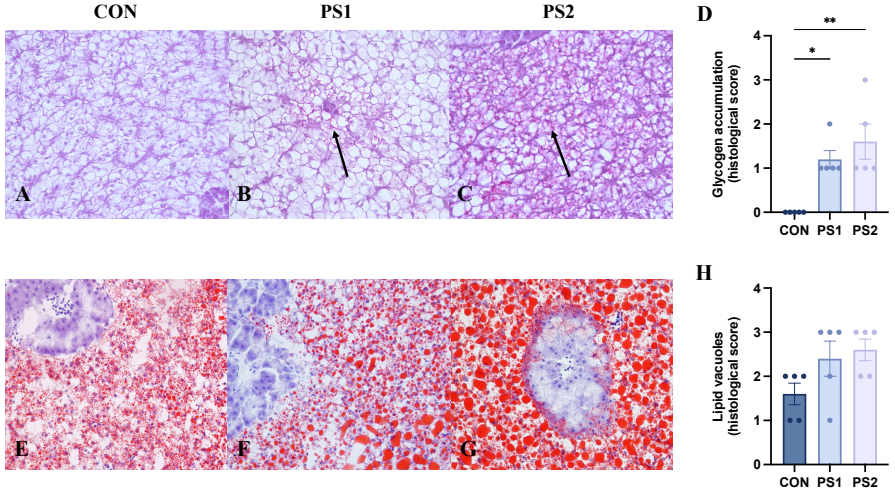

Fig. 6.3.1.1. Impact of PS-MPs on the accumulation of glycogen and lipid vacuoles in the liver of *S. aurata*. Representative PAS-stained sections from liver tissues of gilthead seabream (original magnification, 40×). CON group (A), PS1 group (B), PS2 group (C), histological score of glycogen accumulation (D) (n= 5 for each group). Representative ORO-stained sections from liver tissues of gilthead

seabream (original magnification, 40×). CON group (E), PS1 group (F), PS2 group (G), histological score of lipid vacuoles (H) (n= 5 for each group). All data are shown as mean ± S.E.M. *p < 0.05, **p < 0.01.

Contextually, PS-MPs impacted the gene expression of markers involved in lipid metabolism. Increased mRNA levels of genes related to lipid synthesis (i.e., *PPARγ*, *Srebp1*, and *Fasn*) were revealed in PS2 group (Fig. 6.3.1.2 A-C). However, PS-MPs did not modify the hepatic *Fabp1* gene expression (Fig. 6.3.1.2 D). Moreover, both PS doses raised *Lpl* levels (Fig. 6.3.1.2 E), but the mRNA expression of genes related to lipid catabolism (i.e., *PPARα*, *HL*, and *Pla2*) was not modified among the groups (Fig. 6.3.1.2 F-H).

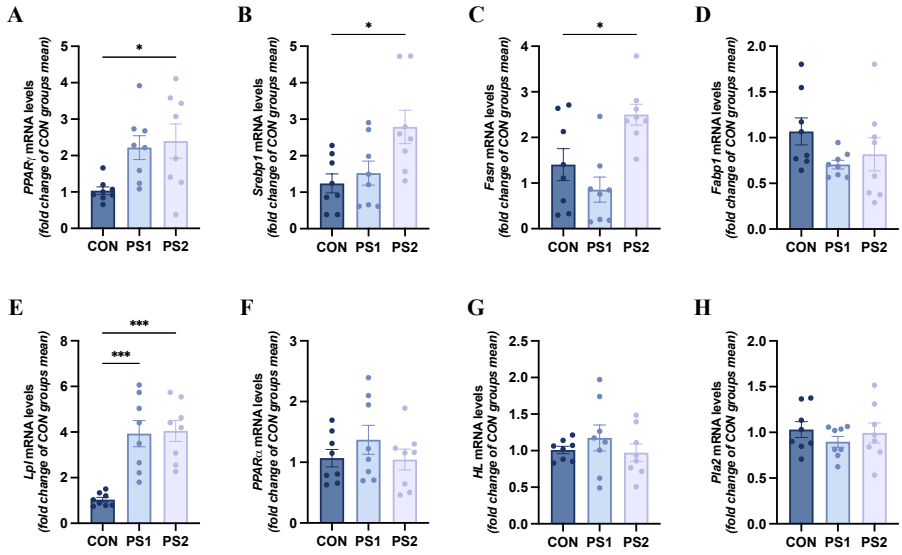

Fig. 6.3.1.2. Impact of PS-MPs on lipid metabolism-related genes in the liver of *S. aurata*. The hepatic mRNA levels of *PPARγ* (A), *Srebp1* (B), *Fasn* (C), *Fabp1* (D), *Lpl* (E), *PPARα* (F), *HL* (G), *Pla2* (H) in the liver of all experimental groups (n= 8 for each group). All data are shown as mean ± S.E.M. *p < 0.05, ***p < 0.001.

6.3.2 PS-MPs activate immune response in the liver

The hepatic immune response was activated by PS-MPs. Specifically, the TLRs-MyD88 signaling pathway was modified after the exposure. PS-MPs increased *TLR2* and *TLR5* gene levels in a dose-dependent manner (Fig. 6.3.2.1 A and B). However, *MyD88* gene expression was not modified among the groups (Fig. 6.3.2.1 C). Regarding *CSF1R*, it was higher than CON in both exposed groups and most significant at the lowest PS-MP dose (Fig. 6.3.2.1 D).

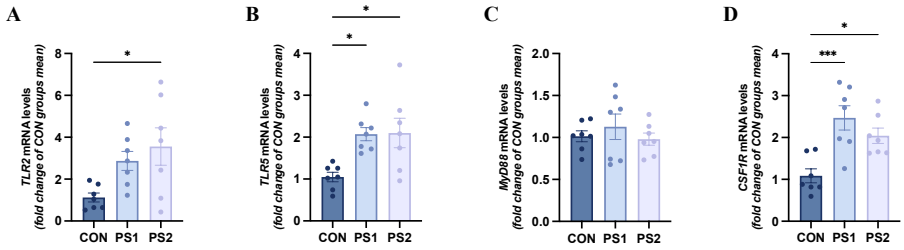

Fig. 6.3.2.1. Impact of PS-MPs on hepatic immune mediators in *S. aurata*. The mRNA levels of *TLR2* (A), *TLR5* (B), *MyD88* (C), *CSF1R* (D) in the liver of all fish groups (n= 7 for each group). All data are shown as mean ± S.E.M. *p < 0.05, ***p < 0.001.

6.3.3 PS-MPs increase levels of hepatic inflammatory mediators

Histological examination with H&E staining evidenced scarce inflammation and absence of necrosis in the fish liver of CON group (Fig. 6.3.3.1 A, D, and E). PS1 showed mild to moderate inflammation, characterized by perivascular lymphocytes and plasma cells inflammatory infiltrate, and presence of multifocal areas of coagulative necrosis (Fig. 6.3.3.1 B, D, and E). PS2 group exhibited a moderate to severe degree of inflammation, mostly defined by a cell infiltrate

consisting of peripancreatic lymphocytes and plasma cells, with the presence of multifocal to coalescing areas of necrosis (Fig. 6.3.3.1 C-E). Moreover, it has been observed severe hepatic congestion in PS2 group (Fig. 6.3.3.1 C-E).

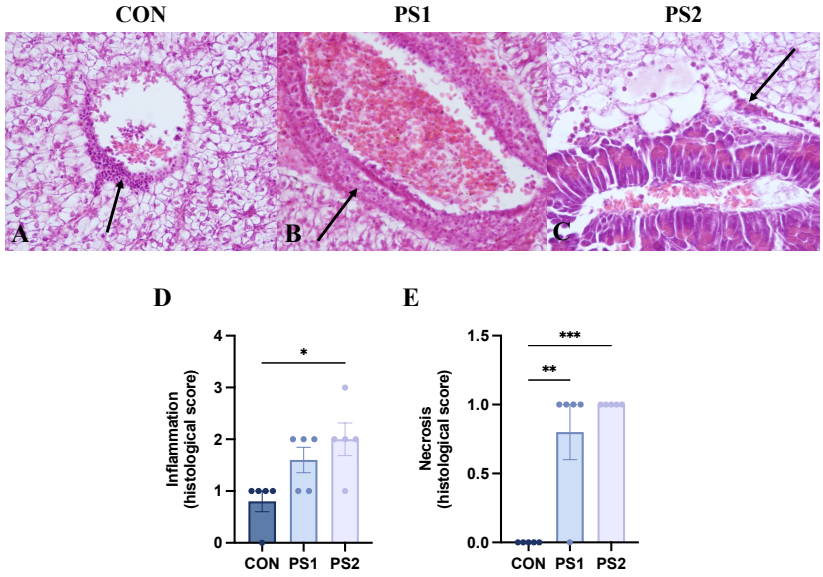

Fig. 6.3.3.1. Impact of PS-MPs on hepatic morphology in *S. aurata*. Representative H&E-stained sections from liver of gilthead seabream (original magnification, 40×). CON group (A), PS1 group (B), PS2 group (C), histological score of inflammation (D), histological score of necrosis (E) (n= 5 for each group). All data are shown as mean ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001.

The mRNA levels of hepatic inflammatory mediators and cytokines were impacted by PS-MP exposure. *TNF-α* and *COX-2* were significantly increased in fish exposed to the highest PS-MP dose (Fig. 6.3.3.2 A and D), while *IL-1β* was augmented in both exposed groups (Fig. 6.3.3.2 B). On the contrary, *IL-6* levels were not significantly modified by PS-MPs (Fig. 6.3.3.2 C). Moreover, the anti-inflammatory cytokine *TGF-β1* gene expression was raised in PS1-exposed group (Fig. 6.3.3.2 E).

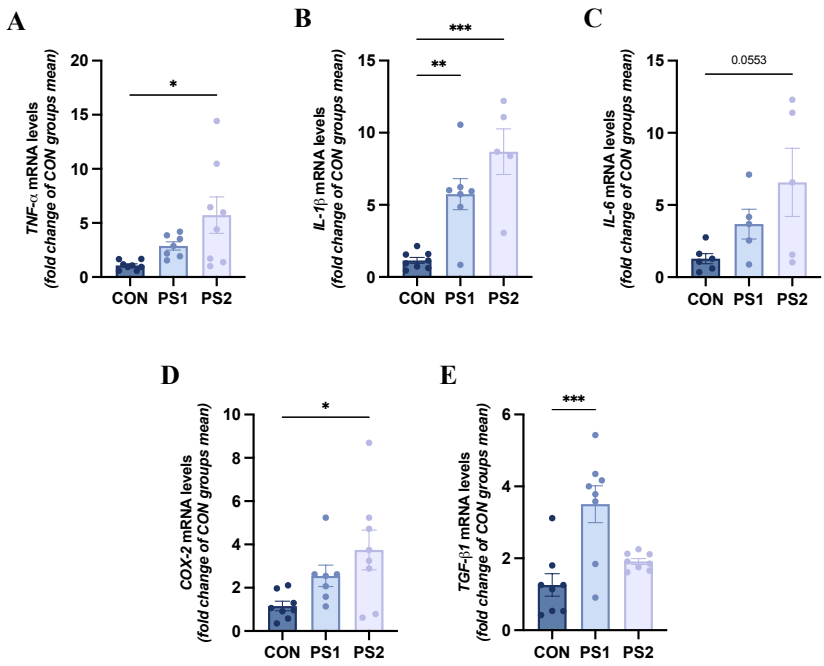

Fig. 6.3.3.2. Impact of PS-MPs on hepatic inflammation in *S. aurata*. The mRNA levels of *TNF-α* (A), *IL-1β* (B), *IL-6* (C), *COX-2* (D), *TGF-β1* (E) in the liver of all experimental groups (n= at least 5 for each treatment). All data are shown as mean ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001.

6.3.4 PS-MPs impair hepatic antioxidant and detoxifying systems

Hepatic defense systems were impacted by PS-MP. As regards the enzymatic antioxidant system, *CuZn/SOD* and *CAT* gene expression were increased in PS2 group (Fig. 6.3.4.1 A and B), while CAT activity remained unchanged (Fig. 6.3.4.1 C). Otherwise, *GR* gene expression was not modified by PS-MPs, but its activity decreased at the highest PS dose (Fig. 6.3.4.1 D and E).

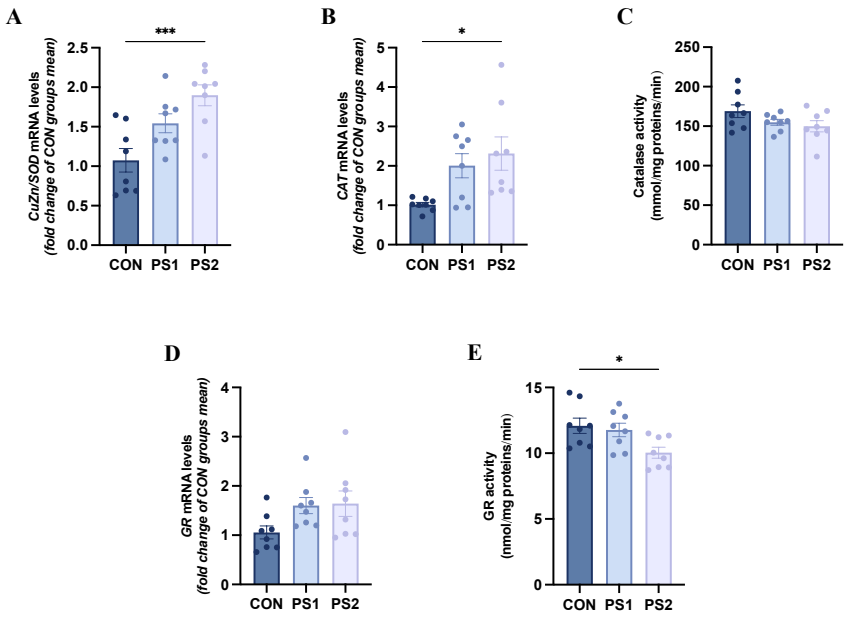

Fig. 6.3.4.1 Impact of PS-MPs on antioxidant enzymes in the liver of *S. aurata*. The mRNA levels of *CuZn/SOD* (A), *CAT* (B), *GR* (D) in the liver of all treatment groups (n= 8 for each group). The enzymatic activity of catalase (C), and GR (E) in the liver of CON, PS1 and PS2 groups (n= 8 for each group). All data are shown as mean ± S.E.M. *$p < 0.05$, ***$p < 0.001$.

The impairment of hepatic antioxidant defense was also evidenced by the altered content of GSH. Specifically, PS-MPs did not modify tGSH levels (Fig. 6.3.4.2 A) but increased the GSSG content in a dose-dependent manner (Fig. 6.3.4.2 B). Thus, the GSSG percentage was augmented in both PS1 and PS2 groups (Fig. 6.3.4.2 C).

At the same time, PS-MPs impacted the activity of detoxifying enzymes. Indeed, the highest PS dose increased the GST activity (Fig. 6.3.4.2 D) and decreased the EROD activity (Fig. 6.3.4.2 E), suggesting a reduced induction of CYP1A.

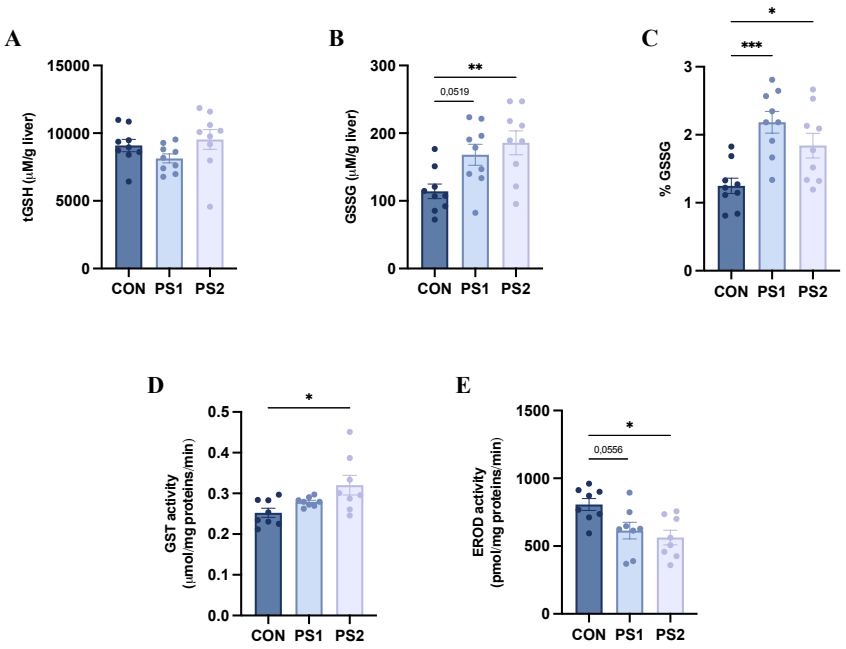

Fig. 6.3.4.2. Impact of PS-MPs on glutathione content and detoxifying enzymes in the liver of *S. aurata*. Hepatic levels of tGSH (A) and GSSG (B), and GSSG percentage (C) in gilthead seabreams (n= 9 for each group). Enzymatic activity of GST (D), and EROD (E) in the liver (n= 8 for each experimental group). All data are shown as mean ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001.

6.3.5 PS-MPs cause hepatic oxidative stress

PS-MP doses differently impacted OS biomarkers in the liver. Indeed, ROS and MDA amounts increased at the lowest and the highest PS dose, respectively (Fig. 6.3.5.1 A and B).

However, no effect on protein carbonyl levels was evidenced in both PS1 and PS2 groups (Fig. 6.3.5.1 C).

