UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO SCIENZE DELLA VITA

E DELL'AMBIENTE

Corso di Laurea Magistrale in Biologia Marina

Il destino delle microplastiche alimentari: un approccio multidisciplinare per valutare la loro localizzazione e gli effetti fisiologici sui giovanili di zebrafish (*Danio rerio*).

The fate of dietary microplastics: a multidisciplinary approach to evaluate their localization and physiological effects on zebrafish (*Danio rerio*) juveniles.

Tesi Magistrale di:

Beniamino Dimichino

Relatore:

Chiar.mo Prof. Ike Olivotto

Correlatore:

Dott. Nico Cattaneo

Dott. Matteo Zarantoniello

Sessione Ottobre

Anno Accademico

2023/2024

Table of Contents

ABSTRACT

L'inquinamento da plastica è ancora un grave problema ambientale che colpisce gli ecosistemi di tutto il mondo. Un numero crescente di studi scientifici recenti sottolinea come i detriti di plastica, in particolare le microplastiche (MPs), particelle con dimensioni minori di 5 mm, stiano avendo un impatto sempre più negativo sia sugli ambienti terrestri che su quelli acquatici. Le MPs possono essere primarie e secondarie, le prime vengono progettate di queste dimensioni per usi industriali, mentre le seconde si originano dalla degradazione di oggetti di plastica più grandi tramite processi naturali. Queste MPs sono immesse da diverse fonti come gli scarichi industriali, gli imballaggi, e prodotti per la cura personale, ponendo una seria minaccia al benessere degli organismi acquatici e potenzialmente all'uomo attraverso la catena alimentare. Il presente studio ha permesso la ricerca degli effetti delle MPs contenute nei mangimi dello zebrafish (*Danio rerio*), il quale viene usato come modello per la tossicologia ambientale, per studiare più nel dettaglio il bioaccumulo e gli effetti tossicologici di questi inquinanti.

Anche il settore dell'acquacoltura non è esente da questo problema, dal momento che sono state riscontrate MPs negli allevamenti e negli animali da allevamento. Le principali fonti di MPs nell'acquacoltura sono l'ambiente, gli utensili usati per praticare acquacoltura, che vanno incontro a degradazione, e i mangimi, che risultano essere contaminati da MPs di varia origine.

Lo scopo della tesi è esplorare l'impatto biologico delle MPs sulla salute dei pesci, concentrandosi in particolare sullo zebrafish come modello di studio.

Vengono indagati l'ingestione, l'assorbimento e l'accumulo di MPs di diverse dimensioni (1-5 μm e 40-47 μm) e concentrazioni (50 mg/kg e 500 mg/kg) in zebrafish giovanili, valutando come questi fattori influenzino la loro possibile

traslocazione in altri tessuti, e il possibile impatto che possono avere sulla crescita e la salute dei pesci.

Gli zebrafish sono stati esposti a diete sperimentali contenenti due tipi di MPs, polimero di amino formaldeide (AFP) e polietilene (POL), per un periodo di 60 giorni. Lo studio ha utilizzato un approccio multidisciplinare, includendo misurazioni biometriche per monitorare la crescita; la digestione chimica per quantificare le MPs nei tessuti e la microscopia confocale per rilevare particelle fluorescenti nei vari organi dei pesci. Le analisi istopatologiche hanno permesso la determinazione dei danni ai tessuti, mentre le tecniche molecolari sono state utilizzate per valutare l'espressione genica relativa allo stress ossidativo e alla risposta immunitaria.

I risultati di questa tesi sottolineano la minaccia emergente dell'inquinamento da plastica, in particolare il ruolo delle MPs negli ambienti acquatici come gli allevamenti. L'assorbimento e l'accumulo differenziale delle MPs in base alla loro dimensione evidenziano la necessità di ulteriori ricerche sugli effetti a lungo termine di questi inquinanti. Le particelle più piccole, che vengono assorbite a livello intestinale e raggiungono altri compartimenti dell'organismo, rappresentano un rischio significativo sia per i pesci che potenzialmente per gli esseri umani attraverso la rete trofica.

Questa tesi pone ulteriore attenzione all'urgenza di trovare soluzioni per mitigare gli effetti negativi da ingestione di MPs nei pesci, come nell'impedirne l'assorbimento.

1. INTRODUCTION

1.1 Environmental MPs

Plastic pollution is still a serious environmental problem that affects ecosystems worldwide (Strungaru et al., 2019). An increasing number of studies show how plastic waste, is harming both land and water environments (Macleod et al., 2021).

Research suggests that if there is no change in the approach to dealing with this issue, there will be a huge increase in plastic waste. By 2060, it is predicted that 155 to 265 million metric tons of badly managed plastic trash will be produced each year. Developing countries, especially in Asia and Africa, will suffer the most from plastic pollution unless waste management systems are improved (Lebreton & Andrady, 2019).

One of the most famous examples of plastic pollution in the ocean is the Great Pacific Garbage Patch, a gyre of plastic debris, is growing fast. Recent studies show that this area has a huge amount of plastic waste, possibly millions of tons, which is threatening ecosystems and marine animals (Lebreton et al., 2018).

Since the beginning of the third millennium, scientists have recognised the presence of microplastics (MPs) in marine waters. Thanks to Thompson's data collection, MPs are defined as particles with a diameter of 5 mm or less that

can accumulate in the environment (Thompson et al., 2004), and for this reason are considered emerging marine pollutants. Nowadays, MPs pollution is a matter of global interest (Gigault et al., 2018; Upendra & Kaur, 2023).

MPs can come from primary or secondary sources. Primary MPs are small particles released directly, like pellets or powders, often used in industrial processes, they are also found in many personal care products, like facial cleansers, where they replace natural exfoliating materials (Conkle et al., 2018; Zhou et al., 2021). After use, these particles often escape water treatment systems and end up in the oceans (Fendall & Sewell, 2009). Secondary MPs come from the breakdown of larger plastic items due to environmental factors like sunlight and waves (Thompson, 2015). Despite the original size of the plastic (> 5 mm), the particles have become smaller because of weathering processes resulting in the massive formation of MPs particles (Ivar Do Sul & Costa, 2014; Lambert & Wagner, 2016).

The main polymers involved in the MPs composition, mostly derived from human activities, can be grouped into (Kedzierski et al., 2022):

- Polyethylene (PE), divided in low and high-density type (LDPE and HDPE);
- Polypropylene (PP);
- Polystyrene (PS);

- Polymethylmethacrylate (PMMA);
- Polyethylene terephthalate (PET);
- Polyamide (PA);
- Polyvinyl chloride (PVC) (Marine Debris Programme).

Global plastic production is extremely high, with different types of plastics used in sectors like packaging, agriculture, and construction (Wang et al., 2020). MPs are formed as a result of poor management and disposal of plastic materials, which come from various sources such as municipal, commercial, and industrial waste (Lebreton & Andrady, 2019). When not properly managed, due to littering or inadequate waste systems, plastics often end up in the environment. Over time, larger plastic objects break down into smaller particles, contributing to the growing problem of MPs (Barnes et al., 2009).

MPs have been found in many different environments including the atmosphere, where they can be carried by the air and settle on both land and sea surfaces (Gasperi et al., 2018).

MPs found in the terrestrial environment have been documented in many scientific publications, noting their potential dispersion rates despite the different environmental matrices: in the atmosphere, terrestrial systems such as lands and soils, freshwater and marine environments (Coyle et al., 2020; Strungaru et al., 2019; Wang et al., 2020). This often happens because of plastic

waste and contaminated fertilizers spreading. (Coyle et al., 2022; Horton et al., 2017; Wang et al., 2020; Zhang et al., 2020).

As regards MPs pollution in marine ecosystems, their concentration largely depends on human activity and the level of impact in the affected areas (Khuyen et al., 2021). Sources of MPs pollution include sewage effluents, river runoff, coastal inputs, marine activities, and the atmospheric transport of particles (Khuyen et al., 2021). Plastic films, such as shopping bags, are particularly prone to dispersal by wind, and the breakdown of larger plastics contributes significantly to the accumulation of MPs in oceans, beaches, and other environments (Khuyen et al., 2021) (Fig. 1). In coastal waters the morphology of MPs affects their movement and accumulation. For example, light particles like polyethylene (PE) and polypropylene (PP) are easily moved by wind and tides, while denser materials such as polyethylene terephthalate (PET) and polyvinyl chloride (PVC) tend to settle in the sand (Khuyen et al., 2021). In a coastal area, the most abundant dominant type of plastic is the polyethylene, this reflects the single-use plastic products that are usually dumped on the beaches (Browne et al., 2015; Strungaru et al., 2019), it makes the coastal environment more vulnerable to the plastic pollution.

Despite of the environmental matrices these particles are found even in living organisms, and MPs can be internalised by terrestrial plants and animals

through contaminated soil and water (Wang et al., 2020). In the same way marine animals, from tiny plankton to fish and marine mammals, can accidentally ingest MPs resulting in their absorption (Desforges et al., 2015; Hurley et al., 2018; Lei et al., 2018; Van Cauwenberghe et al., 2015) This can be harmful and can lead to an accumulation in their bodies (Coyle et al., 2022).

Due to plastic weathering process, even the smallest debris is responsible for contaminating the food web: MPs are found in marine snow and are ingested by all biota, posing a serious environmental problem and a potential risk to ecosystem health (de Bruin et al., 2022). These particles are part of the growing accumulation of plastic debris seen in recent decades, caused by their light weight, durability, and widespread use in single-use items. Even though they break down slowly in UV light, marine conditions slow down the process, allowing them to last for long periods on the ocean surface and in sediments (Barnes et al., 2009). Both primary and secondary marine-water-MPs are vertically distributed following polymers characteristics, such as density and interactions with marine biota, which influence the distribution of plastic at the lowest depths, including the deep sea (Coyle et al., 2022).

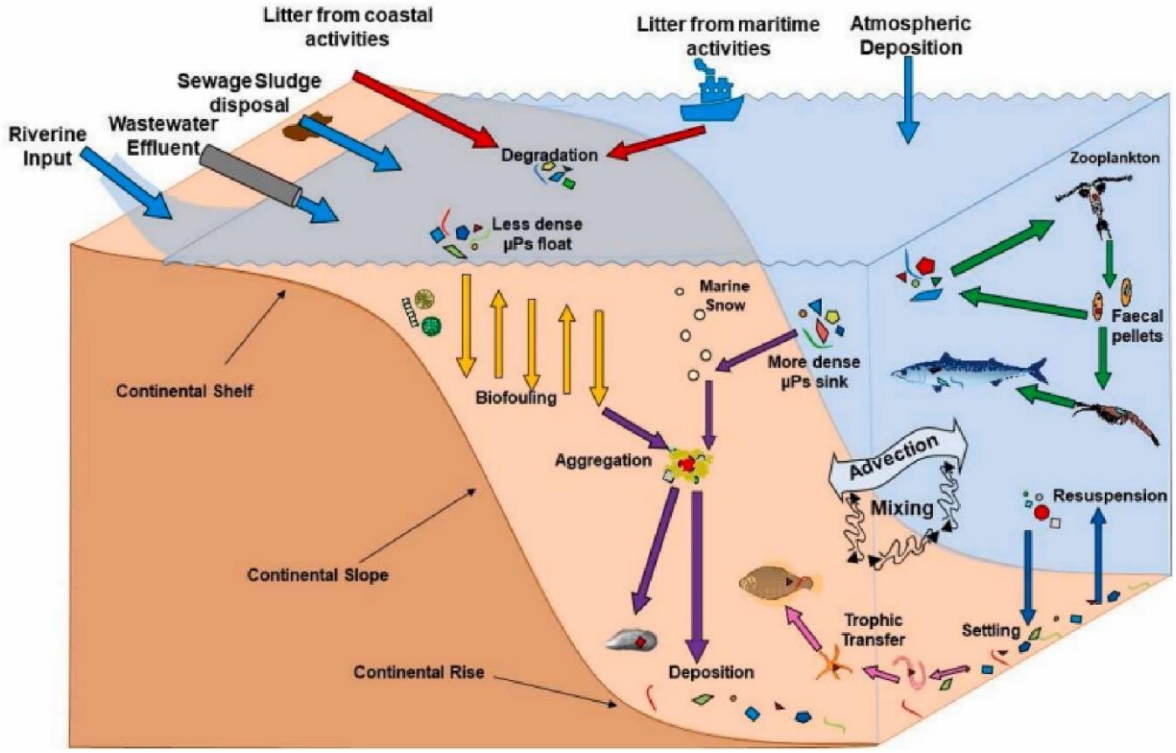


Fig. 1 Marine microplastic pathways with consideration of all possible sources and, secondarily, as a natural consequence, their fate in the environment (*Corrigendµm* to Coyle et al., 2022).