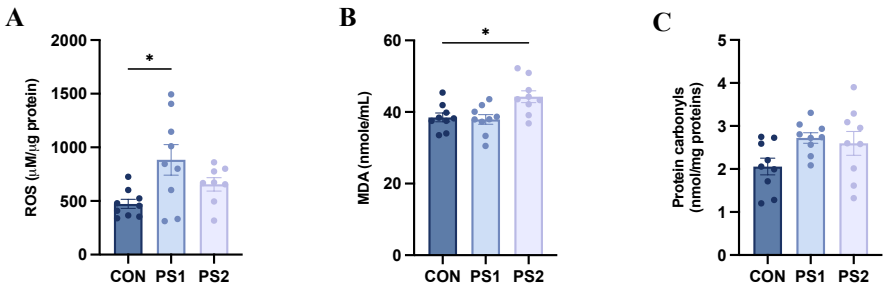

Fig. 6.3.5.1. Impact of PS-MPs on oxidative biomarkers in the liver of *S. aurata*. Hepatic levels of ROS (A), MDA (B), and protein carbonyls (C) in all experimental groups (n= at least 8 for each group). All data are shown as mean ± S.E.M. *p < 0.05.

7. Discussion

7.1 Gut homeostasis is differently affected by PS-MP exposure in the anterior and posterior portions

During the last few years, a growing body of evidence suggested that ingestion is the most relevant uptake route of MPs for aquatic biota, and therefore, MPs first impact the gut after their ingestion and accumulate. The GI tract is a multifunctional and key organ to ensure fish wellness and health status since it has an essential role in osmoregulation and absorption of nutrients/fluids; it is also the largest interface between the organism and the external environmental stressors (Cheng et al., 2010; Sundell and Sundh, 2012). Therefore, intestinal damage and loss of epithelial integrity can lead to nutrient malabsorption and systemic organ failure in fish (Sitja-Bobadilla et al., 2019) as well as in other living beings.

During the first part of PhD programme, research activity focused on the evaluation of the harmful effects induced by PS-MPs on the health status and gut homeostasis of gilthead seabream, used as experimental model.

The 21 days of dietary exposure of gilthead seabreams to 1-20 μm PS-MPs (25 or 250 mg/kg/day) did not affect their growth and physical conditions, revealing a lack of effects on fish wellness. These results are consistent with those previously reported by other authors after the exposure of fish to plastic polymers of several types and sizes under different experimental conditions (Espinosa et al., 2017; Solomando et al., 2020; Alomar et al., 2021; Jacob et al., 2021; Montero et al., 2022).

However, although PS-MPs did not affect fish growth performance, it was revealed an impairment of intestinal homeostasis

that was of different entities in the considered intestinal portions. Gilthead seabream is a carnivorous teleost, and it has a short intestine that can be divided into two main parts with different morphology, physiological function, and diameter that gradually decrease from the AI to the PI. Thus, the AI and PI could differently react to external stressors, such as environmental pollutants. Specifically, the AI is mainly involved in the absorption of lipids and proteins, while the PI in that of macromolecular proteins. Moreover, PI is considered the main site where antigen uptake occurs and triggers the immune response (Cain and Swan, 2010). In fact, the expression of many biomarkers highly involved in the innate immune response is higher in PI than in AI of gilthead seabream. Among these, there are many pattern recognition receptors (PRR), including TLRs, Nucleotide Oligomerization Domain-Containing Proteins (NODs), lectins, and some cytokines (Perez-Sanchez et al., 2015). However, the impact of MPs on different intestinal tracts is still poorly investigated.

Here, the effect of PS-MPs on the innate immune system was evaluated, highlighting the modulation of TLRs-MyD88 signaling pathway in both AI and PI. Specifically, PS-MPs increased the gene expression of TLR2 and TLR5, which are among the main TLR subtypes with corresponding ligands in fish, and they have a key role in the innate and adaptive immune system (Zhang et al., 2014). Similarly, Huang et al. (2020) showed increased levels of TLR4 in the gut of juvenile guppy (*Poecilia reticulata*) after exposure to PS-MPs. The downstream effects of TLRs are mediated by Myd88, a cytosolic adaptor protein that converts extracellular signals to intracellular activity, leading to the direct elimination of the invading pathogens and

the secretion of pro-inflammatory cytokines (Bates et al., 2007). Here, PS-MPs increased the gene expression of Myd88 in AI and PI in a dose-dependent manner. Additionally, PS-MPs modulated the expression of other immune-associated genes, as shown by the increased levels of Lys, receptor of the colony-stimulating factor-1 (CSF1R), and ALP in both intestinal portions.

As mentioned above, the activation of immune-related signaling pathways is strictly linked to the release of pro-inflammatory cytokines. A rise of TNF-α, IL-6, and IL-1β was evidenced in AI of PS2 group, and an increase of IL-6 and IL-1β in PI of PS1 and PS2 groups, respectively. Moreover, PS-MPs augmented the inducible cyclooxygenase (COX-2) levels in both intestinal tracts. On the other hand, the expression of anti-inflammatory protective cytokines TGF-β1 and IL-10 was differently modulated in AI and PI. Indeed, these factors increased overall in AI, suggesting an attempt to counteract the over-activated immune response in this intestinal portion, limiting tissue inflammation. On the contrary, IL-10 was reduced, and TGF-β1 was unchanged in PI. Thus, these results suggest that inflammatory alterations were more severe in PI compared to AI of gilthead seabream, as also confirmed by histological evaluations. Accordingly, more severe histopathological alterations and inflammatory effects were observed in the distal intestine compared to the proximal intestine of goldfish exposed to MPs (Jabeen et al., 2018).

Inflammation is part of a complex biological response of the gut to harmful stimuli and may be strictly associated with intestinal redox unbalance (Circu and Aw, 2012). Under physiological conditions, ROS and RNS are produced in low amounts required to maintain cell

homeostasis. Environmental stressors can cause the overproduction of these reactive oxygen and nitrogen species, and cells trigger complex signaling pathways to counteract this phenomenon. However, in several cases, ROS and RNS overproduction and the antioxidant defense system failure result in redox unbalance and OS. The latter is one of the main features of MP toxicity. Indeed, MPs can induce an unbalance between the production of free radicals and the antioxidant defense systems triggering and sustaining OS and nitrosative stress (Hu and Palic, 2020). The main consequence is the damage to lipids, proteins, and nucleic acids (Ferrante et al., 2022; Subaramaniyam et al., 2023). Lipid peroxidation can alter the structure and functions of cell membranes, endangering cell survival and leading to the production of MDA, a biomarker of OS. Here, the subchronic oral exposure to PS-MPs increased ROS production in both AI and PI of gilthead seabream and augmented MDA levels only in PI, suggesting a greater susceptibility of this intestinal portion to peroxidative damage. Moreover, PS-MPs caused a rise of nitrosylated proteins in both intestinal tracts, revealing the occurrence of also nitrosative stress damage. In agreement with the reported results, some authors showed increased levels of ROS and MDA in the gut of fish exposed to different MP polymer types (Kang et al., 2021; Zhang et al., 2021a; Zhao et al., 2021b; Hou et al., 2022).

To counteract ROS and RNS overproduction, cells activate specific signaling pathways, including the induction of a complex network of antioxidant and detoxifying systems (superoxide dismutase – SOD, CAT, GR, glutathione peroxidase – GPx, GSH and HSPs) and transcription factors such as Nrf2 (Ma, 2013; Shan et al., 2020;

Demirci-Cekic et al., 2022). Several studies have shown that the exposure of fish species to MPs impacts the intestinal antioxidant enzymes efficiency by modulating their activity and/or expression (Kang et al., 2021; Zhang et al., 2021a; Zhao et al., 2021b; Hou et al., 2022).

Here, the exposure of gilthead seabreams to PS-MPs impacts the main enzymes involved in the antioxidant defense, such as SOD and CAT (that are direct scavengers of free radicals) and GR (involved in the inactivation of secondary metabolites, more precisely, the reduction of GSH disulfide to GSH). Specifically, PS-MPs increased SOD levels and decreased those of CAT in AI without affecting GR levels. These results might explain the lower responsiveness of AI to oxidative damage. Indeed, the MDA production may be counteracted by an efficient enough regulation of the SOD expression in this tract. On the contrary, PS-MPs did not change SOD and GR levels but reduced the expression of CAT in PI.

The expression of antioxidant defense systems is controlled by several mechanisms. Among these, Nrf2 is a regulator of cellular resistance to oxidants because it mediates the induction of enzymes and signaling proteins involved in antioxidant defense and oxidant signaling, thereby influencing the physiological and pathological reaction to oxidant agents (Ma, 2013). In this research work, Nrf2 expression was decreased by PS-MPs only in PI, confirming its susceptibility to oxidative damage.

HSPs, including HSP70 and HSP90, are highly conserved molecular chaperones that provide for the maintenance of cellular homeostasis for their involvement in the refolding of misfolded

proteins. HSPs are activated in response to environmental stressors (Shan et al., 2020). The levels of both HSP70 and HSP90 were increased by PS-MPs in the PI of gilthead seabream but were unchanged in the AI, probably because the oxidative and inflammatory damage in AI was not enough to trigger a measurable response. Similarly, the dietary exposure of European sea bass to PVC-MPs for 21 days caused an upregulation of HSP70 expression in the intestine (Espinosa et al., 2019), and the same effect was observed in the intestine of silver carp acutely exposed to PS-MPs (Zhang et al., 2021a).

The intestinal barrier is a crucial structure that receives several stimuli and dynamically reacts to them, protecting the host against harmful microorganisms, antigens, and toxins, and ensuring the correct absorption of nutrients at the same time. Barrier efficiency derives from the interaction between the mucus layer, commensal bacteria, epithelial and immune cells residing in the lamina propria (Barbara et al., 2021). However, several factors, including genetic and environmental ones, can impair intestinal barrier integrity, leading to numerous disorders (Martel et al., 2022). The intestinal paracellular permeability is regulated by intercellular junctions composed of TJs, adherens junctions, desmosomes, and gap junctions (Barbara et al., 2021). The permeability differs along the intestinal tracts, and it has been shown that tight and gap junction genes are more expressed in the PI of gilthead seabream than in AI (Perez-Sanchez et al., 2015). In physiological conditions, only water and solutes can cross the epithelium through the paracellular way. However, several inducers of TJ dysfunction, among which inflammation and OS, can cause

hyperpermeability of the intestinal barrier (Wells et al., 2017). The activation of the MAPKs signaling pathway can be involved in the process of TJ disruption (Gonzalez-Mariscal et al., 2008). Here, the MAPKs pathway was activated by PS-MPs, which increased the phosphorylation of p38 and ERK in the PI of gilthead seabream but not in AI. Therefore, the expression of TJs and integrins (zonula occludens-1, claudin-15, occludin, tricellulin, and B6 subunit of integrin αvβ6) were decreased in the PI, indicating an impairment of intestinal barrier integrity in this tract.

Besides the TJs, the intestinal tract is protected by the mucus secreted by goblet cells, which reside throughout the GI tract. Muc are proteins that give the mucus its gel-like properties; they are encoded by approximately 20 genes (Johansson and Hansson, 2016). As for TJs, Muc are constitutively more expressed in the PI of gilthead seabream (Perez-Sanchez et al., 2015).

PS-MP treatment decreased the number of goblet cells in both AI and PI and reduced the levels of Muc2 and Muc13 in PI. Muc2 is involved in the maintaining of healthy microbiota, while Muc13 plays a crucial role in defense against infections, inflammatory diseases, and cancer development (Sheng et al., 2011; Leon-Coria et al., 2021). These findings further suggest the occurrence of impairment of barrier integrity and functionality determined by PS-MPs in the PI of gilthead seabream. Accordingly, reduced mucus secretion and decreased TJ expression were reported in the intestine of common carp after prolonged exposure to PE-MPs (Chen et al., 2022).

Since the damage to the intestinal barrier integrity leads to a permissive unrestricted passage (Odenwald and Turner, 2017), PS-

MPs of small dimension could translocate across the leaky gut, reaching the gut associated-lymphoid-tissue and other organs, also causing widespread toxic effects. Indeed, literature data suggest that MP/NP particles reach several organs through the translocation process, among which the liver (Ma et al., 2021).

7.2 Exposure to PS-MPs impairs liver health contributing to the onset and progression of NAFLD

The liver is one of the main metabolic and detoxifying organs. It physiologically metabolizes and excretes exogenous chemical substances or toxins that enter the systemic circulation via oral ingestion and absorption. Therefore, the liver is exposed to noteworthy concentrations of xenobiotics potentially able to damage it; hence, hepatoxicity evaluation is a key part in risk assessment of many environmental pollutants (Armstrong and Guo, 2019). In the last few years, a growing body of evidence showed the link between exposure to environmental toxicants and the onset and progression of liver disease, among which NAFLD, in mammals (Armstrong and Guo, 2019; Rajak et al., 2022). NAFLD is a multifactorial disease characterized by excessive accumulation of lipids, mainly triglycerides, in hepatocytes cytoplasm, inflammation, and OS (Karkucinska-Wieckowska et al., 2022). Recently, some studies revealed, also in fish species, the positive correlation between exposure to pollutants (i.e., bisphenols, cadmium, and perchlorate) and the onset or progression of NAFLD (Minicozzi et al., 2019; Qin et al., 2020; Guo et al., 2022a; Chakraborty et al., 2023). However, although MPs

threaten aquatic species, their impact on liver homeostasis is overlooked, and their role in the onset of NAFLD in fish remains almost unknown.

The liver is strictly intertwined with the gut due to the existence of a gut-liver axis, a bidirectional connection between these organs. Consequently, alteration of the intestinal barrier may result in increased portal influx of bacteria and/or their products, as well as toxicants, to the liver, where they induce or worsen several hepatic disorders, among which the NAFLD (Albillos et al., 2020). Given the evidenced alterations induced by PS-MPs in the gut of gilthead seabream, during the second part of PhD programme, the research aimed to evaluate the consequences of oral exposure to PS-MPs on the gut-liver axis balance. The analysis focused on the potential role of PS-MPs in liver health impairment and, specifically, in the onset and progression of NAFLD.

As evidenced above for growth rate and physical conditions index, neither HSI was modified by PS-MPs. This lack of effects may be explained by the relatively short exposure time. Similarly, HSI was not modified in gilthead seabreams exposed for 24 and 96 h to polymethylmethacrylate NPs (Brandts et al., 2021).

Hepatic lipid metabolism is regulated by a combination of signaling pathways involved in the uptake and export of FA, *de novo* lipogenesis (DNL), and fat utilization by β-oxidation. The alteration of these processes can cause the accumulation of lipid droplets in the hepatocytes, resulting in hepatic steatosis and promoting the onset of NAFLD (Mato et al., 2019).

The hepatic DNL is a key metabolic pathway that involves different factors and regulators, and it is firstly controlled by the sterol regulatory element binding proteins (SREBPs). Among these, SREBP1c is a transcription factor that, translocating to the nucleus, upregulates the expression of genes involved in the FA biosynthesis pathway, including the fatty acid synthase (FAS) (Horton et al., 2002). FAS is the main enzyme catalyzing the biosynthesis of saturated FA from simple precursors. The primary product of the reaction is palmitate, which is subsequently modified to generate several FA species (Jensen-Urstad and Semenkovich, 2012). Here, the highest PS-MP dose increased the mRNA levels of both SREBP1c and FAS, indicating the activation of DNL in the liver of exposed gilthead seabreams. Similarly, Lai et al. (2021) showed an up-regulation of DNL mediators in fish hepatocytes after both *in vivo* and *in vitro* exposure to nano PS particles.

Besides those derived from DNL, FA are taken up by hepatocytes via plasma membrane-associated proteins, including fatty acid binding protein 1 (FABP1). This latter regulates cellular uptake, transport, and metabolism of toxic-free FA in the liver of fish and mammals (Xu et al., 2017). In this study, hepatic levels of FABP1 in gilthead seabreams were not modified by exposure to PS-MPs. This result suggests that hepatic lipid metabolism dysregulation does not depend on FA intracellular uptake in these conditions.

PPARs are ligand-activated transcription factors involved in the regulation of lipid and carbohydrate metabolism, as well as in inflammatory processes. Among PPARs, PPARα is highly expressed in the liver, where contributes to the regulation of FA β-oxidation

pathways and plays an important role in inflammatory response; PPARγ regulates fat storage, and its overexpression has been related to lipids accumulation in the liver (Qiu et al., 2023). PPARs are highly expressed in gilthead seabream tissue, i.e., liver, intestine, and adipose tissue (Leaver et al., 2005). Their role in lipid metabolism disruption has been indirectly confirmed in another teleost species, the red tilapia, by a recent study evidencing that treatment with resveratrol reduced lipid hepatic damage in fish exposed to a high-fat diet (Zheng et al., 2022). In the present study, it was observed an up-regulation of PPARγ mRNA levels in the liver of PS2 gilthead seabreams, while PPARα levels were unchanged, suggesting the occurrence of an increased lipid accumulation and not FA β-oxidation. Additionally, PS-MPs did not modify the expression of other lipid catabolism-related factors, such as hepatic lipase (HL*)* and phospholipase A2 (Pla2). Instead, the levels of lipoprotein-lipase (LPL), an enzyme that hydrolyzes triglycerides in chylomicrons and very low-density lipoproteins, were increased by both PS-MP doses. This evidence suggest that the liver tried to reduce the storage of triglycerides but increased the release of FA, which could have a detrimental role, contributing to the progression of liver disease. Overall, the obtained results indicate the presence of lipid accumulation in the liver, which could be a wake-up call of steatosis. The histological evaluations confirm the molecular findings, showing not only an increased accumulation of lipid vacuoles in PS-MP exposed groups, but also the infiltration of inflammatory cells and necrosis. Accordingly, literature data reported lipid droplets, immune and inflammatory infiltration, and necrosis of hepatocytes, as well as

increased levels of LPL and PPARγ in the liver of zebrafish exposed to PS-MPs (Lu et al., 2016; Du et al., 2023; Zhou et al., 2023).

The activation of innate immunity plays an important role in triggering and amplifying hepatic inflammation, a hallmark of the onset and progression of several liver diseases, such as NAFLD (Peiseler et al., 2022). TLR pathways are involved in these processes (Khanmohammadi and Kuchay, 2022). Here, the hepatic immune response was activated by PS-MPs right through these pathways, as shown by the increased levels of TLR2 and TLR5. Similarly, Cui et al. (2023) showed the activation of the TLR2 pathway in the hepatopancreas of carp exposed to PS-MPs for 21 days. Moreover, PS-MPs also augmented CSF1R levels in the liver of exposed gilthead seabream. This is a transmembrane tyrosine kinase receptor found in mononuclear phagocytes of several *S. aurata* immune tissues (Roca et al., 2006) and is involved in the macrophage recruitment by controlling macrophage survival, proliferation, and differentiation.

As a result of the innate immune activation, increased hepatic inflammatory response was revealed with a stronger effect in the PS2 group. Specifically, PS-MPs increased the mRNA levels of TNF-α, IL-1β, and IL-6, which have been described as early and key pro-inflammatory cytokines in the liver of fish exposed to chemical pollutants (Zhang et al., 2019; Hossain et al., 2021; Li et al., 2023). In addition, the highest PS-MP dose determined the increase of hepatic COX-2 levels. Finally, an up-regulation of anti-inflammatory TGF-β1 was highlighted in the liver of PS1 group. This result might explain the less inflammatory damage evidenced in the liver of gilthead seabreams exposed to the lowest PS-MP dose.

In agreement with these results, other authors evidenced the MP-induced modulation of inflammatory cytokines in the liver or hepatopancreas of different fish (Hodkovicova et al., 2021; Cui et al., 2023; Hollerova et al., 2023; Liu et al., 2023; Wei et al., 2023).

Environmental pollutants, including MPs, can trigger OS causing oxidative damage to lipids, proteins, and nucleic acids also in hepatocytes of aquatic organisms (Livingstone, 2001; Alomar et al., 2017; Barboza et al., 2018; Sillero-Ríos et al., 2018). OS underlies the pathophysiology of many liver diseases, including NAFLD (Delli Bovi et al., 2021). In the liver, several antioxidant and detoxifying mechanisms are involved in the regulation of OS, reducing or scavenging ROS production, permitting xenobiotics metabolization, and counteracting or repairing damaged biomolecules (Livingstone, 2001; Biller and Takahashi, 2018). Here, PS-MPs affected the hepatic antioxidant and detoxifying systems, resulting in OS.

The antioxidant defense system is based on the activity of enzymatic and non-enzymatic components. Among them, SOD, CAT, GR, and GSH are widely recognized and applied as biomarkers of OS induced by chemical contaminants. SOD and CAT have an important role in avoiding the production of hydroxyl radicals (HO•), while GSH is the first and most important non-enzymatic component which acts as a radical scavenger, as a cofactor for the enzymatic antioxidant GPx, and as a co-substrate for GST. GSH may react with oxidant species, resulting in the formation of oxidized form GSSG, which is highly cytotoxic and reduced to GSH by GR enzyme (Subaramaniyam et al., 2023). The exposure of gilthead seabreams to PS-MPs increased the gene expression of CAT and SOD in the liver of PS2 group without

modifying CAT activity. On the contrary, GR mRNA levels were unchanged, while its enzymatic activity was reduced in PS2. PS-MPs also impacted the GSH cycle. Indeed, an increased production of GSSG was revealed, while tGSH was unmodified. All these alterations resulted in hepatic OS, as indicated by increased levels of ROS and MDA. The available literature data on this issue report contrasting results, probably due to the different experimental conditions adopted. Indeed, some authors evidenced an increased activity of antioxidant enzymes in the liver of fish exposed to MPs (Rios-Fuster et al., 2021; Choi and Kim, 2023; Felix et al., 2023; Lee et al., 2023), while others observed a reduced or unchanged activity (Hollerova et al., 2023; Kessabi et al., 2023; Yu et al., 2023a; Zhou et al., 2023) or mixed results (Das et al., 2023; Hao et al., 2023) with a lack of homogeneity in gene expression data (Brandts et al., 2021; Espinosa-Ruiz et al., 2023). Conflicting data were also observed on GSH content in fish after subchronic MP or NP treatment (Lai et al., 2021; Yu et al., 2023a; Yu et al., 2023b).

As regards hepatic metabolizing capability, fish have well-developed systems that convert lipophilic toxicants into more water-soluble metabolites to facilitate their excretion. The metabolization process is firstly catalyzed by Phase I enzymes, which oxidize, reduce, or hydrolyze compounds, and then by Phase II enzymes. These last catalyze the conjugation of phase I metabolites to cofactors such as GSH, sulfate or glucuronic acid (Santana et al., 2018).

CYP450s are crucial Phase I enzymes that act in the metabolization processes of several xenobiotics; their activity and amount are important tools to assess environmental disturbances triggered or

amplified by exposure to chemical pollutants. EROD activity, a catalytic function of the CYP1A subfamily, is a popular biomarker for exposure to many organic xenobiotics (Whyte et al., 2000). The effect of MPs on EROD activity was not deeply investigated, and the few performed studies reported contrasting results (Cormier et al., 2021; Capó et al., 2022; Martyniuk et al., 2022; Espinosa-Ruiz et al., 2023; Martyniuk et al., 2023). Here, PS-MPs decreased the EROD activity in the liver of gilthead seabreams in a dose-dependent manner, suggesting the impairment of CYP1A metabolizing function and a weakened detoxifying process. Accordingly, Capó et al. (2022) reported a decreased EROD activity in the liver of *S. aurata* chronically exposed to MPs. Similarly, Peda et al. (2022) observed a decrease in hepatic EROD activity in European seabass (*Dicentrarchus labrax*) exposed for 60 days, via an enriched diet, to virgin and marine incubated PVC particles. Moreover, Martyniuk et al. (2022) also observed a decreased EROD activity in the digestive gland of freshwater bivalves exposed to MPs.

In this study, it was also considered the effect of PS-MPs on GST activity. GST is a family of enzymes that catalyze the conjugation of GSH to xenobiotics (i.e., benzo(a)pyrene, aflatoxin B1, and different classes of pesticides) in Phase II reactions. Moreover, GST enzymes are involved in the detoxification processes of oxidative damage products (Schlenk et al., 2008). GST activity was increased in the liver of PS2 gilthead seabreams, probably due to the effort to counteract OS. Despite this, the impairment of the antioxidant defense system prevailed, rendering the hepatic capability to counteract or prevent oxidative damage ineffective, as proven by the increased ROS and

MDA levels. Similar results regarding GST were observed by other authors in the liver of fish exposed to MPs under experimental or wild conditions (Alomar et al., 2017; Capó et al., 2021; Rios-Fuster et al., 2021; Solomando et al., 2021; Capó et al., 2022; Solomando et al., 2022; Cohen-Sanchez et al., 2023; Jeyavani et al., 2023; Lee et al., 2023; Sahabuddin et al., 2023). Moreover, Capó et al. (2022) observed an increased GST activity along with increased levels of ROS and MDA in the liver of gilthead seabreams exposed to MPs.

Notably, the response evoked by PS-MPs in the liver showed, in most cases, a linear, dose-related pattern. The scientific literature on the topic often reports that the toxic effect appears to be slight or not evident at low doses. It has been explained by the recent "hormesis theory" that might be a positive adaptive biological response of the organism to low levels of stressors like MPs in aquatic organisms (Sun et al., 2021b). When the stressor doses become higher, the negative effects manifest themselves. An adaptive response has also been observed at high concentrations in defined species (i.e., freshwater gastropods), evidencing that the response to MPs may also depend on the species, as well as on the dose.

8. Conclusions

Based on the obtained data, it can be inferred that PS-MPs affect the intestinal and hepatic homeostasis in gilthead seabreams subchronically exposed via contaminated feed. Here, it was highlighted that PS-MPs had inflammatory and immune effects in both AI and PI, also causing oxidative damage. This effect could be expected as, being the intestine the first encountered body district, it is a target organ for ingested MPs. PI showed higher responsiveness than AI which might depend on the different morphology and physiological functions of the analyzed tracts. PS-MPs also damaged, in a dose-dependent manner, PI barrier integrity. The persistence of intestinal inflammatory and immune stimuli may have a key role in the pathogenesis of chronic disorders. At the same time, the loss of intestinal barrier integrity may result in an increased portal influx of bacteria or toxicants to the liver, causing or worsening hepatic diseases. This study evidenced that PS-MPs also affected liver health in gilthead seabreams, inducing lipid metabolism impairment and, thus, steatosis, inflammatory response, and OS. All these detrimental effects cooperate in liver dysfunction and may synergistically promote and influence each other in the onset, development, and progression of NAFLD.

Thus, PS-MPs induced gut-liver axis unbalance in gilthead seabream leading to intestinal and hepatic alterations. The latter may contribute to the onset of diseases (i.e., NAFLD itself and non-alcoholic steatohepatitis, as well as intestinal bowel disease), which can negatively impact health status. It should be noted that PS-MPs did not affect the biometric parameters during the experimental period.

A crucial issue is whether ingested MPs can contribute to the above alterations also in other animal species and humans, focusing on more susceptible categories such as individuals affected by metabolic and endocrine disorders. In this regard, it is also important to consider that MPs adsorb several contaminants, namely endocrine disruptors, that may synergistically work, worsening the above disorders.

The obtained results confirm the usefulness of gilthead seabream as a good experimental model to investigate the toxicity of pollutants widespread in environmental ecosystems. Moreover, they further support the awareness of MPs as a threat to living organisms. The reported findings could be useful for researchers, regulators, legislators, and public opinion, contributing to filling critical knowledge gaps in understanding MP toxicity and potential health risks.

Abbreviations

AB-PAS: Alcian Blue-Periodic Acid Schiff's

AChE: Acetylcholinesterase

AI: Anterior intestine

ALP: Alkaline phosphatase

ALT: Alanine aminotransferase

AMPK: AMP-activated protein kinase

AST: Aspartate aminotransferase

BSA: Bovine Serum Albumin

CAT: Catalase

CDNB: 1-chloro-2, 4-dinitrobenzene

CF: Fulton's condition factor

CON: Control

COX: Cyclooxygenase

CSF1R: Receptor of the colony-stimulating factor-1

CYP: Cytochrome

DNL: *de novo* lipogenesis

DNPH: 2,4-Dinitrophenylhydrazine

DTNB: 5,5'-dithiobis-(2-nitrobenzoic acid)

ERK: Extracellular signal-regulated kinase

EROD: Ethoxyresorufin-O-deethylase

FA: Fatty acid

FABP1: Fatty acid binding protein 1

FAS: Fatty acid synthase

GI: Gastrointestinal

GPx: Glutathione peroxidase

GSH: Glutathione

GSSG: Oxidized Glutathione

GST: Glutathione s-transferase

H&E: Hematoxylin & eosin

HL: Hepatic lipase

HSI: Hepatosomatic index

HSP: Heat shock protein

IL: Interleukin

LPL: Lipoprotein-lipase

Lys: Lysozyme

MAPK: Mitogen-activated protein kinase

MDA: Malondialdehyde

MP: Microplastic

Muc: Mucin

MyD88: Myeloid differentiation primary response 88

NAFLD: Non-alcoholic fatty liver disease

NOD: Nucleotide Oligomerization Domain-Containing Protein

NP: Nanoplastic

Nrf2: Nuclear factor erythroid 2-related factor 2

ORO: Oil Red O

OS: Oxidative stress

PA: Polyamide

PAS: Periodic Acid-Schiff

PE: Polyethylene

PET: Polyethylene terephthalate

PI: Posterior intestine

Pla2: Phospholipase A2

POP: Persistent organic pollutant

PP: Polypropylene

PPAR: Proliferator-activated receptor

PRR: Pattern recognition receptors

PS: Polystyrene

PVC: Polyvinyl chloride

RAS: Recirculating Aquaculture System

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

SOD: Superoxide dismutase

SREBP: Sterol regulatory element binding protein

TJ: Tight junction

TLR: Toll-like receptor

WG: Weight gain

Supplementary data

Table S1. Fish feed composition.

Gross Energy (MJ/kg)	18,75
Digestible energy (MJ/kg)	16,67
Crude Fat (%)	17,90
Crude Protein (%)	42,34
Digestible proteins (%)	36,14
Fish Protein (%)	8,24
Animal Protein (%)	27,34
Fish protein/Total protein (%)	19,45
Digestible protein/Digestible energy (mg/kJ or g/MJ)	21,68
Animal protein/Total protein (%)	64,59
Fiber in Feed (%)	2,81
Nitrogen-free extracts (%)	18,05
Amido (%)	7,71
Non-starch polysaccharides (%)	13,15
Dry Matter (%)	88,31
Starch in feed (%)	7,71
Ash in feed (%)	7,21
Ala (%)	2,78
Arg (%)	2,71
Asp (%)	3,64
Glu (%)	5,93
Gly (%)	3,40
His (%)	1,11
Iso (%)	1,55
Leu (%)	3,28
Lys (%)	2,54
Met (%)	1,04
Phe (%)	1,97
Pro (%)	2,55
Ser (%)	2,20
Thr (%)	1,75

Tyr (%)	1,24
Trp (%)	0,39
Val (%)	2,17
Taurine (%)	0,46
Vit. D (I.U./Kg)	2100
Vit. A (I.U./Kg)	8000
Vit. E (mg/Kg)	389,72
Inositol (mg/Kg)	192,83
Niacin (mg/Kg)	229,17
Pantothenic acid (mg/Kg)	88,97
Vit. B2 (mg/Kg)	33,98
Vit. B1 (mg/Kg)	34,31
Vit. B6 (mg/Kg)	27,87
Folic acid (mg/Kg)	7,93
Vit. K (mg/Kg)	6,56
Biotin (mg/Kg)	1,12
Vit B12 (mg/Kg)	0,06
Choline (mg/Kg)	1698,71
Vit. C (mg/Kg)	250,25
Calcium (mg/Kg)	1,60
Cobalt (mg/Kg)	0,34
Copper (mg/Kg)	45,64
Iron (mg/Kg)	577,18
Magnesium (mg/Kg)	0,18
Manganese (mg/Kg)	47,47
Molybdate (mg/Kg)	0,54
Nichel (mg/Kg)	1,97
Phosphorus (mg/Kg)	1,17
Potassium (mg/Kg)	0,90
Sodium (mg/Kg)	0,21
Selenium (mg/Kg)	0,62
Sulfur (mg/Kg)	0,56
Zinc (mg/Kg)	83,05
Tot myristic acid (C14:0, %)	0,18

Tot pentadecanoic acid (C15:0, %)	0,02
Tot palmitic acid (C16:0, %)	1,36
Tot palmitoleic acid (C16:1, %)	0,27
Tot heptadecanoic acid (C17:0, %)	0,02
Tot heptadecenoic acid (C17:1, %)	0,02
Tot stearic acid (C18:0, %)	0,42
Tot oleic acid (C18:1 n-9, %)	7,75
Tot vaccenic acid (C18:1 n-7, %)	0,23
Tot linoleic acid (C18:2 n-6, LA, %)	3,11
Tot α-linolenic acid (C18:3 n-3, ALA %)	1,01
Tot γ-linolenic acid (C18:3 n-6, %)	0,02
Tot octadecatrienoic acid (C18:4 n-3, %)	0,06
Tot arachidic acid (C20:0, %)	0,07
Tot eicosenoic acid (C20:1 n-9, %)	0,32
Tot eicosadienoic acid (C20:2 n-6, %)	0,05
Tot eicosatrienoic acid (C20:3 n-6, %)	0,01
Tot eicosatrienoic acid (C20:3 n-3, %)	0,01
Tot arachidonic acid (C20:4 n-6, %)	0,04
Tot eicosatetraenoic acid (C20:4 n-3, %)	0,03
Tot eicosapentaenoic acid (C20:5 n-3, EPA, %)	0,29
Tot behenic acid (C22:0, %)	0,06
Tot cetoleic acid (C22:1 n-11, %)	0,15
Tot erucic acid (C22:1 n-9, %)	0,04
Tot docosadienoic acid (C22:2 n-6, %)	0,02
Tot docosapentaenoic acid (C22:5 n-3, DPA, %)	0,06
Tot docosahexanoic acid (C22:6 n-3, DHA, %)	0,40
Tot lignoceric acid (C24:0, %)	0,01
Tot tetracosahexaenoic acid (C24:1 n-9, %)	0,02
Tot pentacosanoic acid (C25:0, %)	0,01
Tot Omega 3 (%)	2,09
Tot Omega 6 (%)	3,94
Omega 3/Omega 6 (ratio)	0,53

*Percentage values are expressed on total feed.

Table S2. Primer sequences used for Real Time-PCR analysis.