The NOAA (National Oceanographic and Atmospheric Agency) suggested a broader definition for MPs, which includes all plastic particles smaller than 5 mm in size. This definition has also been adopted by the European Union as part of the Marine Strategy Framework Directive (MSFD) (Thompson, 2015). Most researchers agree that MPs are plastic particles smaller than 5 mm, however, these particles come in many different sizes and shapes, which can affect how they interact with the environment and living organisms. For

example, Brennecke et al. found MP transfer to organs in crab (Brennecke et al., 2015) and Avio et al. (2015) even found a 400 μm MP particle in the liver of a wild fish (Avio et al., 2015). Moreover, MPs smaller than 15 μm can accumulate more easily in food chains (Batel et al., 2020).

In addition to the size of the MPs, the shape of the particles should also be considered, especially when considering the risk assessment of MPs (Batel et al., 2020), they come in different shapes like beads (small round particles), fragments (irregular pieces), and fibres (thin, thread-like particles). In terms of toxicity, the shape could also make a difference. For instance, MPs fibres were found to stay longer in the intestines and caused higher death rates in amphipods than particles like beads or fragments (Qiao, Deng, et al., 2019). In grass shrimp, fibres showed stronger toxic effects than beads or fragments: it has been demonstrated that MPs fibres accumulate more in tissues, leading to intestinal epithelial cell necrosis in the gut of zebrafish *Danio rerio* (Qiao, Deng, et al., 2019). MPs ingestions by fishes is responsible for bioaccumulation through trophic transfer in the food chain. Bioaccumulation starts with the accumulation of MPs on organic matter and phytoplankton, which are then ingested by zooplankton, middle and top predators, finally MPs then enter the human diet indirectly (Cau et al., 2020; Coyle et al., 2022; Markic et al., 2020).

The main adverse effects of plastic components are reproductive toxicity, mutagenicity, and carcinogenicity, but further studies are needed to evaluate a holistic assessment of these polymers (Avio et al., 2017; Gasperi et al., 2018).

1.2 MPs and animal health status

"At present, the only known biological effect of these particles is that they act as a surface for the growth of hydroids, diatoms and probably bacteria", reads one of the first articles on MPs in the sea, since scientists realised in 1972 that biota could be disturbed by the presence of plastic (Carpenter & Smith, 1972). At present, many studies have investigated the ecotoxicological effect without assessing the panorama of all the possible ways through which MPs can cause damage (Avio et al., 2017). As plastic microparticles become a bigger problem, many studies have investigated their effects on animal health (Jeong et al., 2024). These particles can harm animals in different ways when eaten, affecting them chemically, physically, and biologically. They may also cause problems for animals that eat prey contaminated with MPs, leading to indirect effects on the food chain (Oehlmann et al., 2009).

The intake of MPs poses a risk to life quality in terms of adverse biological effects. The effects of MPs on the health of living organisms depend on various factors in response to defence mechanisms (chemical, physical, and biological barriers). These factors include concentration, the size of the MPs and exposure time, in fact the less time the organisms are exposed, the more efficiently the barriers act. (Lu et al., 2016; Rios-Fuster et al., 2021)

The defence barriers can vary between mammals, fish, and invertebrates (Borrelle et al., 2017).

In mammals and fish the gastrointestinal system is the first line of defence for ingested MPs, the larger particles 20 μm are expelled, but when the smaller ones pass through the intestinal walls they can enter in the bloodstream and spread to various organs (Lu et al., 2016; Qiao, Lu, et al., 2019; Su et al., 2019). At the brain barrier and placenta only the smallest MPs, <10 um, can enter, potentially affecting the brain and fetal development (Bhagat et al., 2020a). Moreover, in fish the gills constitute another important barrier against large MPs assimilation (Bhagat et al., 2020).

A study by Lei et al. (2018) found that tiny microplastics (1 μm can have stronger effects on invertebrates. These tiny plastic particles caused damage to the intestines, disrupted gut bacteria, and led to inflammation in species like *Caenorhabditis elegans* (a type of worm). Smaller particles are more harmful because they can enter tissues more easily and cause more toxic effects on these small organisms.

As regards fish, studies demonstrated that smaller MPs (<20 μm are more likely to enter internal organs (Lu et al., 2016). They bypass physical barriers like mucus and cell walls, collecting in organs like the liver and kidneys (Borrelle et al., 2017). These barriers are more efficient when there are low

concentrations of MPs, but when particles are more concentrated, they become more bioavailable causing worse damages to the organs (Galloway & Lewis, 2016). Small MPs in finfish hatcheries can interfere with the nutritional needs of larvae, however the levels at which this occurs are significantly higher than those documented in marine waters (Campos et al., 2021).

Concerning the MPs toxicity in fish, the literature has a consistent background in alterations of morphology (Pitt et al., 2018), behaviour (Mak et al., 2019); growth (Pitt et al., 2018), histopathology (Qiang & Cheng, 2019), and microbiota, as well as activation of oxidative stress, immune and stress responses (Jin et al., 2018; Qiao, Deng, et al., 2019). For instance, studies on gilthead seabream (*Sparus aurata*) have shown that MPs negatively affect this species, causing the pro-inflammatory response in the liver, intestine and brain, (Capó et al., 2021; Rios-Fuster et al., 2021). Moreover, gilthead seabream exposed to MPs showed an increase in oxidative stress marker activity in fish brain (Capó et al., 2021; Rios-Fuster et al., 2021). In addition, studies show the pro-inflammatory response in the intestine of fish and the possibility of recovery after MPs removal (Solomando et al., 2020). In support of the latter changes, better known polymers have been used to understand the histological and morphological changes in the fish case study, in particular, the microplastics caused intestinal damage, changes to the gut bacteria, and

inflammation. The zebrafish exposed to these particles showed signs of physical stress and inflammation in the intestines, with MPs accumulation in their tissues. (Lei et al., 2018). The MPs adverse effects of the altered microbiome and metabolites may be closely linked to the development of energy metabolism disorders such as oxidative stress and neurotoxicity in larvae, as documented in the model fish *Danio rerio* (Wan et al., 2019). Moreover, dysbiosis in the gut of adults has been shown in the same species, like has been documented in other fishes (Jin et al., 2018; Qiao, Deng, et al., 2019). However, it seems that growth tends to be more affected in the larval stage than in the juvenile or adult stages, as reported in a study conducted on MPs toxicity on zebrafish development (Tarasco et al., 2022), suggesting that the developmental stage is more critical compared to the others.

Research is still important for the aquaculture companies and for citizens: the long-term effects of MPs are still as unclear as their ingestion consequences on animals (Batel et al., 2020; G. Chen et al., 2021; Rios-Fuster et al., 2021), consequently more studies on this topic must be performed.

1.3 Microplastics in Aquaculture

The presence of plastic debris poses significant challenges to the sustainable development of fisheries and aquaculture due to the potential food safety issues, socio-economic concerns, and increased mortality rates (Wu et al., 2023a). The marine environment can be characterized by the presence of mariculture, and in some cases, this specific aquaculture system is affected by the MPs plague (Egea-Corbacho et al., 2023b). Before and during the weathering process, meteorological and oceanographic conditions play an active role in material corrosion, such as the abrasion of ship coatings, leading to massive releases of MPs. These aquaculture facilities are often located in coastal areas, where environmental concentrations of MPs are generally higher (Browne et a, 2015). This is evidenced by the long-term use of plastic fishing gear, which affects the coastal environment in the form of sediment. For the same reason, most aquaculture species appear to be more susceptible to contamination by MPs than wild species (M. Chen et al., 2018; Dibke et al., 2021; Vázquez-Rowe et al., 2021).

MPs can reach the aquaculture systems through different pathways that can be direct or indirect (M. Chen et al., 2018). Among the direct sources fishing tools, water pumps electrical cables and maintenance stuff are included as contaminants (M. Chen et al., 2018), on the other side, external inputs are due

to the flow of water inside the tanks, cages and aquaculture facilities (Zhang et al., 2020). An additional contributor to contamination is fish feed, with fishmeal being particularly abundant in MPs, primarily in fragmentary form (Zhou et al., 2021). Ingredients in the commercial feed are easily contaminated by the different commercial routes that have a direct link with them: like for the fish meal, the MPs particles counting is related to the big amount of various packaging polymers, these materials can degrade over time and release MPs into the fishmeal during storage or transport (Karbalaei et al., 2020). The fishmeal production process itself can contribute to MPs contamination. During grinding, plastics present in the fish or introduced during processing can break down into MPs and become incorporated into the final product (Gündoğdu et al., 2021). The fish feed can also be contaminated with MPs because it is often made from wild-caught fish that have already been exposed to MPs for example, fishmeal from the Yellow Sea near China contained a high concentration of MPs due to the heavy pollution in the area (M. Chen et al., 2018). The types of plastics found include polyethylene (PE) and polypropylene (PP), which are common in ocean surface waters (M. Chen et al., 2018).

Despite the widespread use of plastic in the aquaculture industry, a study reveals that MPs have a more pronounced impact on the surrounding environment than within the industry's water tanks. This disparity is likely

attributed to the efficiency of purification systems implemented at the tank inlets, exemplified by the case of recirculating aquaculture systems (RAS) as discussed by Egea-Corbaco *et al.* (2023b).

In the realm of water-related activities, proactive management of aquaculture is essential to address the issue of MPs. Solutions include the recycling of disposable plastics and the reutilization of fishing gear (Miao et al., 2023). Notably, some companies have taken innovative steps to transform plastic waste into sustainable products for everyday use, such as bags and textiles (Wu et al., 2023). A fundamental approach to mitigate particle pollution involves reducing the usage of packaging materials (Miao et al., 2023; Wu et al., 2023a).

The inherent characteristics of MPs intensify the viral load within aquaculture systems. The resulting infection, coupled with toxicological effects such as oxidative stress, growth depletion, and behavioural alterations, culminates in a reduction of aquaculture products, leading to economic consequences (Wu et al., 2023a). Among aquaculture sources, mussel farms stand out as particularly hazardous contributors to MPs pollution (G. Chen et al., 2021). This heightened risk is attributed to the direct intake of contaminants by consumers who typically consume the whole shellfish. Notably, MPs are predominantly found in the intestinal tract of these species (G. Chen et al., 2021). Moreover, these emerging pollutants can adsorb antibiotics used in aquaculture, acting as carrier

for these contaminants. In the study of Li et al. (2018) there are evidence of how the adsorption could lead to the long-range transport of antibiotics, enhancing their exposure to aquatic organisms and ecosystems, and potentially increasing their bioavailability and accumulation in food chains.

2. AIM OF THE THESIS

The impact of microplastics (MPs) has prompted an increase in research around the world, and the organism model *Danio rerio*, a freshwater fish, is well suited to study their adverse effects on aquatic animals. This teleost is particularly suitable for studies on the bioaccumulation and toxicity of environmental pollutants, including MPs, due to its small size, short life cycle (4-6 months to adulthood) and sustainable maintenance, they are easy to breed, are simple to induce reproduction throughout the year and produce hundreds of transparent-like eggs in a single spawning event (Bhagat et al., 2020; Ribas & Piferrer, 2014; Fishbase).

The present study focused on the adverse effects of MPs on fish growth and welfare, focusing also on the fate of dietary MPs once ingested by fish.

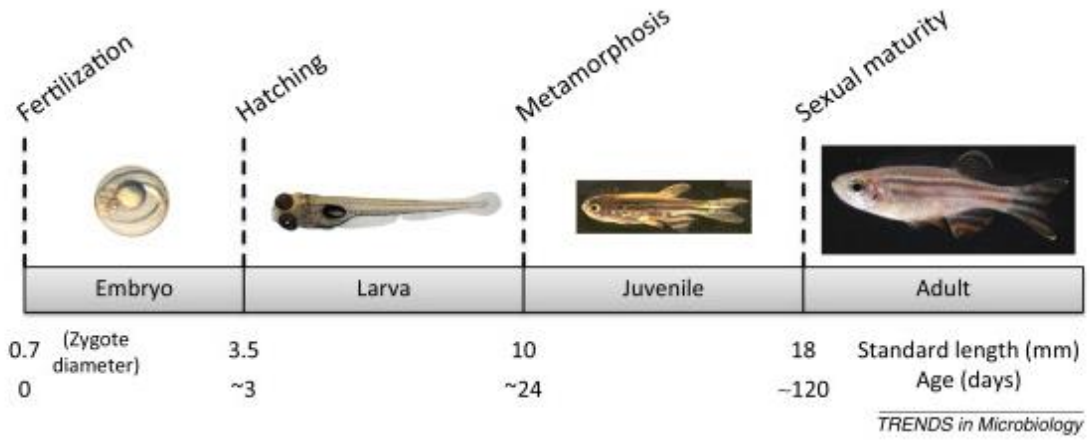
The aim of this project was to (i) produce experimental diets containing two different sized fluorescent-MPs of 1-5 μm and 40-47 μm at implemented at two different concentrations (50 mg/kg and 500 mg/kg) representing an environmental realistic and a 10x higher concentration to intensify possible effects, respectively; (ii) monitor the possible translocation of MPs among tissues and organs depending on their size; (iii) evaluate the potential different biological adverse effects on fish growth and welfare of the contaminated diets on *D. rerio* juveniles in relation to the MPs size and concentration. Zebrafish

were fed from the larval to the juvenile phase with experimental diets supplemented with MPs. At the end of the feeding trial (60 days), survival and growth rates were measured, and samples were collected for further analysis. A multidisciplinary approach consisting in biometric measurement, confocal and fluorescence microscopy, along with histological and molecular analyses, was performed to better elucidate the effects of dietary MPs administration in juvenile zebrafish.

3. MATERIALS AND METHODS

3.1 Experimental model

The zebrafish *Danio rerio*, is a freshwater teleost (3-4 cm) belonging to the family Cyprinidae, native to South-East Asia. The body is slender and laterally compressed, with the mouth pointing upwards and the lower jaw protruding forward. It lives in tropical areas subject to monsoon climate fluctuations, in rivers, small streams, stagnant or slow-moving canals, rice paddies and surrounding areas (McClure et al., 2006). It is an omnivorous organism, feeding on zooplankton, insects, plant and algal compounds (Spence et al., 2006). In captivity, it is fed on rotifers, nauplii of *Artemia salina* and dry granular food (Ribas & Piferrer, 2014).



Danio rerio development *(Levraud et al., 2014)*

The zebrafish is emerging as a model in the field of basic research and aquaculture due to favourable characteristics: ease of breeding, low cost, simplicity in reproduction, production of transparent eggs, embryos and larvae, and reaching adulthood in 3-4 months (Spence et al., 2008). In captivity, females lay eggs at frequent but irregular intervals, and a single female can produce several hundred eggs under optimal conditions (Spence et al., 2006). The eggs, which are non-adhesive and approximately 0.7 mm in diameter, are immediately fertilised by the males. In captivity, measures must be taken to prevent cannibalism of the eggs (Lee et al., 1999). Eighteen hours after fertilisation, the embryos show developed eyes and muscle and brain segments; after about 24 hours, segmentation is complete, and in 48-72 hours hatching occurs. The larva, about 3 mm long at hatching, does not have a fully formed intestine, and the swim bladder develops about two days later (Laale, 1977). In

the first few days, the larvae grow rapidly, consuming the yolk sac, and the intestine folds into an 'S' shape. After the second day, the larvae develop a swim bladder, start breathing with their gills and move their jaws, thus changing their hunting and feeding behaviour (Kimmel et al., 1995).

Apart from the ease of breeding, another reason why the zebrafish is an excellent model organism is that its entire genome has been sequenced, showing a high similarity to that of humans. It shares 70% of the human genetic make-up, and disease-associated genes show 84% homology to human ones. Zebrafish has been useful in understanding the mechanisms of tumour initiation, growth and spread, as the biology of neoplasms in fish and humans is similar. It is widely used in various areas of aquaculture to study genes related to nutrient metabolism, muscle development and stress response (Rahman Khan & Sulaiman Alhewairini, 2019; Reed & Jennings, 2011). A key feature for biological studies is the transparency of embryos and larvae, which allows direct observation of organ development and circulatory flow through the use of fluorescent markers (Ribas & Piferrer, 2014).

3.2 Ethics

All animal experiments were conducted in accordance with the procedures outlined in the European Community Council Directive (86/609/EEC and

2010/63/EU) for the welfare of animals and received approval from the ethics committee of the Marche Polytechnic University (Ancona, Italy) (n.3 24/11/2022) and the Italian Ministry of Health (Aut. n. 391/2023-PR).

Specimens were reared under optimal conditions, and care was taken to minimize fish suffering by using tricaine anaesthetic (MS222; Merck KGaA, Darmstadt, Germany) at a concentration of 0.3 g/L for suppression.

3.3 MPs

In the present study, two different MPs polymers were used, differing in composition (amino formaldehyde polymer, AFP; and polyethylene, POL), size range (1-5 μm and 40-47 μm, respectively), and fluorescence (AFP has an emission peak of 636 nm when excited at 584 nm, POL has an emission peak of 607 nm when excited at 575 nm). Before adding the polymers to the diet, MPs were resuspended in a 0.1% tween-80 solution as a surfactant (Merck KGaA) and rinsed with deionized water three times.

3.4 Diet composition

Thanks to the Department of Agriculture, Food, Environmental and Animal Science of University of Udine (Italy), the experimental diets were obtained

from conventional ingredients according to the nutritional requirements of zebrafish.

A control diet (CTRL) without contamination was prepared with the proximate composition of a standard commercial zebrafish diet (Zebrafeed; Sparos LDA, Olhão, Portugal) and four experimental diets containing MPs were created by adding fluorescent polymer AFP and POL at two different concentrations to the control mixed diet (Table 1). Since the basic diet mixture consisted of ingredients from the same batches, the possible non-fluorescent-MPs contamination in all diets was equal, to refer the observed effect only to the presence of fluorescent MPs. Ingredients (Table 1) were mixed for 20 min and then the polymers were added with water in the mixture. Pellets were produced using a 3 mm die meat grinder, dried at 37 °C for 48 hours in a ventilated heater.

Table 1. Composition of the experimental diets used, and relative polymers concentrations: CTRL; AFP50; AFP500; POL50 and POL500.

	CTRL	**AFP50**	**AFP500**	**POL50**	**POL500**
Ingredients (g/kg)					
Fish meal [1]	490	490	490	490	490
CPSP 90 [2]	123	123	123	123	123
Wheat gluten meal [3]	120	120	120	120	120
Pea protein concentrate [4]	120	120	120	120	120
Wheat starch [5]	55	55	55	55	55
Fish oil	60	60	60	60	60

Soya lecithin	8	8	8	8	8
Mineral and vitamin supplements	14	14	14	14	14
Binder (sodiμm alginate) [6]	10	10	10	10	10
MPs concentrations (mg/kg feed)					
AFP (size: 1–5 μm)	-	50	500	-	-
POL (size: 40–47 μm)	-	-	-	50	500
Proximate composition (%)					
Dry matter	94.2	94.2	94.2	94.2	94.2
Crude protein	58.3	58.3	58.3	58.3	58.3
Crude lipid	14.0	14.0	14.0	14.0	14.0
Ash	12.5	12.5	12.5	12.5	12.5

1 Fish meal (61% CP, 11% CF), kindly provided by Skretting Italia, Mozzecane (VR, Italy). 2 Soluble fish protein concentrate (82% CP) (Sopropêche, France). 3 Wheat gluten meal (CP, 81%), kindly provided by Skretting Italia. 4 Pea protein concentrate (CP 69%) (Lombarda trading srl, Cremona, Italy). 5 Wheat starch: pre-gelatinized wheat starch, kindly provided by Skretting Italia. 6 Sodiμm alginate (Merck KGaA, Darmstadt, Germany). For proximate composition, values are reported as mean ± standard deviation of triplicate analyses.

3.5 Experimental design

Newly hatched zebrafish larvae (wild-type strain AB) were obtained from the broodstock colony of Marche Polytechnic University. Larvae were initially reared in fifteen 20 L tanks (3 tanks per experimental group, 500 larvae for each tank) under optimal water conditions: 28±0.5 °C; pH 7±0.1; ammonia and nitrite concentrations <0.01mg/L; and nitrate concentration <10 mg/L.

At 20 days post-fertilization (dpf) fish from each tank, were transferred to 100 L tanks, three tanks per experimental group, equipped with thermoregulation, mechanical and biological filtration (Panaque, Italy) to maintain optimal chemical-physical parameters of the water throughout the experiment, with the same water conditions as for the larval tanks.

The experimental groups were divided as follows: (i) CTRL group, fed the CTRL diet; (ii) AFP50 group, fed the diet AFP50 containing 50 mg/kg of polymer A (range size: 1–5 μm); (iii) AFP500 group, fed the diet AFP500 containing 500 mg/kg of polymer A (range size: 1–5 μm); (iv) POL50 group, fed the diet POL50 containing 50 mg/kg of polymer POL (range size: 40–47 μm); (v) POL500 group, fed the diet POL500 containing 500 mg/kg of polymer POL (range size: 40–47 μm).

From the 5th dpf to the juveniles stage (60 dpf), fish were fed the experimental diet as described in Table 1. The daily intake was equal to 3% of the total biomass present in each of the tanks and was fed to the fish as a powdered dry pellet, the total amount was divided in 2 daily feedings (one in the morning and one the afternoon).

More specifically, from 5 to 10 dpf, zebrafish larvae across all experimental groups were fed rotifers (*Brachionus plicatilis*, 5 individuals/mL) twice daily. Any uneaten food and dead larvae, if observed, were siphoned from the tanks

30 minutes post-feeding and recorded. Fish from each tank were sampled after euthanasia with MS222 (0.3 g/L) at two developmental stages: 60 dpf (juvenile stage). Biological samples were collected and stored appropriately for subsequent analyses. Specifically, samples were placed in biopsy cassettes and fixed in Blouin's solution at 4°C or 4% paraformaldehyde (PFA) for histological analysis and confocal microscopy, respectively. For chemical and molecular analyses, samples were stored in 1.5 mL Eppendorf tubes at −20°C or −80°C. These storage conditions were deemed suitable, as the study focused specifically on fluorescent MPs.

3.6 Growth and survival

At 3 dpf, ten newly hatched larvae were randomly collected from each tank (30 per experimental group) to measure the initial body weight (IBW), with measurements taken in pools of five larvae. For the final body weight (FBW) assessment, at 60 dpf 20 juveniles were randomly selected from each tank (60 per experimental group) and measurements were performed. The IBW and FBW were assessed by an OHAUS Explorer analytical balance (Greifensee, Switzerland), with an accuracy of 0.1 mg. Specific growth rate (SGR) was calculated using the following formula:

$$SGR\ (\%\ day^{-1}) = [(\ln FBW - \ln IBW)/t] \times 100;$$

the term “t” stands for the number of days post IBW collection (57 days).

Finally, the survival rate was calculated by removing the total number of dead specimens from the initial number of fish.

3.7 Confocal Microscope

For confocal microscopy analysis, three subsamples of feed from each experimental diet were analysed to assess the presence of fluorescent microspheres. Liver, gut, and muscle samples from 15 juveniles per experimental group (5 for each tank) were fixed in 4% PFA for 24 h at 4 °C and then stored in 1% phosphate-buffered saline (PBS) at the same temperature. The samples were then placed on concave slides with a glycerol-PBS solution (90:10) and mounted with a coverslip. The presence of fluorescent microspheres in the collected samples was assessed using a Nikon A1R confocal microscope (Nikon Corporation, Tokyo, Japan) mounted on an Eclipse Ti240 Ei inverted microscope. Samples were simultaneously excited at wavelengths of 561/647 nm, and emissions were collected at 615 and 670 nm

to visualize microspheres (red) and tissue texture (blue), respectively. Images were processed using NIS-Element software (version 5.21.00; Nikon).

3.8 Chemical Digestion

For chemical digestion, livers, intestines and muscles (3 fish per tank, 9 per experimental group) were weighed and stored at -20°C in 1.5 mL Eppendorf tubes until KOH-mediated digestion.

Samples were treated with 10% KOH pre-filtered through a glass fibre filter (0.7 µm pore size, Whatman GF/A, Merck KGaA, Darmstadt, Germany). The solution was added to each sample (1:10 w/v) and incubated at 40 °C for 48 hours. The digestion products were then filtered through glass fibre filters (0.7 µm pore size, Whatman GF/A) using a vacuµm pump connected to a filter funnel. The membrane filters were cooled to room temperature and placed in glass Petri dishes until identification and quantification (Chemello et al., 2023).

Quantification of fluorescent MPs was performed using a Zeiss Axio Imager.A2 microscope (Zeiss, Oberkochen, Germany) with Texas red (561 nm) and FITC (491 nm) beams. MPs were counted manually using ZEN Blue 2.3 software (Zeiss) and images were captured using an Axiocam 503 digital camera (Zeiss).