Gene name	Protein	Primer Forward (5'→3')	Primer Reverse (5'→3')
Rps18	Ribosomal protein S18	AGGGTGTTGGCAGACGTTAC	CGCTCAACCTCCTCATCAGT
TLR2	Toll-like receptor 2	TCCATGCTTTCGTCCAGGAC	ACTGTGTTGAGCAAGGCCTC
TLR5	Toll-like receptor 5	CCTGTCTGCAACTGTCAGGA	TGTGGATCTGGTTCAAGCTG
Myd88	Myeloid differentiation primary response 88	GAGGTTGACAGCTGTCTCCC	GGCAGTAGCAGATGAAGGCA
Lys	Lysozime	CCAGGGCTGGAAATCAACTA	CCAACATCAACACCTGCAAC
CSF1R	Colony-stimulating factor-1 receptor	ACGTCTGGTCCTATGGCATC	AGTCTGGTTGGGACATCTGG
ALP	Alkaline phosphatase	TTACTGGGCCTGTTTGAACC	ATCCTTGATGGCCACTTCCAC
COX-2	Cyclooxygeaase-2	GAGTACTGGAAGCCGAGCAC	GATATCACTGCCGCCTGAGT
TNF-α	Tumor necrosis factor α	CAGGCGTCGTTCAGAGTCTC	CTGTGGCTGAGAGGTGTGTG
IL-1β	Interleukin-1β	GCGACCTACCTGCCACCTACACC	TCGTCCACCGCCTCCAGATGC
IL-6	Interleukin-6	AGGCAGGAGTTTGAAGCTGA	ATGCTGAAGTTGGTGGAAGG
IL-10	Interleukin-10	CTCACATGCAGTCCATCCAG	TGTGATGTCAAACGGTTGCT
TGF-β1	Transforming growth factor-β1	GCATGTGGCAGAGATGAAGA	TTCAGCATGATACGGCAGAG

CuZn/ SOD	Superoxide dismutase	CCATGGTAAGAATCATGGCGG	CGTGGATCACCATGGTTCTG
CAT	Catalase	TTCCCGTCCTTCATTCACTC	CTCCAGAAGTCCCACACCAT
GR	Glutathione reductase	CAAAGCGCAGTGTGATTGTGG	CCACTCCGGAGTTTTGCATTTC
Nrf2	Nuclear factor erythroid 2 –related factor 2	GTTCAGTCGGTGCTTTGACA	CTCTGATGTGCGTCTCTCCA
hsp70	Heat-shock protein 70	AATGTTCTGCGCATCATCAA	GCCTCCACCAAGATCAAAGA
hsp90	Heat-shock protein 90	GTGGATTCTGAGGACCTGCC	GAGAGTCTTCGTGGATGCCC
ZO-1	Zonula occludens-1	ACGACAAGCGCCTGTTAAGT	TCCTGAGCTTCCGACATTTT
Occludin	Occludin	GTGCGCTCAGTACCAGCAG	TGAGGCTCCACCACACAGTA
Tricellulin	Tricellulin	CCAGAGATCAGCTGTGTGGA	TGCTGTTCCTCTTGGCTTTT
Itgb6	B6 subunit of integrin αvβ6	AGCCTCCCAACATCCCTATGATTATTC	CTTCCACACACCCAGCAGAA
Cldn15	Claudin-15	CCGATTGTGGAAGTAGTGGCTCTGGT	CAGCATCACCCAACCGACGAACC
Muc2-like	Mucin 2	GTGTGTGGCTGTGTTCCTTGCTTTGT	GCGAACCAGTCTGGCTTGGACATCA
Muc13-like	Mucin 13	TTCAAACCCGTGTGGTCCAG	GCACAAGCAGACATAGTTCGGATAT
PPARγ	Peroxisome proliferator-activated receptor γ	CGCCGTGGACCTGTCAGAGC	GGAATGGATGGAGGAGGAGGAGATGG

Srebp 1	Sterol regulatory element-binding protein	AGGGCTGACCACAAC GTCTCCTCTCC	GCTGTACGTGGGATGT GATGGTTTGG
Fasn	Fatty acid synthase	TGGCAGCATACACACA GACC	CACACAGGGCTTCAGT TTCA
Fabp 1	Fatty Acid Binding Protein 1	CATGAAGGCGATTGGT CTCC	GTCTCCAAGTCTGCCTC CTT
Lpl	Lipoprotei n lipase	GAGCACGCAGACAAC CAGAA	GGGGTAGATGTCGATG TCGC
PPAR α	Peroxiso me proliferato r-activated receptor α	TCTCTTCAGCCCACCA TCCC	ATCCCAGCGTGTCGTC TCC
HL	Hepatic lipase	TTGTAGAAGGTGAGGA AAACTG	GCTCTCCATCAGACCA TCC
Pla2	Phospholi pase A2	CCAGACCATCTTCACC ATCC	CACCCAATCCACAGGA GTTC

References

ACTION, S.I., 2020. World Fisheries and Aquaculture. Food and Agriculture Organization 2020, 1-244.

Aebi, H., 1974. Catalase. Methods of enzymatic analysis. Elsevier, pp. 673-684.

Afrin, S., Rahman, M.M., Akbor, M.A., Siddique, M.A., Uddin, M.K., Malafaia, G., 2022. Is there tea complemented with the appealing flavor of microplastics? A pioneering study on plastic pollution in commercially available tea bags in Bangladesh. Science of the Total Environment 837.

Ahrendt, C., Perez-Venegas, D.J., Urbina, M., Gonzalez, C., Echeveste, P., Aldana, M., Pulgar, J., Galban-Malagon, C., 2020. Microplastic ingestion cause intestinal lesions in the intertidal fish Girella laevifrons. Mar Pollut Bull 151, 110795.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. Transport into the cell from the plasma membrane: endocytosis. Molecular Biology of the Cell. 4th edition. Garland Science.

Albillos, A., de Gottardi, A., Rescigno, M., 2020. The gut-liver axis in liver disease: Pathophysiological basis for therapy. J Hepatol 72, 558-577.

Alimba, C.G., Faggio, C., 2019. Microplastics in the marine environment: Current trends in environmental pollution and mechanisms of toxicological profile. Environ Toxicol Pharmacol 68, 61-74.

Alimi, O.S., Farner Budarz, J., Hernandez, L.M., Tufenkji, N., 2018. Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. Environ Sci Technol 52, 1704-1724.

Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Jiménez, P.D., Simonneau, A., Binet, S., Galop, D., 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nat Geosci 12, 339-344.

Alomar, C., Sanz-Martin, M., Compa, M., Rios-Fuster, B., Alvarez, E., Ripolles, V., Valencia, J.M., Deudero, S., 2021. Microplastic ingestion in reared aquaculture fish: Biological responses to low-density polyethylene controlled diets in Sparus aurata. Environ Pollut 280, 116960.

Alomar, C., Sureda, A., Capó, X., Guijarro, B., Tejada, S., Deudero, S., 2017. Microplastic ingestion by Linnaeus, 1758 fish and its potential for causing oxidative stress. Environ Res 159, 135-142.

An, R., Wang, X.F., Yang, L., Zhang, J.J., Wang, N.N., Xu, F.B., Hou, Y., Zhang, H.Q., Zhang, L.S., 2021. Polystyrene microplastics cause granulosa cells apoptosis and fibrosis in ovary through oxidative stress in rats. Toxicology 449.

Andrady, A.L., 2017. The plastic in microplastics: A review. Mar Pollut Bull 119, 12-22.

Araújo, A.P.D., Gomes, A.R., Malafaia, G., 2020. Hepatotoxicity of pristine polyethylene microplastics in neotropical tadpoles (Fitzinger, 1826). Journal of Hazardous Materials 386.

Armstrong, L.E., Guo, G.L., 2019. Understanding Environmental Contaminants' Direct Effects on Non-alcoholic Fatty Liver Disease Progression. Curr Environ Health Rep 6, 95-104.

Asmonaite, G., Sundh, H., Asker, N., Carney Almroth, B., 2018. Rainbow Trout Maintain Intestinal Transport and Barrier Functions Following Exposure to Polystyrene Microplastics. Environ Sci Technol 52, 14392-14401.

Au, S.Y., Lee, C.M., Weinstein, J.E., van den Hurk, P., Klaine, S.J., 2017. Trophic transfer of microplastics in aquatic ecosystems: Identifying critical research needs. Integr Environ Asses 13, 505-509.

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environmental Pollution 198, 211-222.

Banaee, M., Soltanian, S., Sureda, A., Gholamhosseini, A., Haghi, B.N., Akhlaghi, M., Derikvandy, A., 2019. Evaluation of single and combined effects of cadmium and micro-plastic particles on biochemical and immunological parameters of common carp (Cyprinus carpio). Chemosphere 236.

Banaei, M., Forouzanfar, M., Jafarinia, M., 2022. Toxic effects of polyethylene microplastics on transcriptional changes, biochemical response, and oxidative stress in common carp (Cyprinus carpio). Comp Biochem Phys C 261.

Banerjee, A., Shelver, W.L., 2021. Micro- and Nanoplastic-Mediated Pathophysiological Changes in Rodents, Rabbits, and Chickens: A Review. J Food Protect 84, 1480-1495.

Barbara, G., Barbaro, M.R., Fuschi, D., Palombo, M., Falangone, F., Cremon, C., Marasco, G., Stanghellini, V., 2021. Inflammatory and Microbiota-Related Regulation of the Intestinal Epithelial Barrier. Front Nutr 8, 718356.

Barboza, L.G.A., Lopes, C., Oliveira, P., Bessa, F., Otero, V., Henriques, B., Raimundo, J., Caetano, M., Vale, C., Guilhermino, L., 2020. Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. Sci Total Environ 717, 134625.

Barboza, L.G.A., Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., Guilhermino, L., 2018. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, Dicentrarchus labrax (Linnaeus, 1758). Aquatic Toxicology 195, 49-57.

Bates, J.M., Akerlund, J., Mittge, E., Guillemin, K., 2007. Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. Cell Host Microbe 2, 371-382.

Beriot, N., Peek, J., Zornoza, R., Geissen, V., Huerta Lwanga, E., 2021. Low density-microplastics detected in sheep faeces and soil: A case study from the intensive vegetable farming in Southeast Spain. Sci Total Environ 755, 142653.

Bhagat, J., Zang, L.Q., Nishimura, N., Shimada, Y., 2020. Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. Science of the Total Environment 728.

Bhatt, V., Chauhan, J.S., 2023. Microplastic in freshwater ecosystem: bioaccumulation, trophic transfer, and biomagnification. Environ Sci Pollut R 30, 9389-9400.

Biller, J.D., Takahashi, L.S., 2018. Oxidative stress and fish immune system: phagocytosis and leukocyte respiratory burst activity. An Acad Bras Cienc 90, 3403-3414.

Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the freshwater amphipod. Environ Sci Pollut R 23, 23522-23532.

Bläsing, M., Amelung, W., 2018. Plastics in soil: Analytical methods and possible sources. Science of the Total Environment 612, 422-435.

Brandts, I., Barría, C., Martins, M.A., Franco-Martínez, L., Barreto, A., Tvarijonaviciute, A., Tort, L., Oliveira, M., Teles, M., 2021. Waterborne exposure of gilthead seabream (Sparus aurata) to polymethylmethacrylate nanoplastics causes effects at cellular and molecular levels. Journal of Hazardous Materials 403.

Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L). Environ Sci Technol 42, 5026-5031.

Burgos-Aceves, M.A., Faggio, C., 2017. An approach to the study of the immunity functions of bivalve haemocytes: Physiology and molecular aspects. Fish Shellfish Immun 67, 513-517.

Cain, K., Swan, C., 2010. Barrier function and immunology. Fish physiology. Elsevier, pp. 111-134.

Camilleri, M., 2019. Leaky gut: mechanisms, measurement and clinical implications in humans. Gut 68, 1516-1526.

Campanale, C., Massarelli, C., Savino, I., Locaputo, V., Uricchio, V.F., 2020. A Detailed Review Study on Potential Effects of Microplastics and Additives of Concern on Human Health. Int J Environ Res Public Health 17.

Cao, J., Xu, R., Geng, Y., Xu, S., Guo, M., 2023. Exposure to polystyrene microplastics triggers lung injury via targeting toll-like receptor 2 and activation of the NF-kappaB signal in mice. Environ Pollut 320, 121068.

Capó, X., Alomar, C., Compa, M., Sole, M., Sanahuja, I., Rojas, D.L.S., González, G.P., Martínez, R.M.G., Deudero, S., 2022. Quantification of differential tissue biomarker responses to microplastic ingestion and plasticizer bioaccumulation in aquaculture reared sea bream. Environ Res 211.

Capó, X., Company, J., Alomar, C., Compa, M., Sureda, A., Grau, A., Hansjosten, B., Lopez-Vazquez, J., Quintana, J., Rodil, R., 2021. Long-term exposure to virgin and seawater exposed microplastic enriched-diet causes liver oxidative stress and inflammation in gilthead seabream Sparus aurata, Linnaeus 1758. Science of The Total Environment 767, 144976.

Carbery, M., O'Connor, W., Thavamani, P., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environment International 115, 400-409.

Carpenter, E.J., Smith, K.L., Jr., 1972. Plastics on the Sargasso sea surface. Science 175, 1240-1241.
Castro, C., Couto, A., Diógenes, A.F., Corraze, G., Panserat, S., Serra, C.R., Oliva-Teles, A., 2019. Vegetable oil and carbohydrate-rich diets marginally affected intestine histomorphology, digestive enzymes activities, and gut microbiota of gilthead sea bream juveniles. Fish Physiol Biochem 45, 681-695.
Chae, Y., An, Y.J., 2020. Nanoplastic ingestion induces behavioral disorders in terrestrial snails: trophic transfer effects via vascular plants. Environ Sci-Nano 7, 975-983.
Chakraborty, S., Dissanayake, M., Godwin, J., Wang, X., Bhandari, R.K., 2023. Ancestral BPA exposure caused defects in the liver of medaka for four generations. Sci Total Environ 856, 159067.
Chang, J.N., Fang, W., Liang, J.S., Zhang, P.Y., Zhang, G.M., Zhang, H.B., Zhang, Y.J., Wang, Q.Y., 2022a. A critical review on interaction of microplastics with organic contaminants in soil and their ecological risks on soil organisms. Chemosphere 306.
Chang, X., Fang, Y., Wang, Y., Wang, F., Shang, L., Zhong, R., 2022b. Microplastic pollution in soils, plants, and animals: A review of distributions, effects and potential mechanisms. Sci Total Environ 850, 157857.
Chen, G.L., Feng, Q.Y., Wang, J., 2020. Mini-review of microplastics in the atmosphere and their risks to humans. Science of the Total Environment 703.
Chen, H., Wan, L., Qiu, Y., Qiu, F., Wen, C., Mao, Y., He, Z., 2024. Microplastics exposure induced and exacerbated the development of systemic lupus erythematosus in mice. Sci Total Environ 909, 168586.
Chen, J.J., Rao, C.Y., Yuan, R.J., Sun, D.D., Guo, S.Q., Li, L.L., Yang, S., Qian, D.D., Lu, R.H., Cao, X.L., 2022. Long-term exposure to polyethylene microplastics and glyphosate interferes with the behavior, intestinal microbial homeostasis, and metabolites of the common carp (Cyprinus carpio L.). Science of the Total Environment 814.
Chen, X.B., Xu, L.Y., Chen, Q.L., Su, S.Y., Zhuang, J.S., Qiao, D.F., 2023. Polystyrene micro- and nanoparticles exposure induced anxiety-like behaviors, gut microbiota dysbiosis and metabolism disorder in adult mice. Ecotox Environ Safe 259.

Cheng, L.K., O'Grady, G., Du, P., Egbuji, J.U., Windsor, J.A., Pullan, A.J., 2010. Gastrointestinal system. Wiley Interdiscip Rev Syst Biol Med 2, 65-79.

Choi, J.H., Kim, J.H., 2023. Toxic effects of sub-acute microplastic (polyamide) exposure on the accumulation, hematological, and antioxidant responses in crucian carp, Carassius carassius. Environ Toxicol Pharmacol 102, 104199.

Circu, M.L., Aw, T.Y., 2012. Intestinal redox biology and oxidative stress. Semin Cell Dev Biol 23, 729-737.

Cohen-Sanchez, A., Solomando, A., Pinya, S., Tejada, S., Valencia, J.M., Box, A., Sureda, A., 2023. Microplastic Presence in the Digestive Tract of Pearly Razorfish Xyrichtys novacula Causes Oxidative Stress in Liver Tissue. Toxics 11.

Cole, M., Galloway, T.S., 2015. Ingestion of Nanoplastics and Microplastics by Pacific Oyster Larvae. Environ Sci Technol 49, 14625-14632.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod Calanus helgolandicus. Environ Sci Technol 49, 1130-1137.

Cormier, B., Le Bihanic, F., Cabar, M., Crebassa, J.C., Blanc, M., Larsson, M., Dubocq, F., Yeung, L., Clérandeau, C., Keiter, S.H., Cachot, J., Bégout, M.L., Cousin, X., 2021. Chronic feeding exposure to virgin and spiked microplastics disrupts essential biological functions in teleost fish. Journal of Hazardous Materials 415.

Cozar, A., Sanz-Martin, M., Marti, E., Gonzalez-Gordillo, J.I., Ubeda, B., Galvez, J.A., Irigoien, X., Duarte, C.M., 2015. Plastic accumulation in the Mediterranean sea. PLoS One 10, e0121762.

Cribb, A.E., Leeder, J.S., Spielberg, S.P., 1989. Use of a Microplate Reader in an Assay of Glutathione-Reductase Using 5,5'-Dithiobis(2-Nitrobenzoic Acid). Anal Biochem 183, 195-196.

Critchell, K., Hoogenboom, M.O., 2018. Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (Acanthochromis polyacanthus). Plos One 13.

Cui, J., Zhang, Y.H., Liu, L., Zhang, Q.R., Xu, S.W., Guo, M.Y., 2023. Polystyrene microplastics induced inflammation with activating the TLR2 signal by excessive accumulation of ROS in hepatopancreas of carp (Cyprinus carpio). Ecotox Environ Safe 251.

da Silva Brito, W.A., Mutter, F., Wende, K., Cecchini, A.L., Schmidt, A., Bekeschus, S., 2022. Consequences of nano and microplastic exposure in rodent models: the known and unknown. Part Fibre Toxicol 19, 28.

Das, B.C., Ramanan, P.A., Gorakh, S.S., Pillai, D., Vattiringal Jayadradhan, R.K., 2023. Sub-chronic exposure of Oreochromis niloticus to environmentally relevant concentrations of smaller microplastics: Accumulation and toxico-physiological responses. J Hazard Mater 458, 131916.