3.9 Histopathology investigations

For histology, fillet , liver and whole intestine samples from 5 juveniles per tank (15 per group) were collected in Bouin's solution (Merck KGaA) at 4°C for histological processing. The fixative solution was used for the first 24 hours then, the samples were stocked in 70% Ethanol (EtOH) solution at 4 °C.

The inclusion process consisted of a first dehydration step of samples through serial baths of increasing EtOH concentration solutions (80, 95, and 100%), then samples were cleared in Xylene (BioOptica, Milano, Italy) and finally embedded in paraffin (BioOptica).

Included samples were ready for cutting 5 µm sections. The sectioning was performed by the Leica model RM2125 RTS (Leica, Nussloch, Germany) microtome. Cuts were adjusted on glass sides including three transversal sections (two slides for fish) collected at a distance from each other of 50 µm. Tissue sections were stained using two distinct methods: i) Mayer's haematoxylin and eosin Y (H&E; Merck KGaA) to evaluate potential tissue architecture alterations and detect any inflammatory event in both intestine and liver; ii) Alcian Blue (Bio-Optica) to quantify the relative abundance of Alcian Blue-positive (Ab+) goblet cells in the intestinal tract. Once the staining step was concluded the slides were closed with the mounting media SafeMount (BioOptica) and finally closed with coverslips.

Each slide was observed under a Zeiss Axio Imager.A2 (Zeiss, Oberkochen, Germany) connected to a combined color digital camera (Axiocam 105, Zeiss), evaluating the transversal sections by the ZEN 2.3 software (Zeiss). Specifically for intestine samples, status and height of mucosal folds were evaluated analysing intestinal sections stained with H&E, while a semi-quantitative analysis of relative Ab+ goblet cells abundance was performed on sections stained with Alcian Blue using the following scores: + = 0 to 3 Ab+ goblet cells per villus; ++ = 3 to 6 Ab+ goblet cells per villus; +++ = more than 6 Ab+ goblet cells per villus.

3.10 Molecular analysis

For molecular biology, 9 livers and 9 intestines per experimental group (3 juveniles for each tank sampled) were collected at the sampling day (60 dpf) and stored in 1.5 mL Eppendorf tubes at -80 °C.

RNA extraction was performed using the TRI Reagent (Merck KGaA). The RNA extracted was eluted in 20 µL of pure water (RNAse-free) (Qiagen, Hilden, Germany). The concentration and the quality of RNA were checked using a NanoPhotometer P-Class (Implen, München, Germany) and by running the samples on a 1% agarose stained with GelRed™. Samples were then stored at -80 °C until next step. The cDNA synthesis was conducted using 1 µg of RNA

that was reverse-transcribed using the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) according to manufacturer instructions.

All quantitative real-time PCR (qPCR) reactions were performed using the iQ5 iCycler thermal cycler (Bio-Rad) with the SYBR green method and the following settings:

- 3 min at 95 °C;
- 45 cycles of 20 s at 95 °C;
- 20 s at the specific annealing temperature (AT) (primer specific);
- 20 s at 72 °C for extension.

The reagents for the polymerase reaction were mixed and spread on a 96-well plate adding to each of the well 1 µL of 1:10 diluted cDNA, 5 µL of fluorescent intercalating agent (2x concentrated iQ™ Sybr Green, Bio-Rad, Milano, Italy), and 0.3 µM of the gene primer (forward and reverse).

In the following table (Table 1) are shown the relative sequences of the primers involved in the analysis. The genes investigated are related to the fish growth, insulin-like growth factor1 (*igf1*); genes involved in immune response (interleukin 1 beta, *il1b*; interleukin 10, *il10*; lipopolysaccharide-induced

TNF factor, *litaf*) and oxidative stress (superoxide dismutase 1, *sod1*; superoxide dismutase 2, *sod2*; catalase, *cat*). To standardize the results have

been used internal reference genes (housekeeping genes), ribosomal protein L13 (*rpl13*) and actin-related protein 2/3 complex subunit 1A (*arpc1a*).

Table 2. List of genes for the quantitative expression. AT = annealing temperature in °C.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	ID Number	AT(°C)
igf1	GGCAAATCTCCACGATCTCTAC	CGGTTTCTCTTGTCTCTCTCAG	ZDB-GENE-010607-2	53
sod1	GTCGTCTGGCTTGTGGAGTG	TGTCAGCGGGCTAGTGCTT	ZDB-GENE-990415-258	60
sod2	CCGGACTATGTTAAGGCCATCT	ACACTCGGTTGCTCTCTTTTCTCT	ZDB-GENE-030131-7742	60
cat	CCAAGGTCTGGTCCCATAA	GCACATGGGTCCATCTCTCT	ZDB-GENE- 000210-20	60
il1b	GCTGGGGATGTGGACTTC	GTGGATTGGGGTTTGATGTG	ZDB-GENE-040702-2	54
il10	ATTTGTGGAGGGCTTTCCTT	AGAGCTGTTGGCAGAATGGT	ZDB-GENE-051111-1	56
litaf	TTGTGGTGGGGTTTGATG	TTGGGGCATTTTATTTTGTAAG	ZDB-GENE-040704-23	53
rpl13	TCTGGAGGACTGTAAGAGGTATGC	AGACGCACAATCTTGAGAGCAG	ZDB-GENE- 031007-1	59
arpc1a	CTGAACATCTCGCCCTTCTC	TAGCCGATCTGCAGACACAC	ZDB-GENE- 040116-1	60

3.11 Data analysis

In this study, the results are shown as the means ± standard deviation. Statistical analyses were performed using the software GraphPad Prism 8 (version 8.0.2, San Diego, CA, USA). Normality of all variables was assessed using the Shapiro–Wilk test, while Levene's test verified the homoscedasticity.

Data concerning biometrics, histology and gene expression were extrapolated through one-way ANOVA followed by Tukey's post-hoc test. Significance was set at $p \leq 0.05$ and the.

4. RESULTS

4.1 Growth and survival

No significant differences among the experimental groups were detected considering SGR data (Fig. 2). The survival rate in all experimental groups was approximately 93 ± 4% at the conclusion of the feeding trial and did not show significant differences.

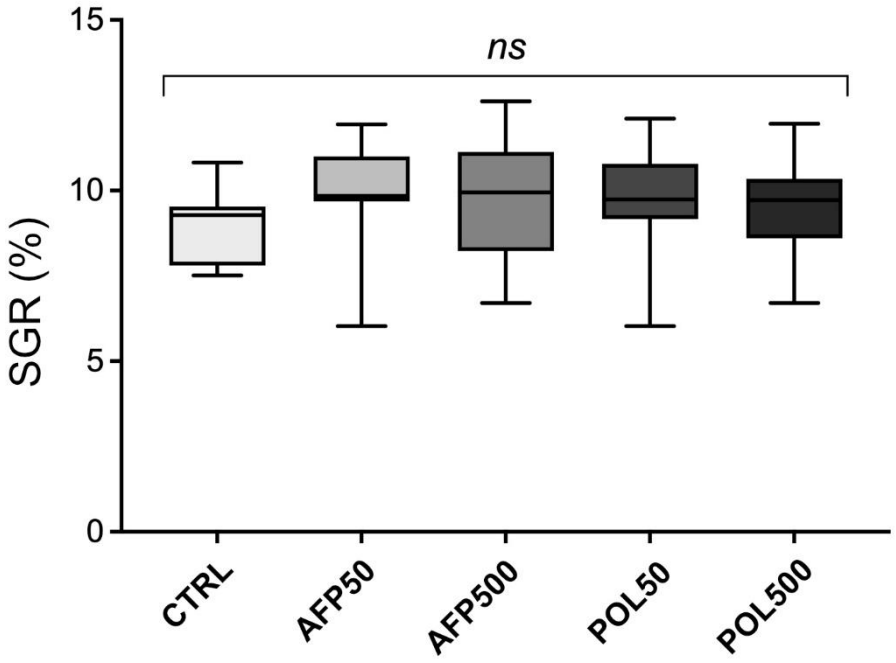


Figure 2. Growth data of *D. rerio* juveniles are represented as specific growth rate in terms of mean percentage ± standard deviation. Significance was set at $p \leq 0.05$.

4.2 Confocal microscopy

The confocal microscope facilitated the observation of the presence of the examined fluorescent MPs in the analysed zebrafish juvenile samples, enabling the assessment of their internalization in various tissues. No fluorescent MPs microbeads were detected in intestine, liver, and muscle of group CTRL and groups fed diets implemented with polymer POL (Fig. 3). Whereas the polymer A was present in all the tissues analysed in both AFP50 and AFP500 groups (Fig. 4).

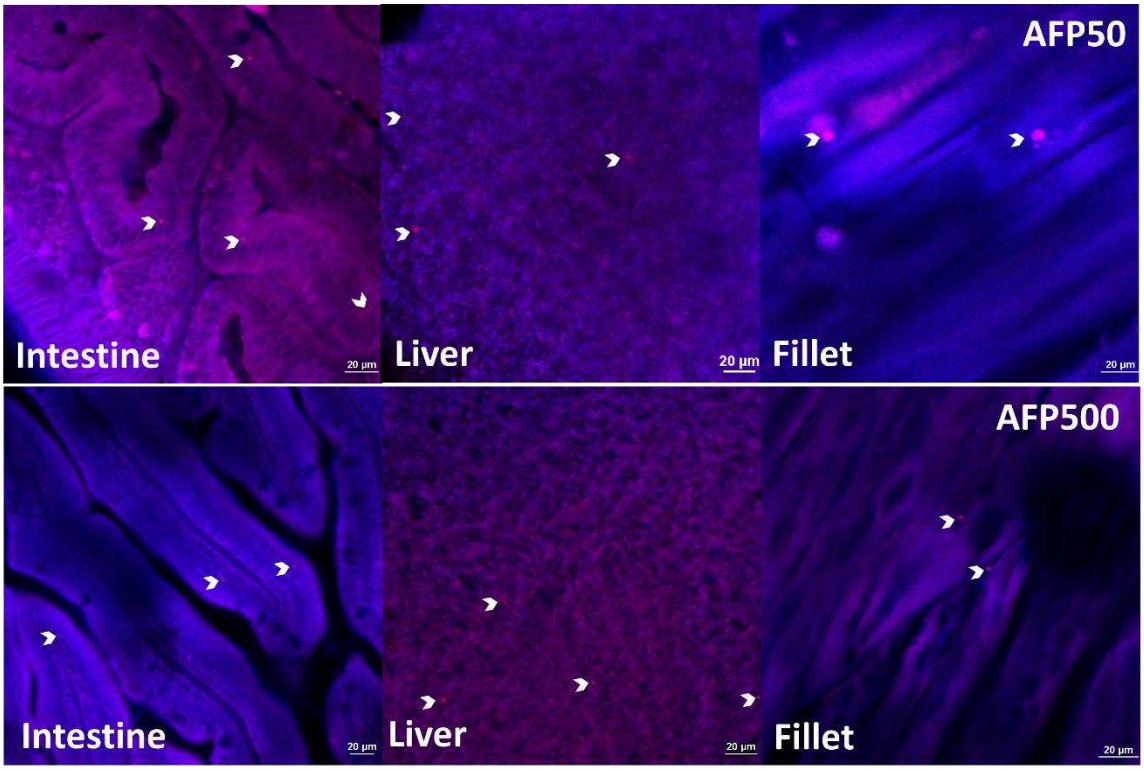


Figure 3. Confocal representative images of intestine, liver and muscle of zebrafish juveniles fed on AFP diets. Evidence of the red-fluorescent MPs are indicated by the arrows. Scale bar = 20 µm.

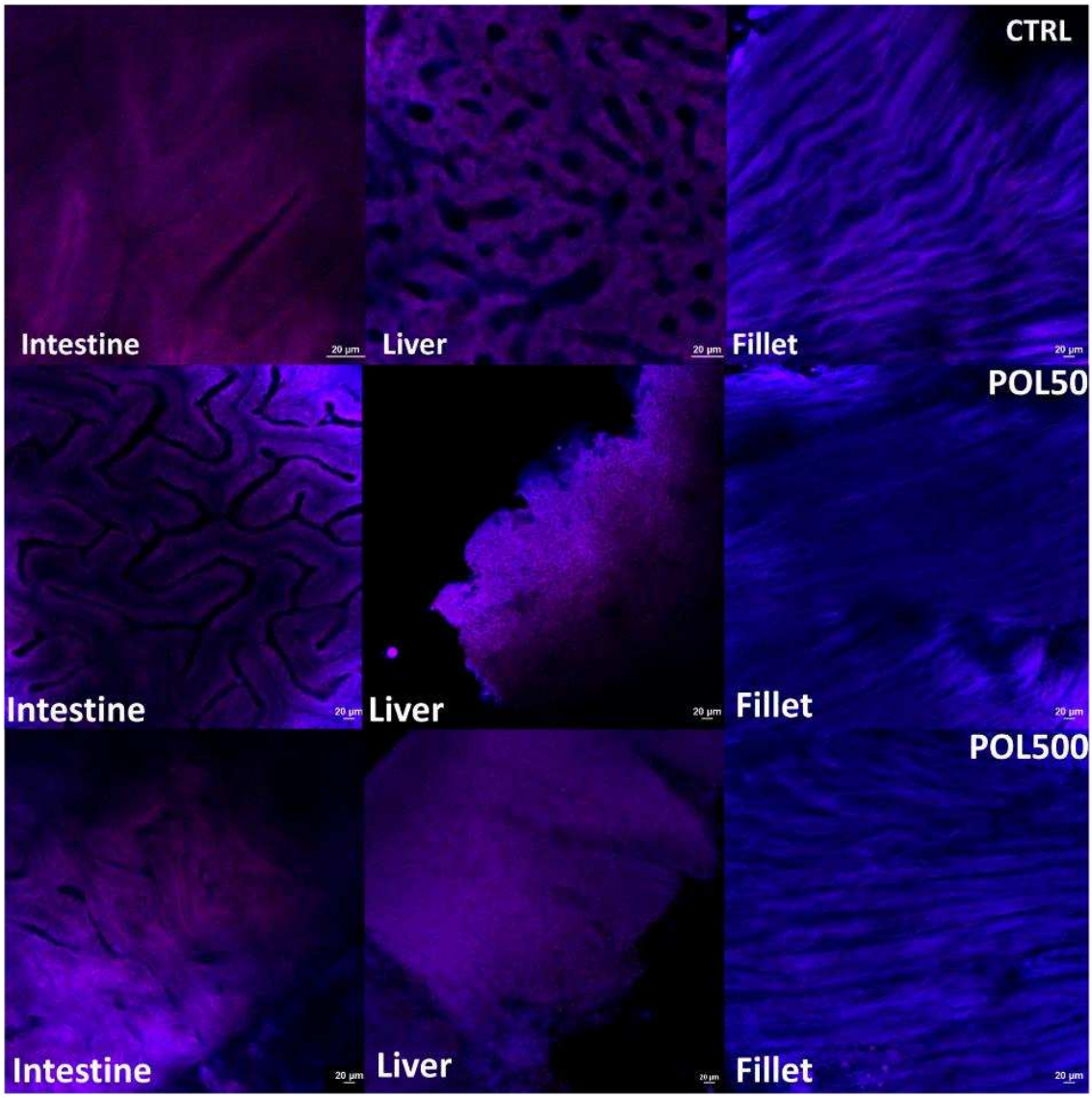


Figure 4. Confocal representative images of intestine, liver and muscle of zebrafish juveniles fend on CTRL, POL50 and POL500 diets. Scale bar = 20 µm.

4.3 MPs quantification

The manual counting of the fluorescent MPs microbeads, after KOH-mediated digestion of the samples and filtration on the membrane, found no MPs in the CTRL group, but they were clearly present in all kinds of samples (intestine, liver, and muscle) of juveniles fed on the polymer AFP, while POL microbeads were detected only (in a scattered way) in intestine samples of the groups POL50 and POL500 (Table 3).

A significant higher number of MPs ($p < 0.05$) was found in the group AFP500 regarding intestine, liver, and muscle samples compared to the other groups. Regarding the polymer POL, no significant difference was detected in the amount between the groups POL50 and POL500.

Table 3. MPs quantification in livers, intestines and fillet of *D. rerio* juveniles.

Sample	**CTRL**	**AFP50**	**AFP500**	**POL50**	**POL500**
Intestine	0	1.15 ± 0.45^{a}	61.93 ± 14.30^{b}	0.14 ± 0.01^{a}	0.64 ± 0.15^{a}
Liver	0	5.4 ± 1.6^{a}	231.1 ± 47.1^{b}	0	0
Muscle	0	0.3 ± 0.1^{a}	4.7 ± 1.2^{b}	0	0

Values are expressed as mean ± standard deviation. Significance was set at $p < 0.05$. The different letters concern to significative differences among data.

4.4 Histology

The histological observations of the intestinal tract and liver parenchyma of the juveniles did not show inflammatory events or pathological alterations (Fig. 5, 6). However, a significant reduction of mucosal folds height was present in POL50 and POL500 groups compared to the others (Table 4). Additionally, these groups fed POL diets, showed a higher relative goblet cells abundance compared to the other groups (Table 4).

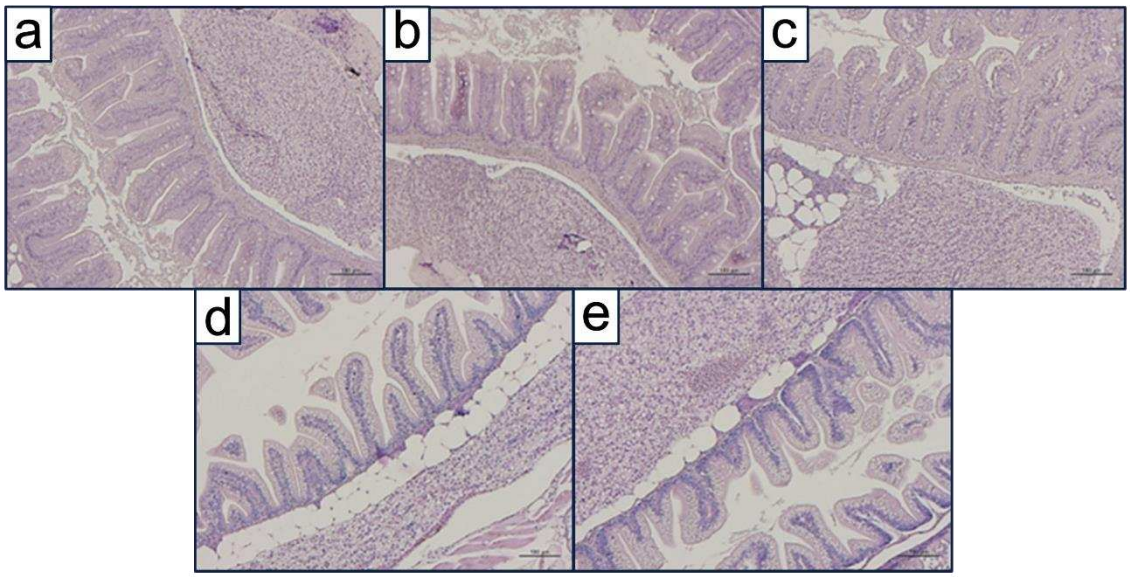


Figure 5. H&E staining. Observations of livers and intestines (mucosal folds) of the 5 experimental groups: (a) CTRL; (b) AFP50; (c) AFP500; (d) POL50; (e) POL500.
Scale bar = 100 μm.

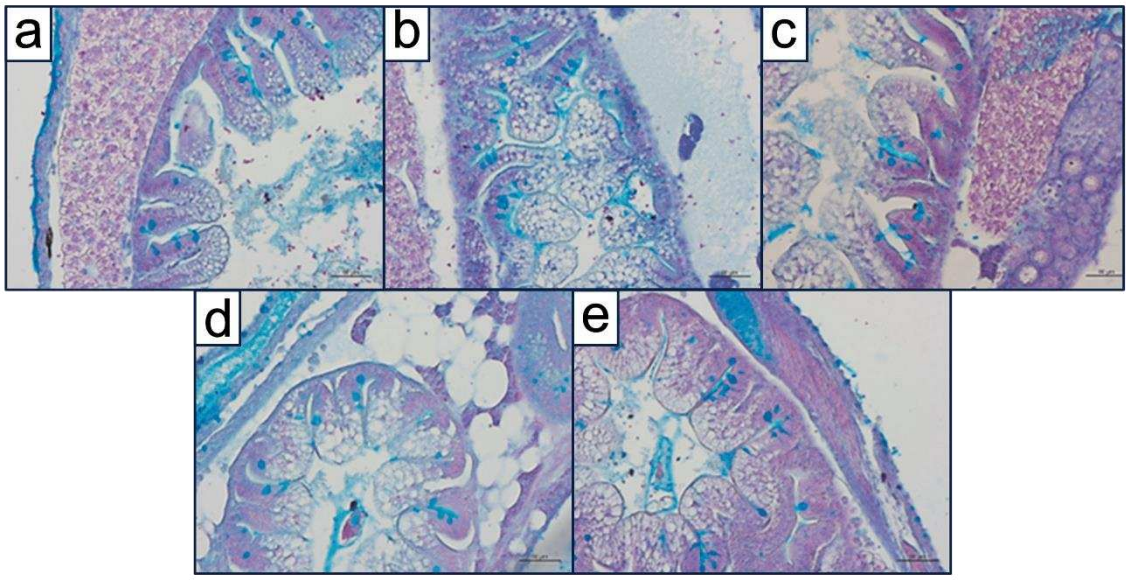


Figure 6. Alcian blue staining of the Ab+ goblet cells (blue spots) in the intestine of the 5 experimental groups: (a) CTRL; (b) AFP50; (c) AFP500; (d) POL50; (e) POL500.
Scale bar = 50 μm.

All histological index measured in the intestine of zebrafish juveniles of each experimental group are reported in Table 4.

Table 4. Schematic visualization of the intestinal and parenchymal tissue characteristics.

	CTRL	**AFP50**	**AFP500**	**POL50**	**POL500**
Inflammatory infiltration (liver)	Absent	Absent	Absent	Absent	Absent
Inflammatory infiltration (intestine)	Absent	Absent	Absent	Absent	Absent
Mucosal fold height (μm)	94.95±5.72 **a**	96.39±12.78 **a**	88.23±9.45 **a**	69.73±12.97 **b**	70.10±13.54 **b**
Ab+ goblet cells	++	++	+++	+++	+++

Significant difference ($p < 0.05$) of the mucosal folds height (denoted by different letters: a e b) and Ab+ goblet cells abundance. Data are reported as mean ± standard deviation. "++" = 4 to 6 Ab+ goblet cells per villus; "+++" = more than 6 per villus.

4.5 Gene expression

Regarding insulin-like growth factor 1 gene expression, *igf1*, a significant upregulation occurred in all the groups fed diets implemented with MPs (AFP50, AFP500, POL50, and POL500) compared to control group (CTRL) (Fig. 8).

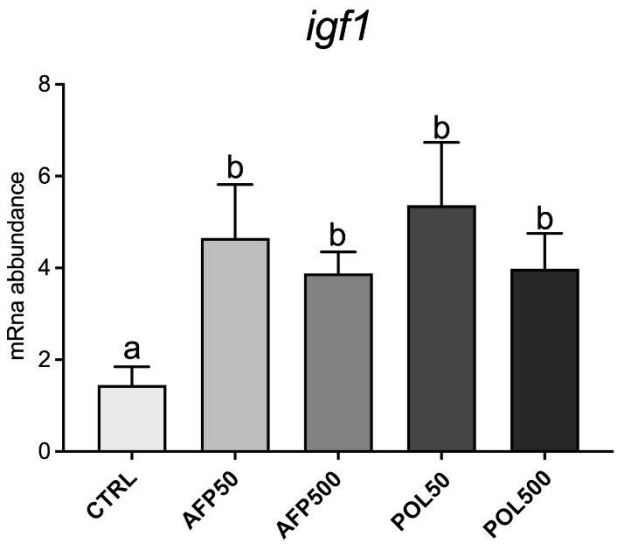


Figure 8. Gene expression of *igf1* in zebrafish. Data are reported as mean ± standard deviation. "a,b" indicate the significant difference ($p<0.05$).

Concerning the immune response at intestinal level, no significant difference in gene expression was detected among all experimental groups in the markers analysed (*il1b*, *il10*, and *litaf*) (Fig. 9).

As regards the expression of the genes involved in oxidative stress response analysed in the liver (Fig. 10), a significant upregulation ($p < 0.05$) was detected in all genes analysed (*sod1*, *sod2*, and *cat*) in the groups AFP50 and AFP500 compared to CTRL group (Fig. 10 a-c). Whereas a significantly higher expression ($p < 0.05$) was found only for the gene *cat* in POL50 and POL500 groups compared to CTRL group (Fig. 10 a).