De Biase, D., Piegari, G., Prisco, F., Cimmino, I., Pirozzi, C., Raso, G.M., Oriente, F., Grieco, E., Papparella, S., Paciello, O., 2020. Autophagy and NLRP3 inflammasome crosstalk in neuroinflammation in aged bovine brains. J Cell Physiol 235, 5394-5403.

De Falco, F., Gullo, M.P., Gentile, G., Di Pace, E., Cocca, M., Gelabert, L., Brouta-Agnesa, M., Rovira, A., Escudero, R., Villalba, R., Mossotti, R., Montarsolo, A., Gavignano, S., Tonin, C., Avella, M., 2018. Evaluation of microplastic release caused by textile washing processes of synthetic fabrics. Environ Pollut 236, 916-925.

de Mesquita, G.C., Menezes, R., da Cunha-Neto, M.A., Dantas-Neto, A.B., da Silva, G.B., 2021. Feeding strategy of pelagic fishes caught in aggregated schools and vulnerability to ingesting anthropogenic items in the western equatorial Atlantic Ocean. Environ Pollut 282, 117021.

Delli Bovi, A.P., Marciano, F., Mandato, C., Siano, M.A., Savoia, M., Vajro, P., 2021. Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. Front Med-Lausanne 8, 165.

Demirci-Cekic, S., Ozkan, G., Avan, A.N., Uzunboy, S., Capanoglu, E., Apak, R., 2022. Biomarkers of Oxidative Stress and Antioxidant Defense. J Pharm Biomed Anal 209, 114477.

Deng, H., Wei, R., Luo, W., Hu, L., Li, B., Di, Y., Shi, H., 2020. Microplastic pollution in water and sediment in a textile industrial area. Environ Pollut 258, 113658.

Deng, Y.F., Zhang, Y., Lemos, B., Ren, H.Q., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci Rep-Uk 7.

Dey, T.K., Rasel, M., Roy, T., Uddin, M.E., Pramanik, B.K., Jamal, M., 2023. Post-pandemic micro/nanoplastic pollution: Toward a sustainable management. Science of the Total Environment 867.

Djouina, M., Vignal, C., Dehaut, A., Caboche, S., Hirt, N., Waxin, C., Himber, C., Beury, D., Hot, D., Dubuquoy, L., Launay, D., Duflos, G., Body-Malapel, M., 2022. Oral exposure to polyethylene microplastics alters gut morphology, immune response, and microbiota composition in mice. Environ Res 212, 113230.

Dong, C.D., Chen, C.W., Chen, Y.C., Chen, H.H., Lee, J.S., Lin, C.H., 2020. Polystyrene microplastic particles: In vitro pulmonary toxicity assessment. J Hazard Mater 385, 121575.

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., Tassin, B., 2017. A first overview of textile fibers, including microplastics, in indoor and outdoor environments. Environ Pollut 221, 453-458.

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic contamination in an urban area: a case study in Greater Paris. Environ Chem 12, 592-599.

Du, J., Hu, Y.Q., Hou, M.M., Zhou, J.Y., Xiang, F.Y., Zheng, H., Zhang, X.K., He, X.L., Xiao, H., 2023. Combined effects of high-fat diet and polystyrene microplastic exposure on microplastic bioaccumulation and lipid metabolism in zebrafish. Fish Shellfish Immun 137.

EFSA, 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. Efsa Journal 14, e04501.

Espinosa, C., Cuesta, A., Esteban, M.A., 2017. Effects of dietary polyvinylchloride microparticles on general health, immune status and expression of several genes related to stress in gilthead seabream (Sparus aurata L.). Fish Shellfish Immun 68, 251-259.

Espinosa, C., Esteban, M.A., Cuesta, A., 2019. Dietary administration of PVC and PE microplastics produces histological damage, oxidative stress and immunoregulation in European sea bass (Dicentrarchus labrax L.). Fish Shellfish Immunol 95, 574-583.

Espinosa-Ruiz, C., Gonzalez-Fernandez, C., Cormier, B., Keiter, S.H., Vieira, L.R., Guilhermino, L., Clerandeau, C., Cachot, J., Esteban, M.A., Cuesta, A., 2023. Immunotoxicological effects of perfluorooctanesulfonic acid on European seabass are reduced by polyethylene microplastics. Fish Shellfish Immunol 137, 108793.

Fackelmann, G., Sommer, S., 2019. Microplastics and the gut microbiome: How chronically exposed species may suffer from gut dysbiosis. Marine Pollution Bulletin 143, 193-203.

Fan, Y., Zheng, K., Zhu, Z., Chen, G., Peng, X., 2019. Distribution, sedimentary record, and persistence of microplastics in the Pearl River catchment, China. Environ Pollut 251, 862-870.

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environmental Pollution 177, 1-3.

Faure, F., Demars, C., Wieser, O., Kunz, M., de Alencastro, L.F., 2015. Plastic pollution in Swiss surface waters: nature and concentrations, interaction with pollutants. Environ Chem 12, 582-591.

Felix, L., Carreira, P., Peixoto, F., 2023. Effects of chronic exposure of naturally weathered microplastics on oxidative stress level, behaviour, and mitochondrial function of adult zebrafish (Danio rerio). Chemosphere 310, 136895.

Ferrante, M.C., Monnolo, A., Del Piano, F., Raso, G.M., Meli, R., 2022. The Pressing Issue of Micro- and Nanoplastic Contamination: Profiling the Reproductive Alterations Mediated by Oxidative Stress. Antioxidants-Basel 11.

Fischer, E.K., Paglialonga, L., Czech, E., Tamminga, M., 2016. Microplastic pollution in lakes and lake shoreline sediments - A case study on Lake Bolsena and Lake Chiusi (central Italy). Environ Pollut 213, 648-657.

Fitri, S., Patria, M., 2019. Microplastic contamination on anadara granosa linnaeus 1758 in pangkal babu mangrove forest area, tanjung jabung barat district, jambi. Journal of Physics: Conference Series. IOP Publishing, p. 012109.

Foley, C.J., Feiner, Z.S., Malinich, T.D., Hook, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci Total Environ 631-632, 550-559.

Förlin, L., Goksøyr, A., Husøy, A., 1994. Cytochrome P450 monooxygenase as indicator of PCB/dioxin like compounds in fish. Biomonitoring of coastal waters and estuaries, 135-150.

Forner-Piquer, I., Mylonas, C.C., Calduch-Giner, J., Maradonna, F., Gioacchini, G., Allarà, M., Piscitelli, F., Di Marzo, V., Pérez-Sánchez, J., Carnevali, O., 2018. Endocrine disruptors in the diet of male Sparus aurata: Modulation of the endocannabinoid system at the hepatic and central level by Di-isononyl phthalate and Bisphenol A. Environment International 119, 54-65.

Franzellitti, S., Canesi, L., Auguste, M., Wathsala, R., Fabbri, E., 2019. Microplastic exposure and effects in aquatic organisms: A

physiological perspective. Environ Toxicol Pharmacol 68, 37-51.
Frias, J., Nash, R., 2019. Microplastics: Finding a consensus on the definition. Mar Pollut Bull 138, 145-147.
Frias, J.P., Otero, V., Sobral, P., 2014. Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. Mar Environ Res 95, 89-95.
Garcimartín, A., López-Oliva, M.E., González, M.P., Sánchez-Muniz, F.J., Benedí, J., 2017. Hydrogen peroxide modifies both activity and isoforms of acetylcholinesterase in human neuroblastoma SH-SY5Y cells. Redox Biology 12, 719-726.
Gasperi, J., Wright, S.L., Dris, R., Collard, F., Mandin, C., Guerrouache, M., Langlois, V., Kelly, F.J., Tassin, B., 2018. Microplastics in air: Are we breathing it in? Curr Opin Env Sci Hl 1, 1-5.
Geremia, E., Tomajoli, M.T.M., Murano, C., Petito, A., Fasciolo, G., 2023. The Impact of Micro- and Nanoplastics on Aquatic Organisms: Mechanisms of Oxidative Stress and Implications for Human Health-A Review. Environments 10.
GESAMP, G., 2016. Sources, fate and effects of microplastics in the marine environment: part two of a global assessment. IMO London, 220.
Gonzalez-Mariscal, L., Tapia, R., Chamorro, D., 2008. Crosstalk of tight junction components with signaling pathways. Biochim Biophys Acta 1778, 729-756.
Gu, W., Liu, S., Chen, L., Liu, Y., Gu, C., Ren, H.Q., Wu, B., 2020. Single-Cell RNA Sequencing Reveals Size-Dependent Effects of Polystyrene Microplastics on Immune and Secretory Cell Populations from Zebrafish Intestines. Environ Sci Technol 54, 3417-3427.
Guo, J.J., Huang, X.P., Xiang, L., Wang, Y.Z., Li, Y.W., Li, H., Cai, Q.Y., Mo, C.H., Wong, M.H., 2020. Source, migration and toxicology of microplastics in soil. Environ Int 137, 105263.
Guo, W.L., Gao, B.B., Zhang, X.Q., Ren, Q.Z., Xie, D.Z., Liang, J.P., Li, H., Wang, X.F., Zhang, Y.R., Liu, S.J., Nie, G.X., 2022a. Distinct responses from triglyceride and cholesterol metabolism in common carp (Cyprinus carpio) upon environmental cadmium exposure. Aquatic Toxicology 249.

Guo, Y., Xia, X., Ruan, J., Wang, Y., Zhang, J., LeBlanc, G.A., An, L., 2022b. Ignored microplastic sources from plastic bottle recycling. Sci Total Environ 838, 156038.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249, 7130-7139.
Hamed, M., Soliman, H.A.M., Osman, A.G.M., Sayed, A.E.D.H., 2019. Assessment the effect of exposure to microplastics in Nile Tilapia (Oreochromis niloticus) early juvenile: I. blood biomarkers. Chemosphere 228, 345-350.
Hao, Y., Sun, Y., Li, M., Fang, X., Wang, Z., Zuo, J., Zhang, C., 2023. Adverse effects of polystyrene microplastics in the freshwater commercial fish, grass carp (Ctenopharyngodon idella): Emphasis on physiological response and intestinal microbiome. Sci Total Environ 856, 159270.
He, D.F., Luo, Y.M., Lu, S.B., Liu, M.T., Song, Y., Lei, L.L., 2018. Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. Trac-Trend Anal Chem 109, 163-172.
He, Y., Li, Z., Xu, T., Luo, D., Chi, Q., Zhang, Y., Li, S., 2022. Polystyrene nanoplastics deteriorate LPS-modulated duodenal permeability and inflammation in mice via ROS drived-NF-kappaB/NLRP3 pathway. Chemosphere 307, 135662.
Heindler, F.M., Alajmi, F., Huerlimann, R., Zeng, C., Newman, S.J., Vamvounis, G., van Herwerden, L., 2017. Toxic effects of polyethylene terephthalate microparticles and Di(2-ethylhexyl)phthalate on the calanoid copepod, Parvocalanus crassirostris. Ecotoxicol Environ Saf 141, 298-305.
Hernandez, L.M., Xu, E.G., Larsson, H.C.E., Tahara, R., Maisuria, V.B., Tufenkji, N., 2019. Plastic Teabags Release Billions of Microparticles and Nanoparticles into Tea. Environmental Science & Technology 53, 12300-12310.
Hirt, N., Body-Malapel, M., 2020. Immunotoxicity and intestinal effects of nano- and microplastics: a review of the literature. Part Fibre Toxicol 17, 57.
Hodkovicova, N., Hollerova, A., Caloudova, H., Blahova, J., Franc, A., Garajova, M., Lenz, J., Tichy, F., Faldyna, M., Kulich, P., Mares, J., Machat, R., Enevova, V., Svobodova, Z., 2021. Do foodborne polyethylene microparticles affect the health of rainbow trout (Oncorhynchus mykiss)? Science of the Total Environment 793.

Hollerova, A., Hodkovicova, N., Blahova, J., Faldyna, M., Franc, A., Pavlokova, S., Tichy, F., Postulkova, E., Mares, J., Medkova, D., Kyllar, M., Svobodova, Z., 2023. Polystyrene microparticles can affect the health status of freshwater fish-Threat of oral microplastics intake. Science of the Total Environment 858.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Science of the Total Environment 586, 127-141.

Horton, J.D., Goldstein, J.L., Brown, M.S., 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 109, 1125-1131.

Horvatits, T., Tamminga, M., Liu, B.B., Sebode, M., Carambia, A., Fischer, L., Püschel, K., Huber, S., Fischer, E.K., 2022. Microplastics detected in cirrhotic liver tissue. Ebiomedicine 82.

Hoseini, S.M., Sinha, R., Fazel, A., Khosraviani, K., Hosseinpour Delavar, F., Arghideh, M., Sedaghat, M., Paolucci, M., Hoseinifar, S.H., Van Doan, H., 2022. Histopathological damage and stress- and immune-related genes' expression in the intestine of common carp, Cyprinus carpio exposed to copper and polyvinyl chloride microparticle. J Exp Zool A Ecol Integr Physiol 337, 181-190.

Hossain, Z., Hossain, M.S., Ema, N.S., Omri, A., 2021. Heavy metal toxicity in Buriganga river alters the immunology of Nile tilapia (Oreochromis niloticus L). Heliyon 7, e08285.

Hou, M., Xu, C., Zou, X., Xia, Z., Su, L., Qiu, N., Cai, L., Yu, F., Wang, Q., Zhao, X., He, Y., Wang, C., Wang, J., 2022. Long-term exposure to microplastics induces intestinal function dysbiosis in rare minnow (Gobiocypris rarus). Ecotoxicol Environ Saf 246, 114157.

Hu, M., Palic, D., 2020. Micro- and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. Redox Biol 37, 101620.

Huang, D.L., Wang, X.Y., Yin, L.S., Chen, S., Tao, J.X., Zhou, W., Chen, H.J., Zhang, G.X., Xiao, R.H., 2022. Research progress of microplastics in soil-plant system: Ecological effects and potential risks. Science of the Total Environment 812.

Huang, J.N., Wen, B., Zhu, J.G., Zhang, Y.S., Gao, J.Z., Chen, Z.Z., 2020. Exposure to microplastics impairs digestive performance,

stimulates immune response and induces microbiota dysbiosis in the gut of juvenile guppy (Poecilia reticulata). Sci Total Environ 733, 138929.

Huang, W., Song, B.A., Liang, J., Niu, Q.Y., Zeng, G.M., Shen, M.C., Deng, J.Q., Luo, Y.A., Wen, X.F., Zhang, Y.F., 2021. Microplastics and associated contaminants in the aquatic environment: A review on their ecotoxicological effects, trophic transfer, and potential impacts to human health. Journal of Hazardous Materials 405.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the Terrestrial Ecosystem: Implications for Lumbricus terrestris (Oligochaeta, Lumbricidae). Environ Sci Technol 50, 2685-2691.

Huerta Lwanga, E., Mendoza Vega, J., Ku Quej, V., Chi, J.L.A., Sanchez Del Cid, L., Chi, C., Escalona Segura, G., Gertsen, H., Salanki, T., van der Ploeg, M., Koelmans, A.A., Geissen, V., 2017. Field evidence for transfer of plastic debris along a terrestrial food chain. Sci Rep 7, 14071.

Hunt, P.R., 2017. The C. elegans model in toxicity testing. J Appl Toxicol 37, 50-59.

Hurley, R.R., Woodward, J.C., Rothwell, J.J., 2017. Ingestion of Microplastics by Freshwater Tubifex Worms. Environmental Science & Technology 51, 12844-12851.

Iannilli, V., Corami, F., Grasso, P., Lecce, F., Buttinelli, M., Setini, A., 2020. Plastic abundance and seasonal variation on the shorelines of three volcanic lakes in Central Italy: can amphipods help detect contamination? Environ Sci Pollut R 27, 14711-14722.

Iheanacho, S.C., Odo, G.E., 2020. Dietary exposure to polyvinyl chloride microparticles induced oxidative stress and hepatic damage in Clarias gariepinus (Burchell, 1822). Environ Sci Pollut R 27, 21159-21173.

Jabeen, K., Li, B., Chen, Q., Su, L., Wu, C., Hollert, H., Shi, H., 2018. Effects of virgin microplastics on goldfish (Carassius auratus). Chemosphere 213, 323-332.

Jacob, H., Besson, M., Oberhaensli, F., Taylor, A., Gillet, B., Hughes, S., Melvin, S.D., Bustamante, P., Swarzenski, P.W., Lecchini, D., Metian, M., 2021. A multifaceted assessment of the effects of polyethylene microplastics on juvenile gilthead seabreams (Sparus aurata). Aquat Toxicol 241, 106004.

Jemec, A., Horvat, P., Kunej, U., Bele, M., Krzan, A., 2016. Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna. Environ Pollut 219, 201-209.

Jensen-Urstad, A.P.L., Semenkovich, C.F., 2012. Fatty acid synthase and liver triglyceride metabolism: Housekeeper or messenger? Bba-Mol Cell Biol L 1821, 747-753.

Jeong, C.B., Kang, H.M., Lee, M.C., Kim, D.H., Han, J., Hwang, D.S., Souissi, S., Lee, S.J., Shin, K.H., Park, H.G., Lee, J.S., 2017. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod Paracyclopina nana. Sci Rep 7, 41323.

Jeong, J., Choi, J., 2019. Adverse outcome pathways potentially related to hazard identification of microplastics based on toxicity mechanisms. Chemosphere 231, 249-255.

Jeyavani, J., Sibiya, A., Bhavaniramya, S., Mahboob, S., Al-Ghanim, K.A., Nisa, Z.U., Riaz, M.N., Nicoletti, M., Govindarajan, M., Vaseeharan, B., 2022. Toxicity evaluation of polypropylene microplastic on marine microcrustacean Artemia salina: An analysis of implications and vulnerability. Chemosphere 296, 133990.