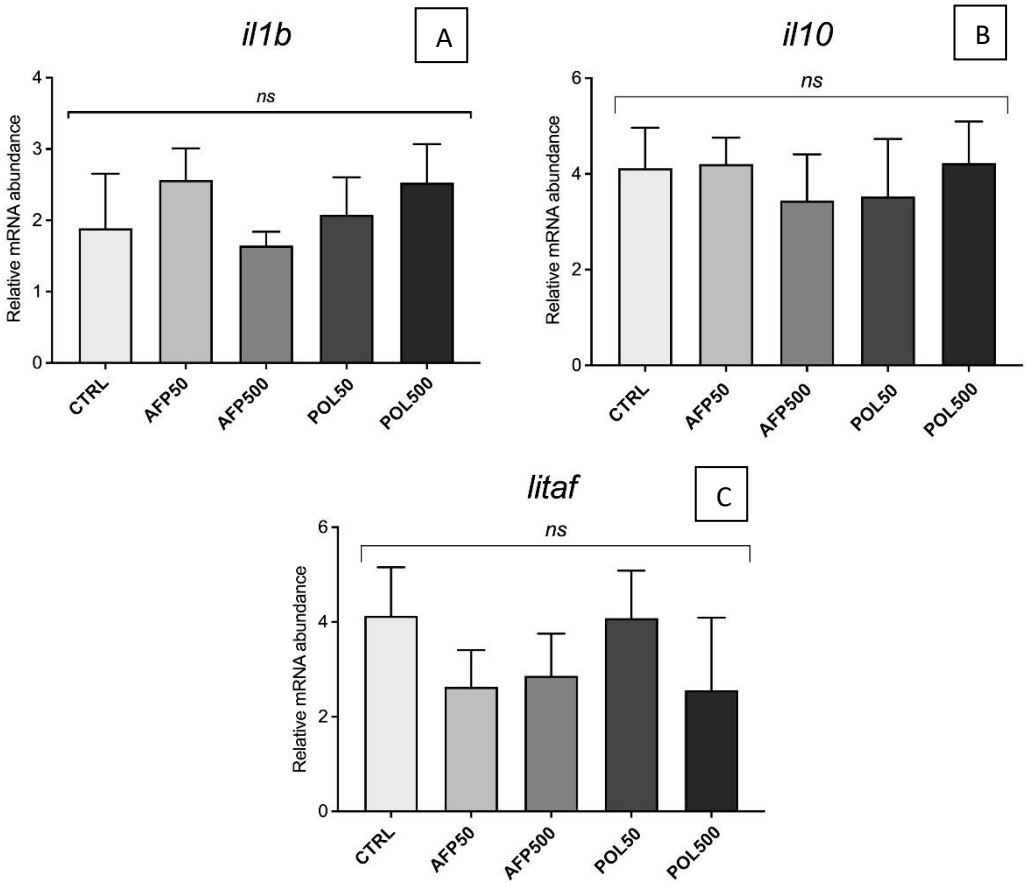


Figure 9. Relative mRNA abundance of immune response genes *il1b* (A), *il10* (B) and *litaf* (C) in zebrafish juveniles' intestines. Data are reported as mean ± standard deviation. No significant differences among the groups ($p<0.05$).

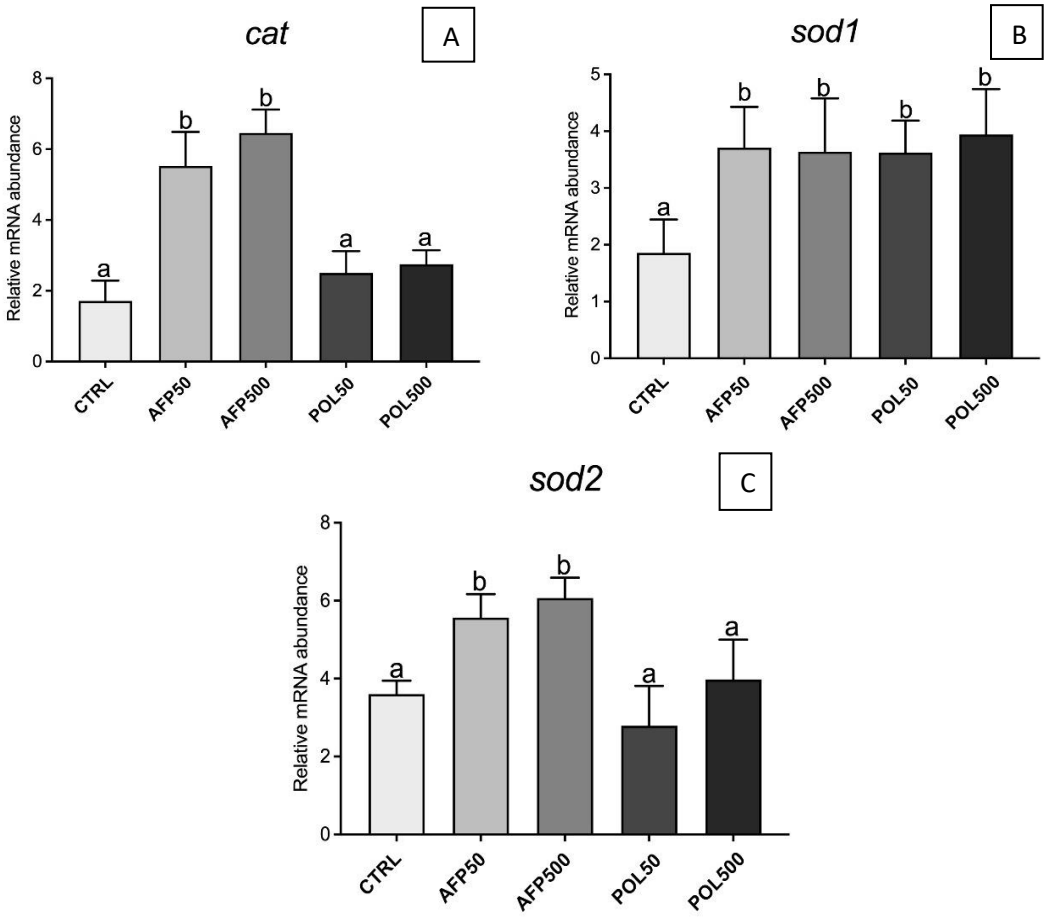


Figure 10. Relative mRNA abundance oxidative stress response genes *cat (A)*; *sod1 (B) and sod2 (C*) expression in zebrafish juveniles' liver. Data are reported as mean ± standard deviation. "a,b" indicate the significant difference (p<0.05).

5. DISCUSSION

MPs pollution is a global issue that afflicts all types of environments (Gasperi et al., 2018; Nunes et al., 2023; Zeb et al., 2024). Furthermore, these contaminants have been detected in living organisms, including aquatic species (Anbumani & Kakkar, 2018; Santana et al., 2017), and have been shown to have detrimental effects on their welfare (Ahrendt et al., 2020; Bhagat et al., 2020b; Santana et al., 2017). The aquaculture sector is also affected by this problematic, as MPs have been found in farmed animals (Wu et al., 2023b; Zhou et al., 2021), with feed identified as one of the primary carriers of these contaminants (Egea-Corbacho et al., 2023a; Matias et al., 2023). However, since different sizes and concentrations of MPs can have different outcomes (Cormier et al., 2021; Hou et al., 2022; Kang et al., 2021), it is fundamental to perform studies that focus on these factors to better understand the issue. On this purpose, the present study aimed to assess the potential different effects of two MPs polymer sizes implemented into fish diets at two different concentrations and administrated to juvenile zebrafish, starting from the larval stage.

The confocal microscopy and quantification analyses showed different fates for the two polymers. The smaller MPs microbeads (AFP) were absorbed at the fish intestinal level and have been detected in liver and muscles of zebrafish

juveniles. The distribution pattern across the different organs and tissues analysed was as follows: relevant amounts were found in the liver, followed by the intestine, and in a considerably less quantity in the muscle. In AFP500 group the number of MPs was significantly higher in all types of samples (intestine, liver, and muscle) analysed compared to those fed AFP50 diet, reflecting the importance for the concentration related effect as suggested by studies on other fish species such as sea bream (Capó et al., 2021; Rios-Fuster et al., 2021), zebrafish larvae (Qiang & Cheng, 2019; Wan et al., 2019) and adults (Mak et al., 2019).

The liver seems to trap a high amount of these MPs functioning as a retaining organ for MPs. These data are consistent with previous studies that observed the accumulation of MPs in fish liver (Lu et al., 2016; Qiao, Lu, et al., 2019; Su et al., 2019). In addition, this retention leaded to a strong increase in oxidative stress response in fish. A previous study, conducted on gilthead seabream, also reported a relation between MPs intake and oxidative stress response in the fish liver (Capó et al., 2021). In contrast, only a small number of polymer AFP microbeads were found in the muscle, reinforcing the hypothesis that the liver appears to be a critical accumulation site for smaller MPs, suggesting a possible defensive mechanism against contamination of the edible parts of the fish (fillet). MPs translocation was also verified through

another study performed on European sea bass (*D. labrax*) in which 1-5 µm MPs have been detected in the fillet after their dietary administration (Zeytin et al., 2020), while another study conducted on red tilapia (*Oreochromis niloticus*) reported the translocation of MPs (size 0.1 µm) from intestine to brain and liver after exposure (Ding et al., 2018).

As regards the larger polymer used in the present study, the quantification through chemical digestion showed a low amount of microbeads (POL), that have been detected only in the intestine for both POL50 and POL500 groups. No presence of these MPs was observed in other tissues through confocal microscopy analysis, suggesting that they were not absorbed at intestinal level. Consequently, polymer POL was absent in both the fillet and liver of the fish.

Despite this, the intestines of POL groups showed shorter mucosal folds and a higher number of Ab+ goblet cells compared to the other experimental groups. These results may be linked to an abrasive effect of the larger polymer microbeads, causing increased intestinal lubrication through a rise in goblet cells to facilitate the elimination of these MPs. Intestinal mucus increasement was also detected in the study of Lei et al. (2018), in which zebrafish exposed to MPs sized 1-5 µm displayed this response as a mechanism to limit intestinal fold disruption. However, no signs of intestinal inflammation or an increase in the expression of markers related to immune response or oxidative stress were

observed (at the exception of *sod1* expression) in POL groups. On the contrary the AFP groups showed an upregulation of the oxidative stress genes, suggesting that smaller MPs microbeads, have more harmful effects compared to larger ones. This hypothesis is supported by other studies performed on aquatic organisms using different MPs sizes, in which as been demonstrated that the smaller MPs sizes have a more significant negative impact of their welfare (Rist et al., 2017; Yang et al., 2020).

Despite the different toxicological effects observed in fish caused by MPs ingestion the growth and survival rate of juvenile zebrafish was not affected by the consumption of either type of polymer at both concentrations. Although the expression of the *igf1* gene was significantly higher in the groups exposed to MPs, the biometric analysis did not show differences in growth. This suggests that the diets containing MPs in this study did not impact fish growth. This result contrasts with a previous study, conducted by Critchell & Hoogenboom (2018) on *Acanthochromis polyacanthus*, which found a reduction in growth in the presence of MPs. Nevertheless, this finding was consistent with another study that also found no difference in the growth rate of fish fed with MPs (Tarasco et al., 2022). This discrepancy might be due to differences in species, MPs size, concentration, and morphology (Batel et al., 2020; Mak et al., 2019). Therefore, more research is needed to clarify the factors affecting growth in

fish exposed to MPs, such as shape, concentration, and chemical composition of the particles.

6. CONCLUSIONS

This study highlights that zebrafish presents biological barriers against MPs. Polymer POL (size 40-47 µm) was not able to cross the intestinal barrier due to its size, thus did not reach other tissues, while polymer AFP (size 1-5 µm) was absorbed at intestinal level, translocated to other organs and tissues of the fish, and mainly accumulated in the liver. This accumulation led to an increase in oxidative stress response by the fish. Nevertheless, the liver served as a retaining organ for MPs, significantly reducing the translocation of these contaminants to the muscle of the fish. This may offer some protection for fish consumers, but it also raises questions about the fish liver's ability to handle continuous exposure to MPs, which consequently impacts fish welfare. As regards, the bigger polymer (POL), the main effect detected was a shortening of the mucosal folds in the fish intestine due to an abrasive action performed by this MPs size. The organisms responded with an increase in the amount of goblet cells in order to enhance the secretion of mucus to promote the transit of this polymer through the digestive tract. Although the different adverse effects detected in the groups fed the diets implemented with MPs, the growth and

survival of the juvenile zebrafish were not negatively affected by the MPs ingestion.

To further understand the effects of MPs, future studies should evaluate the impact of irregularly shaped MPs, investigate the cumulative effects of chronic MP exposure, study the role of MPs made of different chemical composition, since different polymers may interact with biological tissues in distinct ways, as well as to explore solutions aimed at mitigating the negative effects and absorption of MPs in fish.