Jeyavani, J., Sibiya, A., Stalin, T., Vigneshkumar, G., Al-Ghanim, K.A., Riaz, M.N., Govindarajan, M., Vaseeharan, B., 2023. Biochemical, Genotoxic and Histological Implications of Polypropylene Microplastics on Freshwater Fish Oreochromis mossambicus: An Aquatic Eco-Toxicological Assessment. Toxics 11.

Jia, R., Han, J., Liu, X.H., Li, K., Lai, W.Q., Bian, L.P., Yan, J., Xi, Z.G., 2023. Exposure to Polypropylene Microplastics via Oral Ingestion Induces Colonic Apoptosis and Intestinal Barrier Damage through Oxidative Stress and Inflammation in Mice. Toxics 11.

Jin, H.B., Yang, C., Jiang, C.Y., Li, L.X., Pan, M.G., Li, D.M., Han, X.D., Ding, J., 2022. Evaluation of Neurotoxicity in BALB/c Mice following Chronic Exposure to Polystyrene Microplastics (vol 130, 107002, 2022). Environ Health Persp 130.

Jin, M., Wang, X., Ren, T., Wang, J., Shan, J., 2021. Microplastics contamination in food and beverages: Direct exposure to humans. J Food Sci 86, 2816-2837.

Jin, Y., Lu, L., Tu, W., Luo, T., Fu, Z., 2019. Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism of mice. Sci Total Environ 649, 308-317.

Jin, Y., Wu, S., Zeng, Z., Fu, Z., 2017. Effects of environmental pollutants on gut microbiota. Environ Pollut 222, 1-9.

Jin, Y., Xia, J., Pan, Z., Yang, J., Wang, W., Fu, Z., 2018. Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. Environ Pollut 235, 322-329.

Johansson, M.E., Hansson, G.C., 2016. Immunological aspects of intestinal mucus and mucins. Nat Rev Immunol 16, 639-649.

Jovanovic, B., 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integr Environ Assess Manag 13, 510-515.

Jovanovic, B., Gokdag, K., Guven, O., Emre, Y., Whitley, E.M., Kideys, A.E., 2018. Virgin microplastics are not causing imminent harm to fish after dietary exposure. Mar Pollut Bull 130, 123-131.

Ju, H., Zhu, D., Qiao, M., 2019. Effects of polyethylene microplastics on the gut microbial community, reproduction and avoidance behaviors of the soil springtail,. Environmental Pollution 247, 890-897.

Jung, J.W., Park, J.W., Eo, S., Choi, J., Song, Y.K., Cho, Y., Hong, S.H., Shim, W.J., 2021. Ecological risk assessment of microplastics in coastal, shelf, and deep sea waters with a consideration of environmentally relevant size and shape. Environ Pollut 270, 116217.

Kang, H.M., Byeon, E., Jeong, H., Kim, M.S., Chen, Q., Lee, J.S., 2021. Different effects of nano- and microplastics on oxidative status and gut microbiota in the marine medaka Oryzias melastigma. J Hazard Mater 405, 124207.

Karkucinska-Wieckowska, A., Simoes, I.C.M., Kalinowski, P., Lebiedzinska-Arciszewska, M., Zieniewicz, K., Milkiewicz, P., Gorska-Ponikowska, M., Pinton, P., Malik, A.N., Krawczyk, M., Oliveira, P.J., Wieckowski, M.R., 2022. Mitochondria, oxidative stress and nonalcoholic fatty liver disease: A complex relationship. Eur J Clin Invest 52, e13622.

Kessabi, K., Abbassi, A., Lahmar, S., Casado, M., Banni, M., Piña, B., Messaoudi, I., 2023. Combined toxic effects of cadmium and environmental microplastics in Aphanius fasciatus (Pisces, Cyprinodontidae). Marine Environmental Research 189.

Khalid, N., Aqeel, M., Noman, A., 2020. Microplastics could be a threat to plants in terrestrial systems directly or indirectly. Environmental Pollution 267.

Khanmohammadi, S., Kuchay, M.S., 2022. Toll-like receptors and metabolic (dysfunction)-associated fatty liver disease. Pharmacol Res 185.

Kibria, G., 2023. Impacts of microplastic on fisheries and seafood security - Global analysis and synthesis. Science of the Total Environment 904.

Klein, S., Worch, E., Knepper, T.P., 2015. Occurrence and Spatial Distribution of Microplastics in River Shore Sediments of the Rhine-Main Area in Germany. Environ Sci Technol 49, 6070-6076.

Kogel, T., Bjoroy, O., Toto, B., Bienfait, A.M., Sanden, M., 2020. Micro- and nanoplastic toxicity on aquatic life: Determining factors. Sci Total Environ 709, 136050.

Konig Kardgar, A., Ghosh, D., Sturve, J., Agarwal, S., Carney Almroth, B., 2023. Chronic poly(l-lactide) (PLA)- microplastic ingestion affects social behavior of juvenile European perch (Perca fluviatilis). Sci Total Environ 881, 163425.

Kord, M.I., Srour, T.M., Omar, E.A., Farag, A.A., Nour, A.A.M., Khalil, H.S., 2021. The Immunostimulatory Effects of Commercial Feed Additives on Growth Performance, Non-specific Immune Response, Antioxidants Assay, and Intestinal Morphometry of Nile tilapia,. Frontiers in Physiology 12.

Kvale, K., Prowe, A.E.F., Chien, C.T., Landolfi, A., Oschlies, A., 2021. Zooplankton grazing of microplastic can accelerate global loss of ocean oxygen. Nature Communications 12.

Lacerda, A., Rodrigues, L.D.S., van Sebille, E., Rodrigues, F.L., Ribeiro, L., Secchi, E.R., Kessler, F., Proietti, M.C., 2019. Plastics in sea surface waters around the Antarctic Peninsula. Sci Rep 9, 3977.

Lai, W.C., Xu, D., Li, J.M., Wang, Z., Ding, Y., Wang, X.N., Li, X.S., Xu, N., Mai, K.S., Ai, Q.H., 2021. Dietary polystyrene nanoplastics exposure alters liver lipid metabolism and muscle nutritional quality in carnivorous marine fish large yellow croaker (Larimichthys crocea). Journal of Hazardous Materials 419.

Latchere, O., Audroin, T., Hétier, J., Métais, I., Châtel, A., 2021. The need to investigate continuums of plastic particle diversity,

brackish environments and trophic transfer to assess the risk of micro and nanoplastics on aquatic organisms. Environmental Pollution 273.
Leaver, M.J., Boukouvala, E., Antonopoulou, E., Diez, A., Favre-Krey, L., Ezaz, M.T., Bautista, J.M., Tocher, D.R., Krey, G., 2005. Three peroxisome proliferator-activated receptor isotypes from each of two species of marine fish. Endocrinology 146, 3150-3162.
Lebreton, L.C.M., van der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat Commun 8, 15611.
Lee, J.H., Kang, J.C., Kim, J.H., 2023. Toxic effects of microplastic (Polyethylene) on fish: Accumulation, hematological parameters and antioxidant responses in Korean Bullhead, Pseudobagrus fulvidraco. Sci Total Environ 877, 162874.
Lehel, J., Murphy, S., 2021. Microplastics in the Food Chain: Food Safety and Environmental Aspects. Rev Environ Contam Toxicol 259, 1-49.
Lei, K., Qiao, F., Liu, Q., Wei, Z.L., Qi, H.L., Cui, S., Yue, X., Deng, Y.X., An, L.H., 2017. Microplastics releasing from personal care and cosmetic products in China. Marine Pollution Bulletin 123, 122-126.
Leiser, R., Schumann, M., Dadi, T., Wendt-Potthoff, K., 2021. Burial of microplastics in freshwater sediments facilitated by iron-organo flocs. Sci Rep 11, 24072.
Leon-Coria, A., Kumar, M., Workentine, M., Moreau, F., Surette, M., Chadee, K., 2021. Muc2 Mucin and Nonmucin Microbiota Confer Distinct Innate Host Defense in Disease Susceptibility and Colonic Injury. Cell Mol Gastroenterol Hepatol 11, 77-98.
Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S., Stadtman, E.R., 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186, 464-478.
Li, B.Q., Ding, Y.F., Cheng, X., Sheng, D.D., Xu, Z., Rong, Q.Y., Wu, Y.L., Zhao, H.L., Ji, X.F., Zhang, Y., 2020a. Polyethylene microplastics affect the distribution of gut microbiota and inflammation development in mice. Chemosphere 244.
Li, J., Song, Y., Cai, Y., 2020b. Focus topics on microplastics in soil: Analytical methods, occurrence, transport, and ecological risks. Environ Pollut 257, 113570.

Li, S., Xie, J., Li, K., Bai, Y., Jiang, Z., Xiong, X., 2023. Mulberrin alleviates triclocarban induced hepatic apoptosis and inflammation by regulating the ROS/NF-kappaB pathway in grass carp. Comp Biochem Physiol C Toxicol Pharmacol 273, 109734.

Li, S.W., Shi, M., Wang, Y.L., Xiao, Y.X., Cai, D.H., Xiao, F., 2021. Keap1-Nrf2 pathway up-regulation via hydrogen sulfide mitigates polystyrene microplastics induced-hepatotoxic effects. Journal of Hazardous Materials 402.

Li, X.X., Feng, L.X., Kuang, Q.H., Wang, X., Yang, J., Niu, X., Gao, L.K., Huang, L.Z., Luo, P.C., Li, L.L., 2024. Microplastics cause hepatotoxicity in diabetic mice by disrupting glucolipid metabolism via PP2A/AMPK/HNF4A and promoting fibrosis via the Wnt/β-catenin pathway. Environ Toxicol 39, 1018-1030.

Li, Z., Zhu, S., Liu, Q., Wei, J., Jin, Y., Wang, X., Zhang, L., 2020c. Polystyrene microplastics cause cardiac fibrosis by activating Wnt/beta-catenin signaling pathway and promoting cardiomyocyte apoptosis in rats. Environ Pollut 265, 115025.

Lillicrap, A., Belanger, S., Burden, N., Du Pasquier, D., Embry, M.R., Halder, M., Lampi, M.A., Lee, L., Norberg-King, T., Rattner, B.A., Schirmer, K., Thomas, P., 2016. Alternative Approaches to Vertebrate Ecotoxicity Tests in the 21st Century: A Review of Developments Over the Last 2 Decades and Current Status. Environ Toxicol Chem 35, 2637-2646.

Limonta, G., Mancia, A., Benkhalqui, A., Bertolucci, C., Abelli, L., Fossi, M.C., Panti, C., 2019. Microplastics induce transcriptional changes, immune response and behavioral alterations in adult zebrafish. Sci Rep 9, 15775.

Lithner, D., Larsson, A., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci Total Environ 409, 3309-3324.

Liu, K., Wang, X., Fang, T., Xu, P., Zhu, L., Li, D., 2019a. Source and potential risk assessment of suspended atmospheric microplastics in Shanghai. Sci Total Environ 675, 462-471.

Liu, S., Li, H., Wang, J., Wu, B., Guo, X., 2022a. Polystyrene microplastics aggravate inflammatory damage in mice with intestinal immune imbalance. Sci Total Environ 833, 155198.

Liu, X., Liang, C., Zhou, M., Chang, Z., Li, L., 2023. Exposure of Cyprinus carpio var. larvae to PVC microplastics reveals

significant immunological alterations and irreversible histological organ damage. Ecotoxicol Environ Saf 249, 114377.
Liu, Y., Jia, X.W., Zhu, H., Zhang, Q.J., He, Y., Shen, Y.B., Xu, X.Y., Li, J.L., 2022b. The effects of exposure to microplastics on grass carp (Ctenopharyngodon idella) at the physiological, biochemical, and transcriptomic levels. Chemosphere 286.
Liu, Z.Q., Yu, P., Cai, M.Q., Wu, D.L., Zhang, M., Chen, M.H., Zhao, Y.L., 2019b. Effects of microplastics on the innate immunity and intestinal microflora of juvenile. Science of the Total Environment 685, 836-846.
Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollution Bulletin 42, 656-666.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193, 265-275.
Lu, J.W., Yang, W.Y., Lin, Y.M., Jin, S.L.C., Yuh, C.H., 2013. Hepatitis B virus X antigen and aflatoxin B1 synergistically cause hepatitis, steatosis and liver hyperplasia in transgenic zebrafish. Acta Histochem 115, 728-739.
Lu, L., Wan, Z.Q., Luo, T., Fu, Z.W., Jin, Y.X., 2018. Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. Science of the Total Environment 631-632, 449-458.
Lu, X., Deng, D.F., Huang, F., Casu, F., Kraco, E., Newton, R.J., Zohn, M., Teh, S.J., Watson, A.M., Shepherd, B., Ma, Y., Dawood, M.A.O., Mendoza, L.M.R., 2022. Chronic exposure to high-density polyethylene microplastic through feeding alters the nutrient metabolism of juvenile yellow perch (Perca flavescens). Anim Nutr 9, 143-158.
Lu, Y.F., Zhang, Y., Deng, Y.F., Jiang, W., Zhao, Y.P., Geng, J.J., Ding, L.L., Ren, H.Q., 2016. Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (Danio rerio) and Toxic Effects in Liver. Environmental Science & Technology 50, 4054-4060.
Luo, H.W., Tu, C.L., He, D.Q., Zhang, A.P., Sun, J.Q., Li, J., Xu, J., Pan, X.L., 2023. Interactions between microplastics and contaminants: A review focusing on the effect of aging process. Science of the Total Environment 899.

Luo, T., Wang, C.Y., Pan, Z.H., Jin, C.Y., Fu, Z.W., Jin, Y.X., 2019a. Maternal Polystyrene Microplastic Exposure during Gestation and Lactation Altered Metabolic Homeostasis in the Dams and Their F1 and F2 Offspring. Environmental Science & Technology 53, 10978-10992.

Luo, T., Zhang, Y., Wang, C., Wang, X., Zhou, J., Shen, M., Zhao, Y., Fu, Z., Jin, Y., 2019b. Maternal exposure to different sizes of polystyrene microplastics during gestation causes metabolic disorders in their offspring. Environ Pollut 255, 113122.

Lusher, A., 2015. Microplastics in the Marine Environment: Distribution, Interactions and Effects. Marine Anthropogenic Litter, 245-307.

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal Methods-Uk 9, 1346-1360.

Ma, C., Chen, Q., Li, J., Li, B., Liang, W., Su, L., Shi, H., 2021. Distribution and translocation of micro- and nanoplastics in fish. Crit Rev Toxicol 51, 740-753.

Ma, M., Wu, Z., An, L., Xu, Q., Wang, H., Zhang, Y., Kang, Y., 2023. Microplastics transferring from abiotic to biotic in aquatic ecosystem: A mini review. Sci Total Environ 893, 164686.

Ma, Q., 2013. Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol 53, 401-426.

Martel, J., Chang, S.H., Ko, Y.F., Hwang, T.L., Young, J.D., Ojcius, D.M., 2022. Gut barrier disruption and chronic disease. Trends Endocrinol Metab 33, 247-265.

Martyniuk, V., Khoma, V., Matskiv, T., Baranovsky, V., Orlova-Hudim, K., Gylyte, B., Symchak, R., Matciuk, O., Gnatyshyna, L., Manusadzianas, L., Stoliar, O., 2022. Indication of the impact of environmental stress on the responses of the bivalve mollusk Unio tumidus to ibuprofen and microplastics based on biomarkers of reductive stress and apoptosis. Comp Biochem Phys C 261.

Martyniuk, V., Khoma, V., Matskiv, T., Yunko, K., Gnatyshyna, L., Stoliar, O., Faggio, C., 2023. Combined effect of microplastic, salinomycin and heating on. Environ Toxicol Phar 98.

Mato, J.M., Alonso, C., Noureddin, M., Lu, S.C., 2019. Biomarkers and subtypes of deranged lipid metabolism in non-alcoholic fatty liver disease. World J Gastroentero 25, 3009-3020.

Matsuguma, Y., Takada, H., Kumata, H., Kanke, H., Sakurai, S., Suzuki, T., Itoh, M., Okazaki, Y., Boonyatumanond, R., Zakaria, M.P., Weerts, S., Newman, B., 2017. Microplastics in Sediment Cores from Asia and Africa as Indicators of Temporal Trends in Plastic Pollution. Arch Environ Con Tox 73, 230-239.

Miller, M.E., Hamann, M., Kroon, F.J., 2020. Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. Plos One 15.

Minicozzi, M.R., von Hippel, F.A., Furin, C.G., Buck, C.L., 2019. Sodium perchlorate induces non-alcoholic fatty liver disease in developing stickleback. Environ Pollut 251, 390-399.

Mistri, M., Sfriso, A.A., Casoni, E., Nicoli, M., Vaccaro, C., Munari, C., 2022. Microplastic accumulation in commercial fish from the Adriatic Sea. Mar Pollut Bull 174, 113279.

Mohana, A.A., Islam, M.M., Rahman, M., Pramanik, S.K., Haque, N., Gao, L., Pramanik, B.K., 2023. Generation and consequence of nano/microplastics from medical waste and household plastic during the COVID-19 pandemic. Chemosphere 311, 137014.

Montero, D., Rimoldi, S., Torrecillas, S., Rapp, J., Moroni, F., Herrera, A., Gomez, M., Fernandez-Montero, A., Terova, G., 2022. Impact of polypropylene microplastics and chemical pollutants on European sea bass (Dicentrarchus labrax) gut microbiota and health. Sci Total Environ 805, 150402.

Morgana, S., Ghigliotti, L., Estevez-Calvar, N., Stifanese, R., Wieckzorek, A., Doyle, T., Christiansen, J.S., Faimali, M., Garaventa, F., 2018. Microplastics in the Arctic: A case study with sub-surface water and fish samples off Northeast Greenland. Environ Pollut 242, 1078-1086.

Mu, Y.W., Sun, J.Y., Li, Z.Y., Zhang, W.X., Liu, Z.D., Li, C., Peng, C., Cui, G.Q., Shao, H., Du, Z.J., 2022. Activation of pyroptosis and ferroptosis is involved in the hepatotoxicity induced by polystyrene microplastics in mice. Chemosphere 291.

Murano, C., Agnisola, C., Caramiello, D., Castellano, I., Casotti, R., Corsi, I., Palumbo, A., 2020. How sea urchins face microplastics: Uptake, tissue distribution and immune system response. Environmental Pollution 264.

Naidoo, T., Glassom, D., 2019. Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. Mar Pollut Bull 145, 254-259.

Nash, R.D.M., Valencia, A.H., Geffen, A.J., 2006. The origin of Fulton's condition factor - Setting the record straight. Fisheries 31, 236-238.

Nawab, J., Khan, H., Ghani, J., Zafar, M.I., Khan, S., Toller, S., Fatima, L., Hamza, A., 2023. New insights into the migration, distribution and accumulation of micro-plastic in marine environment: A critical mechanism review. Chemosphere 330, 138572.

Nizzetto, L., Langaas, S., Futter, M., 2016. Do microplastics spill on to farm soils? Nature 537, 488-488.

Noventa, S., Boyles, M.S., Seifert, A., Belluco, S., Jiménez, A.S., Johnston, H.J., Tran, L., Fernandes, T.F., Mughini-Gras, L., Orsini, M., 2021. Paradigms to assess the human health risks of nano-and microplastics. Microplastics and Nanoplastics 1, 1-27.

Odenwald, M.A., Turner, J.R., 2017. The intestinal epithelial barrier: a therapeutic target? Nat Rev Gastroenterol Hepatol 14, 9-21.

Pan, L.G., Yu, D.K., Zhang, Y., Zhu, C.Y., Yin, Q.N., Hu, Y., Zhang, X.Q., Yue, R.M., Xiong, X., 2021. Polystyrene microplastics-triggered mitophagy and oxidative burst via activation of PERK pathway. Science of the Total Environment 781.

Pan, Z., Guo, H., Chen, H., Wang, S., Sun, X., Zou, Q., Zhang, Y., Lin, H., Cai, S., Huang, J., 2019. Microplastics in the Northwestern Pacific: Abundance, distribution, and characteristics. Sci Total Environ 650, 1913-1922.

Pannetier, P., Morin, B., Le Bihanic, F., Dubreil, L., Clérandeau, C., Chouvellon, F., Van Arkel, K., Danion, M., Cachot, J., 2020. Environmental samples of microplastics induce significant toxic effects in fish larvae. Environment International 134.

Paul-Pont, I., Lacroix, C., Gonzalez Fernandez, C., Hegaret, H., Lambert, C., Le Goic, N., Frere, L., Cassone, A.L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A., Soudant, P., 2016. Exposure of marine mussels Mytilus spp. to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation. Environ Pollut 216, 724-737.

Peda, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., Perdichizzi, A., Romeo, T., Maricchiolo, G., 2016. Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environ Pollut 212, 251-256.

Peda, C., Romeo, T., Panti, C., Caliani, I., Casini, S., Marsili, L., Campani, T., Baini, M., Limonta, G., de Rysky, E., Caccamo, L., Perdichizzi, A., Gai, F.C.S., Maricchiolo, G., Consoli, P., Fossi, M.C., 2022. Integrated biomarker responses in European seabass Dicentrarchus labrax (Linnaeus, 1758) chronically exposed to PVC microplastics. Journal of Hazardous Materials 438.

Peiseler, M., Schwabe, R.F., Hampe, J., Kubes, P., Heikenwaelder, M., Tacke, F., 2022. Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease - novel insights into cellular communication circuits. Journal of Hepatology 77, 1136-1160.

Pelegrini, K., Pereira, T.C.B., Maraschin, T.G., Teodoro, L.S., Basso, N.R.S., De Galland, G.L.B., Ligabue, R.A., Bogo, M.R., 2023. Micro- and nanoplastic toxicity: A review on size, type, source, and test-organism implications. Sci Total Environ 878, 162954.

Pereira, J.M., Rodriguez, Y., Blasco-Monleon, S., Porter, A., Lewis, C., Pham, C.K., 2020. Microplastic in the stomachs of open-ocean and deep-sea fishes of the North-East Atlantic. Environ Pollut 265, 115060.

Perez-Sanchez, J., Benedito-Palos, L., Estensoro, I., Petropoulos, Y., Calduch-Giner, J.A., Browdy, C.L., Sitja-Bobadilla, A., 2015. Effects of dietary NEXT ENHANCE(R)150 on growth performance and expression of immune and intestinal integrity related genes in gilthead sea bream (Sparus aurata L.). Fish Shellfish Immunol 44, 117-128.

Petersen, F., Hubbart, J.A., 2021. The occurrence and transport of microplastics: The state of the science. Sci Total Environ 758, 143936.

Pironti, C., Notarstefano, V., Ricciardi, M., Motta, O., Giorgini, E., Montano, L., 2022. First Evidence of Microplastics in Human Urine, a Preliminary Study of Intake in the Human Body. Toxics 11.

Pirozzi, C., Lama, A., Simeoli, R., Paciello, O., Pagano, T.B., Mollica, M.P., Di Guida, F., Russo, R., Magliocca, S., Canani, R.B., Raso, G.M., Calignano, A., Meli, R., 2016. Hydroxytyrosol prevents metabolic impairment reducing hepatic inflammation and restoring duodenal integrity in a rat model of NAFLD. J Nutr Biochem 30, 108-115.

Pirsaheb, M., Hossini, H., Makhdoumi, P., 2020. Review of microplastic occurrence and toxicological effects in marine

environment: Experimental evidence of inflammation. Process Safety and Environmental Protection 142, 1-14.

Prata, J.C., 2018. Airborne microplastics: Consequences to human health? Environmental Pollution 234, 115-126.

Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2020. Environmental exposure to microplastics: An overview on possible human health effects. Sci Total Environ 702, 134455.

Priya, A.K., Muruganandam, M., Imran, M., Gill, R., Vasudeva Reddy, M.R., Shkir, M., Sayed, M.A., AlAbdulaal, T.H., Algarni, H., Arif, M., Jha, N.K., Sehgal, S.S., 2023. A study on managing plastic waste to tackle the worldwide plastic contamination and environmental remediation. Chemosphere 341, 139979.

Prokic, M.D., Gavrilovic, B.R., Radovanovic, T.B., Gavric, J.P., Petrovic, T.G., Despotovic, S.G., Faggio, C., 2021. Studying microplastics: Lessons from evaluated literature on animal model organisms and experimental approaches. Journal of Hazardous Materials 414.

Qiao, R., Deng, Y., Zhang, S., Wolosker, M.B., Zhu, Q., Ren, H., Zhang, Y., 2019a. Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. Chemosphere 236, 124334.

Qiao, R., Sheng, C., Lu, Y., Zhang, Y., Ren, H., Lemos, B., 2019b. Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. Sci Total Environ 662, 246-253.

Qin, J., Ru, S., Wang, W., Hao, L., Ru, Y., Wang, J., Zhang, X., 2020. Long-term bisphenol S exposure aggravates non-alcoholic fatty liver by regulating lipid metabolism and inducing endoplasmic reticulum stress response with activation of unfolded protein response in male zebrafish. Environ Pollut 263, 114535.

Qiu, Y.Y., Zhang, J., Zeng, F.Y., Zhu, Y.Z., 2023. Roles of the peroxisome proliferator-activated receptors (PPARs) in the pathogenesis of nonalcoholic fatty liver disease (NAFLD). Pharmacol Res 192.

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M.C.A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., Giorgini, E., 2021. Plasticenta: First evidence of microplastics in human placenta. Environ Int 146, 106274.

Rajak, S., Raza, S., Tewari, A., Sinha, R.A., 2022. Environmental Toxicants and NAFLD: A Neglected yet Significant Relationship. Dig Dis Sci 67, 3497-3507.

Ren, Z.F., Gui, X.Y., Xu, X.Y., Zhao, L., Qiu, H., Cao, X.D., 2021. Microplastics in the soil-groundwater environment: Aging, migration, and co-transport of contaminants-A critical review. Journal of Hazardous Materials 419.

Revel, M., Lagarde, F., Perrein-Ettajani, H., Bruneau, M., Akcha, F., Sussarellu, R., Rouxel, J., Costil, K., Decottignies, P., Cognie, B., Châtel, A., Mouneyrac, C., 2019. Tissue-Specific Biomarker Responses in the Blue Mussel Mytilus spp. Exposed to a Mixture of Microplastics at Environmentally Relevant Concentrations. Front Env Sci-Switz 7.

Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M., Bebianno, M.J., 2017. Microplastics effects in Scrobicularia plana. Mar Pollut Bull 122, 379-391.

Rios-Fuster, B., Alomar, C., Paniagua Gonzalez, G., Garcinuno Martinez, R.M., Soliz Rojas, D.L., Fernandez Hernando, P., Deudero, S., 2022. Assessing microplastic ingestion and occurrence of bisphenols and phthalates in bivalves, fish and holothurians from a Mediterranean marine protected area. Environ Res 214, 114034.

Rios-Fuster, B., Arechavala-Lopez, P., Garcia-Marcos, K., Alomar, C., Compa, M., Alvarez, E., Julia, M.M., Solomando Marti, A., Sureda, A., Deudero, S., 2021. Experimental evidence of physiological and behavioral effects of microplastic ingestion in Sparus aurata. Aquat Toxicol 231, 105737.

Roca, F.J., Sepulcre, P., López-Castejón, G., Meseguer, J., Mulero, V., 2006. The colony-stimulating factor-1 receptor is a specific marker of macrophages from the bony fish gilthead seabream. Mol Immunol 43, 1418-1423.

Rochman, C.M., Browne, M.A., Halpern, B.S., Hentschel, B.T., Hoh, E., Karapanagioti, H.K., Rios-Mendoza, L.M., Takada, H., Teh, S., Thompson, R.C., 2013. Policy: Classify plastic waste as hazardous. Nature 494, 169-171.

Rochman, C.M., Parnis, J.M., Browne, M.A., Serrato, S., Reiner, E.J., Robson, M., Young, T., Diamond, M.L., Teh, S.J., 2017. Direct and indirect effects of different types of microplastics on

freshwater prey (Corbicula fluminea) and their predator (Acipenser transmontanus). Plos One 12.

Sahabuddin, E.S., Noreen, A., Daabo, H.M.A., Kandeel, M., Saleh, M.M., Al-Qaim, Z.H., Jawad, M.A., Sivaraman, R., Fenjan, M.N., Mustafa, Y.F., Heidary, A., Abarghouei, S., Norbakhsh, M., 2023. Microplastic and oil pollutant agglomerates synergistically intensify toxicity in the marine fish, Asian seabass, Lates calcalifer. Environ Toxicol Pharmacol 98, 104059.

Sangkham, S., Faikhaw, O., Munkong, N., Sakunkoo, P., Arunlertaree, C., Chavali, M., Mousazadeh, M., Tiwari, A., 2022. A review on microplastics and nanoplastics in the environment: Their occurrence, exposure routes, toxic studies, and potential effects on human health. Marine Pollution Bulletin 181.

Santana, M.F.M., Moreira, F.T., Turra, A., 2017. Trophic transference of microplastics under a low exposure scenario: Insights on the likelihood of particle cascading along marine food-webs. Marine Pollution Bulletin 121, 154-159.

Santana, M.S., Sandrini-Neto, L., Filipak Neto, F., Oliveira Ribeiro, C.A., Di Domenico, M., Prodocimo, M.M., 2018. Biomarker responses in fish exposed to polycyclic aromatic hydrocarbons (PAHs): Systematic review and meta-analysis. Environ Pollut 242, 449-461.

Santini, S., De Beni, E., Martellini, T., Sarti, C., Randazzo, D., Ciraolo, R., Scopetani, C., Cincinelli, A., 2022. Occurrence of Natural and Synthetic Micro-Fibers in the Mediterranean Sea: A Review. Toxics 10.

Sarkar, D.J., Das Sarkar, S., Das, B.K., Manna, R.K., Behera, B.K., Samanta, S., 2019. Spatial distribution of meso and microplastics in the sediments of river Ganga at eastern India. Science of the Total Environment 694.

Schirinzi, G.F., Perez-Pomeda, I., Sanchis, J., Rossini, C., Farre, M., Barcelo, D., 2017. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environ Res 159, 579-587.

Schlenk, D., Celander, M., Gallagher, E.P., George, S., James, M., Kullman, S.W., van den Hurk, P., Willett, K., 2008. Biotransformation in fishes. The toxicology of fishes 1.

Schymanski, D., Goldbeck, C., Humpf, H.U., Fürst, P., 2018. Analysis of microplastics in water by micro-Raman spectroscopy: Release

of plastic particles from different packaging into mineral water. Water Res 129, 154-162.
Seeley, M.E., Song, B., Passie, R., Hale, R.C., 2020. Microplastics affect sedimentary microbial communities and nitrogen cycling. Nature Communications 11.
Seginer, I., 2016. Growth models of gilthead sea bream (Sparus aurata L.) for aquaculture: A review. Aquacult Eng 70, 15-32.
Shan, Q., Ma, F., Wei, J., Li, H., Ma, H., Sun, P., 2020. Physiological Functions of Heat Shock Proteins. Curr Protein Pept Sci 21, 751-760.
Shashoua, Y., 2008. Conservation of Plastics: Materials Science, Degradation and Preservation Butterworth. Heinemann, Oxford.
Shen, R., Yang, K., Cheng, X., Guo, C., Xing, X., Sun, H., Liu, D., Liu, X., Wang, D., 2022. Accumulation of polystyrene microplastics induces liver fibrosis by activating cGAS/STING pathway. Environ Pollut 300, 118986.
Sheng, Y.H., Lourie, R., Linden, S.K., Jeffery, P.L., Roche, D., Tran, T.V., Png, C.W., Waterhouse, N., Sutton, P., Florin, T.H., McGuckin, M.A., 2011. The MUC13 cell-surface mucin protects against intestinal inflammation by inhibiting epithelial cell apoptosis. Gut 60, 1661-1670.
Shi, W., Han, Y., Sun, S., Tang, Y., Zhou, W., Du, X., Liu, G., 2020. Immunotoxicities of microplastics and sertraline, alone and in combination, to a bivalve species: size-dependent interaction and potential toxication mechanism. J Hazard Mater 396, 122603.
Siddiqui, S.A., Singh, S., Bahmid, N.A., Shyu, D.J.H., Dominguez, R., Lorenzo, J.M., Pereira, J.A.M., Camara, J.S., 2023. Polystyrene microplastic particles in the food chain: Characteristics and toxicity - A review. Sci Total Environ 892, 164531.
Sillero-Ríos, J., Sureda, A., Capó, X., Oliver-Codorniú, M., Arechavala-Lopez, P., 2018. Biomarkers of physiological responses of Octopus vulgaris to different coastal environments in the western Mediterranean Sea. Marine Pollution Bulletin 128, 240-247.
Sitja-Bobadilla, A., Gil-Solsona, R., Estensoro, I., Piazzon, M.C., Martos-Sitcha, J.A., Picard-Sanchez, A., Fuentes, J., Sancho, J.V., Calduch-Giner, J.A., Hernandez, F., Perez-Sanchez, J., 2019. Disruption of gut integrity and permeability contributes to enteritis in a fish-parasite model: a story told from serum metabolomics. Parasit Vectors 12, 486.

Solomando, A., Capo, X., Alomar, C., Alvarez, E., Compa, M., Valencia, J.M., Pinya, S., Deudero, S., Sureda, A., 2020. Long-term exposure to microplastics induces oxidative stress and a pro-inflammatory response in the gut of Sparus aurata Linnaeus, 1758. Environ Pollut 266, 115295.

Solomando, A., Capo, X., Alomar, C., Compa, M., Valencia, J.M., Sureda, A., Deudero, S., 2021. Assessment of the effect of long-term exposure to microplastics and depuration period in Sparus aurata Linnaeus, 1758: Liver and blood biomarkers. Sci Total Environ 786, 147479.

Solomando, A., Cohen-Sánchez, A., Box, A., Montero, I., Pinya, S., Sureda, A., 2022. Microplastic presence in the pelagic fish, Seriola dumerili, from Balearic Islands (Western Mediterranean), and assessment of oxidative stress and detoxification biomarkers in liver. Environ Res 212.

Song, Y.K., Hong, S.H., Eo, S., Jang, M., Han, G.M., Isobe, A., Shim, W.J., 2018. Horizontal and Vertical Distribution of Microplastics in Korean Coastal Waters. Environ Sci Technol 52, 12188-12197.

Souza-Silva, T.G., Oliveira, I.A., Silva, G.G.D., Giusti, F.C.V., Novaes, R.D., Paula, H.A.A., 2022. Impact of microplastics on the intestinal microbiota: A systematic review of preclinical evidence. Life Sci 294, 120366.

Stankus, A., 2021. State of world aquaculture 2020 and regional reviews: FAO webinar series. FAO aquaculture newsletter, 17-18.

Stephensen, E., Svavarsson, J., Sturve, J., Ericson, G., Adolfsson-Erici, M., Forlin, L., 2000. Biochemical indicators of pollution exposure in shorthorn sculpin (Myoxocephalus scorpius), caught in four harbours on the southwest coast of Iceland. Aquat Toxicol 48, 431-442.

Sturve, J., Hultman, M.T., Wassmur, B., Almroth, B.C., 2017. Determining oxidative stress and EROD activity in dab (Limanda limanda) in the North and Baltic Seas. Marine environmental research 124, 46-53.

Subaramaniyam, U., Allimuthu, R.S., Vappu, S., Ramalingam, D., Balan, R., Paital, B., Panda, N., Rath, P.K., Ramalingam, N., Sahoo, D.K., 2023. Effects of microplastics, pesticides and nano-materials on fish health, oxidative stress and antioxidant defense mechanism. Front Physiol 14, 1217666.