REFERENCES

Bibliography

Ahrendt, C., Perez-Venegas, D. J., Urbina, M., Gonzalez, C., Echeveste, P., Aldana, M., Pulgar, J., & Galbán-Malagón, C. (2020). Microplastic ingestion cause intestinal lesions in the intertidal fish Girella laevifrons. *Marine Pollution Bulletin*, *151*, 110795. https://doi.org/https://doi.org/10.1016/j.marpolbul.2019.110795

Anbumani, S., & Kakkar, P. (2018). Ecotoxicological effects of microplastics on biota: a review. *Environmental Science and Pollution Research*, *25*(15), 14373–14396. https://doi.org/10.1007/s11356-018-1999-x

Avio, C. G., Gorbi, S., & Regoli, F. (2015). Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. *Marine Environmental Research*, *111*, 18–26. https://doi.org/10.1016/j.marenvres.2015.06.014

Avio, C. G., Gorbi, S., & Regoli, F. (2017). Plastics and microplastics in the oceans: From emerging pollutants to emerged threat. *Marine Environmental Research*, *128*, 2–11. https://doi.org/https://doi.org/10.1016/j.marenvres.2016.05.012

Barnes, D. K. A., Galgani, F., Thompson, R. C., & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), 1985–1998. https://doi.org/10.1098/rstb.2008.0205

Batel, A., Baumann, L., Carteny, C. C., Cormier, B., Keiter, S. H., & Braunbeck, T. (2020). Histological, enzymatic and chemical analyses of the potential effects of differently

sized microplastic particles upon long-term ingestion in zebrafish (Danio rerio). *Marine Pollution Bulletin*, *153*. https://doi.org/10.1016/j.marpolbul.2020.111022

Bhagat, J., Zang, L., Nishimura, N., & Shimada, Y. (2020a). Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. *Science of The Total Environment*, *728*, 138707. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.138707

Bhagat, J., Zang, L., Nishimura, N., & Shimada, Y. (2020b). Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. *Science of The Total Environment*, *728*, 138707. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.138707

Borrelle, S. B., Rochman, C. M., Liboiron, M., Bond, A. L., Lusher, A., Bradshaw, H., & Provencher, J. F. (2017). Why we need an international agreement on marine plastic pollution. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 114, Issue 38, pp. 9994–9997). National Academy of Sciences. https://doi.org/10.1073/pnas.1714450114

Brennecke, D., Ferreira, E. C., Costa, T. M. M., Appel, D., da Gama, B. A. P., & Lenz, M. (2015). Ingested microplastics (>100μm) are translocated to organs of the tropical fiddler crab Uca rapax. *Marine Pollution Bulletin*, *96*(1–2), 491–495. https://doi.org/10.1016/j.marpolbul.2015.05.001

Browne, M. A., Chapman, M. G., Thompson, R. C., Amaral Zettler, L. A., Jambeck, J., & Mallos, N. J. (2015). Spatial and Temporal Patterns of Stranded Intertidal Marine Debris: Is There a Picture of Global Change? *Environmental Science & Technology*, *49*(12), 7082–7094. https://doi.org/10.1021/es5060572

Campos, D., Rodrigues, A. C. M., Rocha, R. J. M., Martins, R., Candeias-Mendes, A., Castanho, S., Soares, F., Pousão-Ferreira, P., Soares, A. M. V. M., Gravato, C., & Patrício Silva, A. L.

(2021). Are Microplastics Impairing Marine Fish Larviculture?—Preliminary Results with Argyrosomus regius. *Water*, *13*(1). https://doi.org/10.3390/w13010104

Capó, X., Company, J. J., Alomar, C., Compa, M., Sureda, A., Grau, A., Hansjosten, B., López-Vázquez, J., Quintana, J. B., Rodil, R., & Deudero, S. (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. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.144976

Carpenter, E. J., & Smith, K. L. (1972). Plastics on the Sargasso Sea Surface. In *Source: Science* (Vol. 175, Issue 4027).

Cau, A., Avio, C. G., Dessì, C., Moccia, D., Pusceddu, A., Regoli, F., Cannas, R., & Follesa, M. C. (2020). Benthic Crustacean Digestion Can Modulate the Environmental Fate of Microplastics in the Deep Sea. *Environmental Science & Technology*, *54*(8), 4886–4892. https://doi.org/10.1021/acs.est.9b07705

Chemello, G., Faraoni, V., Notarstefano, V., Maradonna, F., Carnevali, O., & Gioacchini, G. (2023). First Evidence of Microplastics in the Yolk and Embryos of Common Cuttlefish (Sepia officinalis) from the Central Adriatic Sea: Evaluation of Embryo and Hatchling Structural Integrity and Development. *Animals*, *13*(1). https://doi.org/10.3390/ani13010095

Chen, G., Li, Y., & Wang, J. (2021). Occurrence and ecological impact of microplastics in aquaculture ecosystems. *Chemosphere*, *274*, 129989. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.129989

Chen, M., Jin, M., Tao, P., Wang, Z., Xie, W., Yu, X., & Wang, K. (2018). Assessment of microplastics derived from mariculture in Xiangshan Bay, China. *Environmental Pollution*, *242*, 1146–1156. https://doi.org/https://doi.org/10.1016/j.envpol.2018.07.133

Conkle, J. L., Báez Del Valle, C. D., & Turner, J. W. (2018). Are We Underestimating Microplastic Contamination in Aquatic Environments? *Environmental Management*, *61*(1), 1–8. https://doi.org/10.1007/s00267-017-0947-8

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*, 125626. https://doi.org/https://doi.org/10.1016/j.jhazmat.2021.125626

Coyle, R., Hardiman, G., & Driscoll, K. O. (2020). Microplastics in the marine environment: A review of their sources, distribution processes, uptake and exchange in ecosystems. *Case Studies in Chemical and Environmental Engineering*, *2*, 100010. https://doi.org/10.1016/j.cscee.2020.100010

Coyle, R., Hardiman, G., & O'Driscoll, K. (2022). Corrigendum to "Microplastics in the marine environment: A review of their sources, distribution processes, uptake and exchange in ecosystems" [Case Studies in Chem. and Environ. Eng. 2 (2020) 1–7]. *Case Studies in Chemical and Environmental Engineering*, *5*, 100146. https://doi.org/https://doi.org/10.1016/j.cscee.2021.100146

Critchell Kay AND Hoogenboom, M. O. (2018). Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (Acanthochromis polyacanthus). *PLOS ONE*, *13*(3), 1–19. https://doi.org/10.1371/journal.pone.0193308

de Bruin, C., de Rijke, E., van Wezel, A. P., & Astefanei, A. (2022). Methodologies to characterize, identify and quantify nano- and sub-micron sized plastics in relevant media for human exposure: a critical review. *Environ. Sci.: Adv.*, *1*(3), 238–258. https://doi.org/10.1039/D1VA00024A

Desforges, J.-P. W., Galbraith, M., & Ross, P. S. (2015). Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean. *Archives of Environmental Contamination and Toxicology*, *69*(3), 320–330. https://doi.org/10.1007/s00244-015-0172-5

Dibke, C., Fischer, M., & Scholz-Böttcher, B. M. (2021). Microplastic Mass Concentrations and Distribution in German Bight Waters by Pyrolysis–Gas Chromatography–Mass Spectrometry/Thermochemolysis Reveal Potential Impact of Marine Coatings: Do Ships Leave Skid Marks? *Environmental Science & Technology*, *55*(4), 2285–2295. https://doi.org/10.1021/acs.est.0c04522

Ding, J., Zhang, S., Razanajatovo, R. M., Zou, H., & Zhu, W. (2018). Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (Oreochromis niloticus). *Environmental Pollution*, *238*, 1–9. https://doi.org/https://doi.org/10.1016/j.envpol.2018.03.001

Egea-Corbacho, A., Martín-García, A. P., Franco, A. A., Albendín, G., Arellano, J. M., Rodríguez-Barroso, R., Coello, M. D., Quiroga, J. M., Cabello, J. F., Iglesias Prado, I., & Malta, E. (2023a). Microplastic in industrial aquaculture: Occurrence in the aquatic environment, feed and organisms (Dicentrarchus labrax). *Science of The Total*

Environment, *904*, 166774. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.166774

Egea-Corbacho, A., Martín-García, A. P., Franco, A. A., Albendín, G., Arellano, J. M., Rodríguez-Barroso, R., Coello, M. D., Quiroga, J. M., Cabello, J. F., Iglesias Prado, I., & Malta, E. jan. (2023b). Microplastic in industrial aquaculture: Occurrence in the aquatic environment, feed and organisms (Dicentrarchus labrax). *Science of the Total Environment*, *904*. https://doi.org/10.1016/j.scitotenv.2023.166774

Fendall, L. S., & Sewell, M. A. (2009). Contributing to marine pollution by washing your face: Microplastics in facial cleansers. *Marine Pollution Bulletin*, *58*(8), 1225–1228. https://doi.org/10.1016/j.marpolbul.2009.04.025

Galloway, T. S., & Lewis, C. N. (2016). Marine microplastics spell big problems for future generations. *Proceedings of the National Academy of Sciences*, *113*(9), 2331–2333. https://doi.org/10.1073/pnas.1600715113

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? *Current Opinion in Environmental Science & Health*, *1*, 1–5. https://doi.org/https://doi.org/10.1016/j.coesh.2017.10.002

Gigault, J., Halle, A. ter, Baudrimont, M., Pascal, P. Y., Gauffre, F., Phi, T. L., El Hadri, H., Grassl, B., & Reynaud, S. (2018). Current opinion: What is a nanoplastic? In *Environmental Pollution* (Vol. 235, pp. 1030–1034). Elsevier Ltd. https://doi.org/10.1016/j.envpol.2018.01.024

Gündoğdu, S., Eroldoğan, O. T., Evliyaoğlu, E., Turchini, G. M., & Wu, X. G. (2021). Fish out, plastic in: Global pattern of plastics in commercial fishmeal. *Aquaculture*, *534*. https://doi.org/10.1016/j.aquaculture.2020.736316

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. https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.01.190

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). *Ecotoxicology and Environmental Safety*, *246*, 114157. https://doi.org/https://doi.org/10.1016/j.ecoenv.2022.114157

Hurley, R. R., Lusher, A. L., Olsen, M., & Nizzetto, L. (2018). Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices. *Environmental Science & Technology*, *52*(13), 7409–7417. https://doi.org/10.1021/acs.est.8b01517

Ivar Do Sul, J. A., & Costa, M. F. (2014). The present and future of microplastic pollution in the marine environment. In *Environmental Pollution* (Vol. 185, pp. 352–364). https://doi.org/10.1016/j.envpol.2013.10.036

Jeong, E., Lee, J.-Y., & Redwan, M. (2024). Animal exposure to microplastics and health effects: A review. *Emerging Contaminants*, *10*(4), 100369. https://doi.org/10.1016/j.emcon.2024.100369

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. *Environmental Pollution*, *235*, 322–329. https://doi.org/10.1016/j.envpol.2017.12.088

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. *Journal of Hazardous Materials*, *405*, 124207. https://doi.org/https://doi.org/10.1016/j.jhazmat.2020.124207

Karbalaei, S., Golieskardi, A., Watt, D. U., Boiret, M., Hanachi, P., Walker, T. R., & Karami, A. (2020). Analysis and inorganic composition of microplastics in commercial Malaysian fish meals. *Marine Pollution Bulletin*, *150*, 110687. https://doi.org/https://doi.org/10.1016/j.marpolbul.2019.110687

Kedzierski, M., Palazot, M., Soccalingame, L., Falcou-Préfol, M., Gorsky, G., Galgani, F., Bruzaud, S., & Pedrotti, M. L. (2022). Chemical composition of microplastics floating on the surface of the Mediterranean Sea. *Marine Pollution Bulletin*, *174*, 113284. https://doi.org/10.1016/j.marpolbul.2021.113284

Khuyen, V. T. K., Le, D. V., Fischer, A. R., & Dornack, C. (2021). Comparison of Microplastic Pollution in Beach Sediment and Seawater at UNESCO Can Gio Mangrove Biosphere Reserve. *Global Challenges*, *5*(11). https://doi.org/10.1002/gch2.202100044

Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics*, *203*(3), 253–310. https://doi.org/10.1002/aja.1002030302

Laale, H. W. (1977). The biology and use of zebrafish, Brachydanio rerio in fisheries research. *Journal of Fish Biology*, *10*(2), 121–173. https://doi.org/10.1111/j.1095-8649.1977.tb04049.x