Sun, L., Liao, K., Wang, D., 2021a. Comparison of transgenerational reproductive toxicity induced by pristine and amino modified nanoplastics in Caenorhabditis elegans. Sci Total Environ 768, 144362.

Sun, T., Zhan, J., Li, F., Ji, C., Wu, H., 2021b. Effect of microplastics on aquatic biota: a hormetic perspective. Environ. Pollut. 285, 117206.

Sundell, K.S., Sundh, H., 2012. Intestinal fluid absorption in anadromous salmonids: importance of tight junctions and aquaporins. Front Physiol 3, 388.

Suzuki, G., Uchida, N., Tuyen, L.H., Tanaka, K., Matsukami, H., Kunisue, T., Takahashi, S., Viet, P.H., Kuramochi, H., Osako, M., 2022. Mechanical recycling of plastic waste as a point source of microplastic pollution. Environmental Pollution 303.

Suzuki, T., Hidaka, T., Kumagai, Y., Yamamoto, M., 2020. Environmental pollutants and the immune response. Nat Immunol 21, 1486-1495.

Takeyama, K., Dabbagh, K., Shim, J.J., Dao-Pick, T., Ueki, I.F., Nadel, J.A., 2000. Oxidative stress causes mucin synthesis via transactivation of epidermal growth factor receptor: Role of neutrophils. Journal of Immunology 164, 1546-1552.

Tang, Y., Rong, J., Guan, X., Zha, S., Shi, W., Han, Y., Du, X., Wu, F., Huang, W., Liu, G., 2020. Immunotoxicity of microplastics and two persistent organic pollutants alone or in combination to a bivalve species. Environ Pollut 258, 113845.

Tao, J., Deng, P., Lin, M., Chen, C., Ma, Q., Yang, L., Zhang, W., Luo, Y., Chen, S., Pi, H., Zhou, Z., Yu, Z., 2024. Long-term exposure to polystyrene microplastics induces hepatotoxicity by altering lipid signatures in C57BL/6J mice. Chemosphere 347, 140716.

Thompson, R.C., Moore, C.J., vom Saal, F.S., Swan, S.H., 2009. Plastics, the environment and human health: current consensus and future trends. Philos Trans R Soc Lond B Biol Sci 364, 2153-2166.

Tilg, H., Adolph, T.E., Trauner, M., 2022. Gut-liver axis: Pathophysiological concepts and clinical implications. Cell Metab 34, 1700-1718.

Turola, E., Petta, S., Vanni, E., Milosa, F., Valenti, L., Critelli, R., Miele, L., Maccio, L., Calvaruso, V., Fracanzani, A.L., Bianchini, M., Raos, N., Bugianesi, E., Mercorella, S., Di Giovanni, M., Craxì, A., Fargion, S., Grieco, A., Cammà, C.,

Cotelli, F., Villa, E., 2015. Ovarian senescence increases liver fibrosis in humans and zebrafish with steatosis. Dis Model Mech 8, 1037-1046.

Ugwu, K., Herrera, A., Gomez, M., 2021. Microplastics in marine biota: A review. Mar Pollut Bull 169, 112540.

Usman, S., Razis, A.F.A., Shaari, K., Amal, M.N.A., Saad, M.Z., Isa, N.M., Nazarudin, M.F., 2021. Polystyrene Microplastics Exposure: An Insight into Multiple Organ Histological Alterations, Oxidative Stress and Neurotoxicity in Javanese Medaka Fish (Oryzias javanicus Bleeker, 1854). Int J Env Res Pub He 18.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats. Environ Pollut 199, 10-17.

Vethaak, A.D., Legler, J., 2021. Microplastics and human health. Science 371, 672-674.

Wan, Z.Q., Wang, C.Y., Zhou, J.J., Shen, M.L., Wang, X.Y., Fu, Z.W., Jin, Y.X., 2019. Effects of polystyrene microplastics on the composition of the microbiome and metabolism in larval zebrafish. Chemosphere 217, 646-658.

Wang, C., Zhao, J., Xing, B., 2021. Environmental source, fate, and toxicity of microplastics. J Hazard Mater 407, 124357.

Wang, C.L., Hou, M.M., Shang, K.Y., Wang, H.S., Wang, J.W., 2022a. Microplastics (Polystyrene) Exposure Induces Metabolic Changes in the Liver of Rare Minnow (Gobiocypris rarus). Molecules 27.

Wang, Q., Wu, Y.L., Zhang, W.J., Shen, T., Li, H.Z., Wu, J.W., Zhang, L., Qin, L., Chen, R.C., Gu, W.J., Sun, Q.H., Liu, C.Q., Li, R., 2022b. Lipidomics and transcriptomics insight into impacts of microplastics exposure on hepatic lipid metabolism in mice. Chemosphere 308.

Wang, S.W., Han, Q., Wei, Z.L., Wang, Y.Y., Xie, J., Chen, M.Q., 2022c. Polystyrene microplastics affect learning and memory in mice by inducing oxidative stress and decreasing the level of acetylcholine. Food Chem Toxicol 162.

Wang, W., Ndungu, A.W., Li, Z., Wang, J., 2017. Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China. Sci Total Environ 575, 1369-1374.

Wang, W.F., Do, A.T.N., Kwon, J.H., 2022d. Ecotoxicological effects of micro-and nanoplastics on terrestrial food web from plants to human beings. Science of the Total Environment 834.

Wang, Y., Mao, Z., Zhang, M., Ding, G., Sun, J., Du, M., Liu, Q., Cong, Y., Jin, F., Zhang, W., Wang, J., 2019. The uptake and elimination of polystyrene microplastics by the brine shrimp, Artemia parthenogenetica, and its impact on its feeding behavior and intestinal histology. Chemosphere 234, 123-131.

Watts, A.J.R., Urbina, M.A., Corr, S., Lewis, C., Galloway, T.S., 2015. Ingestion of Plastic Microfibers by the Crab Carcinus maenas and Its Effect on Food Consumption and Energy Balance. Environmental Science & Technology 49, 14597-14604.

Wei, W., Yang, Q., Xiang, D., Chen, X., Wen, Z., Wang, X., Xu, X., Peng, C., Yang, L., Luo, M., Xu, J., 2023. Combined impacts of microplastics and cadmium on the liver function, immune response, and intestinal microbiota of crucian carp (Carassius carassius). Ecotoxicol Environ Saf 261, 115104.

Wei, Z., Wang, Y., Wang, S., Xie, J., Han, Q., Chen, M., 2022. Comparing the effects of polystyrene microplastics exposure on reproduction and fertility in male and female mice. Toxicology 465, 153059.

Wells, J.M., Brummer, R.J., Derrien, M., MacDonald, T.T., Troost, F., Cani, P.D., Theodorou, V., Dekker, J., Meheust, A., de Vos, W.M., Mercenier, A., Nauta, A., Garcia-Rodenas, C.L., 2017. Homeostasis of the gut barrier and potential biomarkers. Am J Physiol Gastrointest Liver Physiol 312, G171-G193.

Wen, B., Zhang, N., Jin, S.R., Chen, Z.Z., Gao, J.Z., Liu, Y., Liu, H.P., Xu, Z., 2018. Microplastics have a more profound impact than elevated temperatures on the predatory performance, digestion and energy metabolism of an Amazonian cichlid. Aquatic Toxicology 195, 67-76.

Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillitt, D.E., 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Critical Reviews in Toxicology 30, 347-570.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ Pollut 178, 483-492.

Wu, F.Z., Wang, Y.J., Leung, O.N.H., Huang, W., Zeng, J.N., Tang, Y.B., Chen, J.F., Shi, A.Q., Yu, X., Xu, X.Q., Zhang, H.G., Cao,

L., 2020. Accumulation of microplastics in typical commercial aquatic species: A case study at a productive aquaculture site in China. Science of the Total Environment 708.

Xie, X.M., Deng, T., Duan, J.F., Xie, J., Yuan, J.L., Chen, M.Q., 2020. Exposure to polystyrene microplastics causes reproductive toxicity through oxidative stress and activation of the p38 MAPK signaling pathway. Ecotox Environ Safe 190.

Xiong, X., Gao, L., Chen, C., Zhu, K., Luo, P., Li, L., 2023. The microplastics exposure induce the kidney injury in mice revealed by RNA-seq. Ecotoxicol Environ Saf 256, 114821.

Xu, H.G., Zhang, Y.Q., Wang, C.Q., Wei, Y.L., Zheng, K.K., Liang, M.Q., 2017. Cloning and characterization of fatty acid-binding proteins (fabps) from Japanese seabass (Lateolabraxjaponicus) liver, and their gene expressions in response to dietary arachidonic acid (ARA). Comp Biochem Phys B 204, 27-34.

Xu, M.K., Halimu, G., Zhang, Q.R., Song, Y.B., Fu, X.H., Li, Y.Q., Li, Y.S., Zhang, H.W., 2019. Internalization and toxicity: A preliminary study of effects of nanoplastic particles on human lung epithelial cell. Science of the Total Environment 694.

Xu, S., Ma, J., Ji, R., Pan, K., Miao, A.J., 2020. Microplastics in aquatic environments: Occurrence, accumulation, and biological effects. Sci Total Environ 703, 134699.

Yacobi, N.R., Malmstadt, N., Fazlollahi, F., DeMaio, L., Marchelletta, R., Hamm-Alvarez, S.F., Borok, Z., Kim, K.J., Crandall, E.D., 2010. Mechanisms of alveolar epithelial translocation of a defined population of nanoparticles. Am J Respir Cell Mol Biol 42, 604-614.

Yan, Z.H., Liu, Y.F., Zhang, T., Zhang, F.M., Ren, H.Q., Zhang, Y., 2022. Analysis of Microplastics in Human Feces Reveals a Correlation between Fecal Microplastics and Inflammatory Bowel Disease Status. Environmental Science & Technology 56, 414-421.

Yang, H., Xiong, H.R., Mi, K.H., Xue, W., Wei, W.Z., Zhang, Y.Y., 2020. Toxicity comparison of nano-sized and micron-sized microplastics to Goldfish Carassius auratus Larvae. Journal of Hazardous Materials 388.

Yang, W., Jannatun, N., Zeng, Y., Liu, T., Zhang, G., Chen, C., Li, Y., 2022a. Impacts of microplastics on immunity. Front Toxicol 4, 956885.

Yang, X., Man, Y.B., Wong, M.H., Owen, R.B., Chow, K.L., 2022b. Environmental health impacts of microplastics exposure on structural organization levels in the human body. Sci Total Environ 825, 154025.

Ye, G.Z., Zhang, X., Liu, X.Y., Liao, X., Zhang, H., Yan, C.Z., Lin, Y., Huang, Q.S., 2021. Polystyrene microplastics induce metabolic disturbances in marine medaka (Oryzias melastigmas) liver. Science of the Total Environment 782.

Yin, J.L., Ju, Y., Qian, H.H., Wang, J., Miao, X.H., Zhu, Y., Zhou, L.T., Ye, L., 2022. Nanoplastics and Microplastics May Be Damaging Our Livers. Toxics 10.

Yin, K., Wang, Y., Zhao, H.J., Wang, D.X., Guo, M.H., Mu, M.Y., Liu, Y.C., Nie, X.P., Li, B.Y., Li, J.Y., Xing, M.W., 2021. A comparative review of microplastics and nanoplastics: Toxicity hazards on digestive, reproductive and nervous system. Science of the Total Environment 774.

Yin, L., Chen, B., Xia, B., Shi, X., Qu, K., 2018. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (Sebastes schlegelii). J Hazard Mater 360, 97-105.

Yin, Z., 2023. The pollution of microplastics in sediments: The ecological risk assessment and pollution source analysis. Sci Total Environ 859, 160323.

Yu, F., Yang, C., Zhu, Z., Bai, X., Ma, J., 2019. Adsorption behavior of organic pollutants and metals on micro/nanoplastics in the aquatic environment. Sci Total Environ 694, 133643.

Yu, Q., Hu, X., Yang, B., Zhang, G., Wang, J., Ling, W., 2020a. Distribution, abundance and risks of microplastics in the environment. Chemosphere 249, 126059.

Yu, Y., Chen, H., Hua, X., Dang, Y., Han, Y., Yu, Z., Chen, X., Ding, P., Li, H., 2020b. Polystyrene microplastics (PS-MPs) toxicity induced oxidative stress and intestinal injury in nematode Caenorhabditis elegans. Sci Total Environ 726, 138679.

Yu, Y.B., Choi, J.H., Choi, C.Y., Kang, J.C., Kim, J.H., 2023a. Toxic effects of microplastic (polyethylene) exposure: Bioaccumulation, hematological parameters and antioxidant responses in crucian carp, Carassius carassius. Chemosphere 332.

Yu, Z., Yan, C., Qiu, D., Zhang, X., Wen, C., Dong, S., 2023b. Accumulation and ecotoxicological effects induced by combined

exposure of different sized polyethylene microplastics and oxytetracycline in zebrafish. Environ Pollut 319, 120977.

Yuan, Y., Sepulveda, M.S., Bi, B., Huang, Y., Kong, L., Yan, H., Gao, Y., 2023. Acute polyethylene microplastic (PE-MPs) exposure activates the intestinal mucosal immune network pathway in adult zebrafish (Danio rerio). Chemosphere 311, 137048.

Zhang, C., Wang, J., Pan, Z., Wang, S., Zhang, L., Wang, Q., Ye, Q., Zhou, A., Xie, S., Zeng, F., Xu, G., Zou, J., 2021a. A dosage-effect assessment of acute toxicology tests of microplastic exposure in filter-feeding fish. Fish Shellfish Immunol 113, 154-161.

Zhang, D., Tang, J., Zhang, J., Zhang, L., Hu, C.X., 2019. Responses of pro- and anti-inflammatory cytokines in zebrafish liver exposed to sublethal doses of Aphanizomenon flosaquae DC-1 aphantoxins. Aquat Toxicol 215, 105269.

Zhang, J., Kong, X., Zhou, C., Li, L., Nie, G., Li, X., 2014. Toll-like receptor recognition of bacteria in fish: ligand specificity and signal pathways. Fish Shellfish Immunol 41, 380-388.

Zhang, K., Su, J., Xiong, X., Wu, X., Wu, C., Liu, J., 2016. Microplastic pollution of lakeshore sediments from remote lakes in Tibet plateau, China. Environ Pollut 219, 450-455.

Zhang, X., Wen, K., Ding, D., Liu, J., Lei, Z., Chen, X., Ye, G., Zhang, J., Shen, H., Yan, C., Dong, S., Huang, Q., Lin, Y., 2021b. Size-dependent adverse effects of microplastics on intestinal microbiota and metabolic homeostasis in the marine medaka (Oryzias melastigma). Environ Int 151, 106452.

Zhang, Y., Pu, S.Y., Lv, X., Gao, Y., Ge, L., 2020. Global trends and prospects in microplastics research: A bibliometric analysis. Journal of Hazardous Materials 400.

Zhang, Y., Yin, K., Wang, D., Wang, Y., Lu, H., Zhao, H., Xing, M., 2022a. Polystyrene microplastics-induced cardiotoxicity in chickens via the ROS-driven NF-kappaB-NLRP3-GSDMD and AMPK-PGC-1alpha axes. Sci Total Environ 840, 156727.

Zhang, Y.K., Yang, B.K., Zhang, C.N., Xu, S.X., Sun, P., 2022b. Effects of polystyrene microplastics acute exposure in the liver of swordtail fish (Xiphophorus helleri) revealed by LC-MS metabolomics. Science of the Total Environment 850.

Zhao, L.T., Shi, W.N., Hu, F.F., Song, X.J., Cheng, Z.J., Zhou, J.H., 2021a. Prolonged oral ingestion of microplastics induced inflammation in the liver tissues of C57BL/6J mice through

polarization of macrophages and increased infiltration of natural killer cells. Ecotox Environ Safe 227.
Zhao, Y., Bao, Z.W., Wan, Z.Q., Fu, Z.W., Jin, Y.X., 2020. Polystyrene microplastic exposure disturbs hepatic glycolipid metabolism at the physiological, biochemical, and transcriptomic levels in adult zebrafish. Science of the Total Environment 710.
Zhao, Y., Qiao, R., Zhang, S., Wang, G., 2021b. Metabolomic profiling reveals the intestinal toxicity of different length of microplastic fibers on zebrafish (Danio rerio). J Hazard Mater 403, 123663.
Zheng, H.B., Wang, J., Wei, X.Y., Chang, L., Liu, S., 2021. Proinflammatory properties and lipid disturbance of polystyrene microplastics in the livers of mice with acute colitis. Science of the Total Environment 750.
Zheng, Y., Shi, Y.L., Yang, X.X., Gao, J.C., Nie, Z.J., Xu, G.C., 2022. Effects of resveratrol on lipid metabolism in liver of red tilapia Oreochromis niloticus. Comp Biochem Phys C 261.
Zhou, W.S., Shi, W., Du, X.Y., Han, Y., Tang, Y., Ri, S., Ju, K., Kim, T., Huang, L., Zhang, W.X., Yu, Y.H., Tian, D.D., Yu, Y.Y., Chen, L.B., Wu, Z.C., Liu, G.X., 2023. Assessment of Nonalcoholic Fatty Liver Disease Symptoms and Gut-Liver Axis Status in Zebrafish after Exposure to Polystyrene Microplastics and Oxytetracycline, Alone and in Combination. Environ Health Persp 131.
Zhu, J.M., Zhang, Q., Li, Y.P., Tan, S.D., Kang, Z.J., Yu, X.Y., Lan, W.L., Cai, L., Wang, J.Z., Shi, H.H., 2019. Microplastic pollution in the Maowei Sea, a typical mariculture bay of China. Science of the Total Environment 658, 62-68.
Zoabi, A., Touitou, E., Margulis, K., 2021. Recent Advances in Nanomaterials for Dermal and Transdermal Applications. Colloid Interfac 5.