Lambert, S., & Wagner, M. (2016). Formation of microscopic particles during the degradation of different polymers. *Chemosphere*, *161*, 510–517. https://doi.org/10.1016/j.chemosphere.2016.07.042

Lebreton, L., & Andrady, A. (2019). Future scenarios of global plastic waste generation and disposal. *Palgrave Communications*, *5*(1). https://doi.org/10.1057/s41599-018-0212-7

Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., Hajbane, S., Cunsolo, S., Schwarz, A., Levivier, A., Noble, K., Debeljak, P., Maral, H., Schoeneich-Argent, R., Brambini, R., & Reisser, J. (2018). Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-22939-w

Lee, K. W., Webb, S. E., & Miller, A. L. (1999). A Wave of Free Cytosolic Calcium Traverses Zebrafish Eggs on Activation. *Developmental Biology*, *214*(1), 168–180. https://doi.org/10.1006/dbio.1999.9396

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K. M., & He, D. (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans. *Science of The Total Environment*, *619–620*, 1–8. https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.11.103

Levraud, J.-P., Palha, N., Langevin, C., & Boudinot, P. (2014). Through the looking glass: witnessing host–virus interplay in zebrafish. *Trends in Microbiology*, *22*(9), 490–497. https://doi.org/10.1016/j.tim.2014.04.014

Li, J., Zhang, K., & Zhang, H. (2018). Adsorption of antibiotics on microplastics. *Environmental Pollution*, *237*, 460–467. https://doi.org/10.1016/j.envpol.2018.02.050

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., & Ren, H. (2016). Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (Danio rerio) and Toxic Effects in Liver. *Environmental Science & Technology*, *50*(7), 4054–4060. https://doi.org/10.1021/acs.est.6b00183

Macleod, M., Peter, H., Arp, H., Tekman, M. B., & Jahnke, A. (n.d.). *The global threat from plastic pollution*. https://www.science.org

Mak, C. W., Ching-Fong Yeung, K., & Chan, K. M. (2019). Acute toxic effects of polyethylene microplastic on adult zebrafish. *Ecotoxicology and Environmental Safety*, *182*, 109442. https://doi.org/https://doi.org/10.1016/j.ecoenv.2019.109442

Markic, A., Gaertner, J. C., Gaertner-Mazouni, N., & Koelmans, A. A. (2020). Plastic ingestion by marine fish in the wild. *Critical Reviews in Environmental Science and Technology*, *50*(7), 657–697. https://doi.org/10.1080/10643389.2019.1631990

Matias, R. S., Gomes, S., Barboza, L. G. A., Salazar-Gutierrez, D., Guilhermino, L., & Valente, L. M. P. (2023). Microplastics in water, feed and tissues of European seabass reared in a recirculation aquaculture system (RAS). *Chemosphere*, *335*, 139055. https://doi.org/https://doi.org/10.1016/j.chemosphere.2023.139055

McClure, M. M., McIntyre, P. B., & McCune, A. R. (2006). Notes on the natural diet and habitat of eight danionin fishes, including the zebrafish *Danio rerio*. *Journal of Fish Biology*, *69*(2), 553–570. https://doi.org/10.1111/j.1095-8649.2006.01125.x

Miao, C., Zhang, J., Jin, R., Li, T., Zhao, Y., & Shen, M. (2023). Microplastics in aquaculture systems: Occurrence, ecological threats and control strategies. *Chemosphere*, *340*, 139924. https://doi.org/https://doi.org/10.1016/j.chemosphere.2023.139924

Nunes, B. Z., Huang, Y., Ribeiro, V. V., Wu, S., Holbech, H., Moreira, L. B., Xu, E. G., & Castro, I. B. (2023). Microplastic contamination in seawater across global marine protected areas boundaries. *Environmental Pollution*, *316*, 120692. https://doi.org/https://doi.org/10.1016/j.envpol.2022.120692

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K. O., Wollenberger, L., Santos, E. M., Paull, G. C., VanLook, K. J. W., & Tyler, C. R. (2009). A critical analysis of the biological impacts of plasticizers on wildlife. In *Philosophical Transactions of the Royal Society B: Biological Sciences* (Vol. 364, Issue 1526, pp. 2047–2062). Royal Society. https://doi.org/10.1098/rstb.2008.0242

Pitt, J. A., Kozal, J. S., Jayasundara, N., Massarsky, A., Trevisan, R., Geitner, N., Wiesner, M., Levin, E. D., & Di Giulio, R. T. (2018). Uptake, tissue distribution, and toxicity of polystyrene nanoparticles in developing zebrafish (Danio rerio). *Aquatic Toxicology*, *194*, 185–194. https://doi.org/https://doi.org/10.1016/j.aquatox.2017.11.017

Qiang, L., & Cheng, J. (2019). Exposure to microplastics decreases swimming competence in larval zebrafish (Danio rerio). *Ecotoxicology and Environmental Safety*, *176*, 226–233. https://doi.org/https://doi.org/10.1016/j.ecoenv.2019.03.088

Qiao, R., Deng, Y., Zhang, S., Wolosker, M. B., Zhu, Q., Ren, H., & Zhang, Y. (2019). Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. *Chemosphere*, *236*, 124334. https://doi.org/https://doi.org/10.1016/j.chemosphere.2019.07.065

Qiao, R., Lu, K., Deng, Y., Ren, H., & Zhang, Y. (2019). Combined effects of polystyrene microplastics and natural organic matter on the accumulation and toxicity of copper in zebrafish. *Science of The Total Environment*, *682*, 128–137. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.05.163

Rahman Khan, F., & Sulaiman Alhewairini, S. (2019). Zebrafish (*Danio rerio*) as a Model Organism. In *Current Trends in Cancer Management*. IntechOpen. https://doi.org/10.5772/intechopen.81517

Reed, B. T., & Jennings, M. (2011). *Guidance on the housing and care of zebrafish Danio rerio.* https://api.semanticscholar.org/CorpusID:83444853

Ribas, L., & Piferrer, F. (2014). The zebrafish (anio rerio) as a model organism, with emphasis on applications for finfish aquaculture research. *Reviews in Aquaculture*, *6*(4), 209–240. https://doi.org/https://doi.org/10.1111/raq.12041

Rios-Fuster, B., Arechavala-Lopez, P., García-Marcos, K., Alomar, C., Compa, M., Álvarez, E., Julià, M. M., Solomando Martí, A., Sureda, A., & Deudero, S. (2021). Experimental evidence of physiological and behavioral effects of microplastic ingestion in Sparus aurata. *Aquatic Toxicology*, *231*, 105737. https://doi.org/https://doi.org/10.1016/j.aquatox.2020.105737

Rist, S., Baun, A., & Hartmann, N. B. (2017). Ingestion of micro- and nanoplastics in Daphnia magna – Quantification of body burdens and assessment of feeding rates and reproduction. *Environmental Pollution*, *228*, 398–407. https://doi.org/https://doi.org/10.1016/j.envpol.2017.05.048

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*(1), 154–159. https://doi.org/https://doi.org/10.1016/j.marpolbul.2017.05.061

Solomando, A., Capó, X., Alomar, C., Álvarez, 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. *Environmental Pollution*, *266*, 115295. https://doi.org/https://doi.org/10.1016/j.envpol.2020.115295

Spence, R., Gerlach, G., Lawrence, C., & Smith, C. (2008). The behaviour and ecology of the zebrafish, Danio rerio. *Biological Reviews*, *83*(1), 13–34. https://doi.org/https://doi.org/10.1111/j.1469-185X.2007.00030.x

Spence, R., Jordan, W. C., & Smith, C. (2006). Genetic analysis of male reproductive success in relation to density in the zebrafish, Danio rerio. *Frontiers in Zoology*, *3*(1), 5. https://doi.org/10.1186/1742-9994-3-5

Strungaru, S.-A., Jijie, R., Nicoara, M., Plavan, G., & Faggio, C. (2019). Micro- (nano) plastics in freshwater ecosystems: Abundance, toxicological impact and quantification methodology. *TrAC Trends in Analytical Chemistry*, *110*, 116–128. https://doi.org/https://doi.org/10.1016/j.trac.2018.10.025

Su, L., Deng, H., Li, B., Chen, Q., Pettigrove, V., Wu, C., & Shi, H. (2019). The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China. *Journal of Hazardous Materials*, *365*, 716–724. https://doi.org/https://doi.org/10.1016/j.jhazmat.2018.11.024

Tarasco, M., Gavaia, P. J., Bensimon-Brito, A., Cordelières, F. P., Santos, T., Martins, G., de Castro, D. T., Silva, N., Cabrita, E., Bebianno, M. J., Stainier, D. Y. R., Cancela, M. L., &

Laizé, V. (2022). Effects of pristine or contaminated polyethylene microplastics on zebrafish development. *Chemosphere*, *303*, 135198. https://doi.org/https://doi.org/10.1016/j.chemosphere.2022.135198

Thompson, R. C. (2015). Microplastics in the Marine Environment: Sources, Consequences and Solutions. In L. and K. M. Bergmann Melanie and Gutow (Ed.), *Marine Anthropogenic Litter* (pp. 185–200). Springer International Publishing. https://doi.org/10.1007/978-3-319-16510-3_7

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., McGonigle, D., & Russell, A. E. (2004). Lost at Sea: Where Is All the Plastic? *Science*, *304*(5672), 838. https://doi.org/10.1126/science.1094559

Upendra, S., & Kaur, J. (2023). Microplastic Pollution in Seawater: A Review Study. *Nature Environment and Pollution Technology*, *22*(3), 1635–1641. https://doi.org/10.46488/NEPT.2023.v22i03.050

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. *Environmental Pollution*, *199*, 10–17. https://doi.org/10.1016/j.envpol.2015.01.008

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

Wan, Z., Wang, C., Zhou, J., Shen, M., Wang, X., Fu, Z., & Jin, Y. (2019). Effects of polystyrene microplastics on the composition of the microbiome and metabolism in larval zebrafish.

Chemosphere, *217*, 646–658. https://doi.org/https://doi.org/10.1016/j.chemosphere.2018.11.070

Wang, W., Ge, J., Yu, X., & Li, H. (2020). Environmental fate and impacts of microplastics in soil ecosystems: Progress and perspective. *Science of The Total Environment*, *708*, 134841. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.134841

Wu, H., Hou, J., & Wang, X. (2023a). A review of microplastic pollution in aquaculture: Sources, effects, removal strategies and prospects. *Ecotoxicology and Environmental Safety*, *252*, 114567. https://doi.org/https://doi.org/10.1016/j.ecoenv.2023.114567

Wu, H., Hou, J., & Wang, X. (2023b). A review of microplastic pollution in aquaculture: Sources, effects, removal strategies and prospects. *Ecotoxicology and Environmental Safety*, *252*, 114567. https://doi.org/https://doi.org/10.1016/j.ecoenv.2023.114567

Yang, H., Xiong, H., Mi, K., Xue, W., Wei, W., & Zhang, Y. (2020). Toxicity comparison of nano-sized and micron-sized microplastics to Goldfish Carassius auratus Larvae. *Journal of Hazardous Materials*, *388*, 122058. https://doi.org/https://doi.org/10.1016/j.jhazmat.2020.122058

Zeb, A., Liu, W., Ali, N., Shi, R., Wang, Q., Wang, J., Li, J., Yin, C., Liu, J., Yu, M., & Liu, J. (2024). Microplastic pollution in terrestrial ecosystems: Global implications and sustainable solutions. *Journal of Hazardous Materials*, *461*, 132636. https://doi.org/https://doi.org/10.1016/j.jhazmat.2023.132636

Zeytin, S., Wagner, G., Mackay-Roberts, N., Gerdts, G., Schuirmann, E., Klockmann, S., & Slater, M. (2020). Quantifying microplastic translocation from feed to the fillet in European sea bass Dicentrarchus labrax. *Marine Pollution Bulletin*, *156*, 111210. https://doi.org/https://doi.org/10.1016/j.marpolbul.2020.111210

Zhang, Y., Kang, S., Allen, S., Allen, D., Gao, T., & Sillanpää, M. (2020). Atmospheric microplastics: A review on current status and perspectives. *Earth-Science Reviews*, *203*, 103118. https://doi.org/https://doi.org/10.1016/j.earscirev.2020.103118

Zhou, A., Zhang, Y., Xie, S., Chen, Y., Li, X., Wang, J., & Zou, J. (2021). Microplastics and their potential effects on the aquaculture systems: a critical review. *Reviews in Aquaculture*, *13*(1), 719–733. https://doi.org/https://doi.org/10.1111/raq.12496

Web References

Marine Debris Programme : https://marinedebris.noaa.gov/

Fishbase: https://fishbase.mnhn.fr/search.php